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REVIEW

Netosis, Myeloperoxidase and Halogenative Stress: Interrelationship and Role in Pathology

Oleg M. Panasenko[1,a]*, Irina V. Gorudko[2], Daria V. Grigorieva[2], Tatyana V. Vakhrusheva[1], Alexey V. Sokolov[1,3], and Sergey A. Gusev[1]

[1]*Lopukhin Federal Research and Clinical Center of Physical-Chemical Medicine of Federal Medical-Biological Agency, 119435 Moscow, Russia*

[2]*Belarusian State University, 220030 Minsk, Belarus*

[3]*Federal State Budgetary Scientific Institution "Institute of Experimental Medicine", 197022 Saint-Petersburg, Russia*

[a]*e-mail: o-panas@mail.ru*

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Abstract—This review offers an analysis of the signaling pathways for suicidal and vital NETosis, with a focus on myeloperoxidase and the reactive halogen species generated in the reactions it catalyzes. We also consider the diversity of neutrophil extracellular traps formed through NETosis and capable of performing both protective (bactericidal) and pathological (proinflammatory) functions. Particular attention is paid to the mechanisms by which myeloperoxidase, released from azurophilic granules during NETosis, facilitates halogenative stress in non-infectious diseases accompanied by inflammation. The interconnection between NETosis and halogenative stress is demonstrated. On the one hand, in halogenative stress, reactive halogen species and products of their reactions with biomolecules stimulate neutrophils to trigger NETosis. On the other hand, active myeloperoxidase within neutrophil extracellular traps or released during their destruction produces reactive halogen species, promoting the development of halogenative stress.

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INTRODUCTION

Neutrophils are the most abundant leukocytes in human blood, accounting for up to 70% of the total leukocyte count, and an essential cellular component of the innate immune system. They exert their antimicrobial function by phagocytosis, degranulation, generation of reactive oxygen, nitrogen, and halogen species (ROS, RNS, and RHS, respectively), and by NETosis [1]. NETosis, a bactericidal strategy of neutrophils, was first observed by Takei et al. in 1996 [2], who showed that phorbol 12-myristate 13-acetate (PMA)-activated neutrophils acquire morphological features of programmed cell death, but distinct from those of previously known apoptosis and necrosis. Brinkmann et al. [3] subsequently characterized this process in detail; and it was later termed NETosis [4]. NETosis is defined by the extracellular release of neutrophil extracellular traps (NETs), which are web-like scaffold structures composed of decondensed DNA embedded with histones and other bactericidal proteins, including myeloperoxidase (MPO), neutrophil elastase (NE), cathepsin G, lactoferrin (LF), gelatinase, etc. NETs capture pathogenic microorganisms, restrict their spread, and kill them with these bactericidal agents.

NETosis was initially regarded exclusively as a form of programmed cell death (so-called suicidal NETosis) [5]. It was later shown, however, that NETosis could proceed without membrane disruption and cell death (so-called vital NETosis) [6], and that NETs could be formed from mitochondrial DNA (mtDNA)

* To whom correspondence should be addressed.

as well as nuclear DNA [7]. Although the Committee on Cell Death Nomenclature recommended, in 2018, against applying the term "NETosis" to processes not associated with cell death [8], we, like other authors, use the term "vital NETosis" for convenience.

MPO, an enzyme stored in azurophilic granules, plays a key role in all antimicrobial mechanisms of neutrophils. This is primarily because MPO catalyzes the oxidation of halides (Hal: chloride, bromide and iodide) by hydrogen peroxide to form highly reactive hypohalous acids (hypochlorous acid (HOCl), hypobromous acid (HOBr), and hypoiodous acid (HOI)), which are collectively called RHS [9] (see reaction (1)):

$$Hal^- + H_2O_2 + H^+ \rightarrow HOHal + H_2O. \quad (1)$$

It is precisely this property that enables MPO to exert its bactericidal function, which is realized during phagocytosis and degranulation, as well as during NETosis [10]. Excessive RHS generation arising from elevated levels of MPO degranulation or activity can produce halogenative stress, which leads to damage to biologically important molecules (nucleic acids, proteins, lipids, carbohydrates, etc.), cells, and tissues, thereby provoking or aggravating inflammatory diseases of diverse etiology [9, 11, 12].

Up to 30% of the total cellular MPO can be released into the extracellular space through degranulation, leakage during phagocytosis, or NETosis, with the majority (80%) bound to NETs [10]. This substantially revises the understanding of how MPO exerts bactericidal activity and promotes halogenative stress in the extracellular compartment. Whereas free extracellular MPO is distributed uniformly in the blood, NET-associated MPO attains far higher local concentrations in close proximity to trapped microbes, ensuring high enzymatic activity and antibacterial efficacy [10]. MPO plasma levels normally do not exceed 170 ng/ml (~ 1.2 nM) [13] and can increase (for example, in acute coronary syndrome) to 1.1 μg/ml (~ 7.6 nM) [14], while the local MPO concentration produced by accumulated phagocytes at inflammatory sites can reach 1-2 mM [15].

Excessive NET formation is known to perturb immune homeostasis and facilitate inflammation across a range of diseases [16, 17]. If NETosis indeed contributes substantially to the release of enzymatically active MPO from neutrophils, a question arises: can NETosis promote halogenative stress and so be implicated in pathological processes? This review therefore examines, on the one hand, the roles of MPO and RHS in NETosis and, on the other hand, the contribution of NETosis to halogenative stress as a factor that accompanies and aggravates diseases associated with chronic non-infectious inflammation.

INTRACELLULAR SIGNALING MECHANISMS OF SUICIDAL AND VITAL NETOSIS

Neutrophils produce NETs in response to diverse stimuli, including PMA, cytokines, autoantibodies, cholesterol crystals, activated platelets, and microbial agents (bacteria, fungi, parasites, and viruses) together with their virulence factors [18]. Each stimulus, however, triggers specific intracellular signaling pathways that lead to a distinct NETosis type [19]. Several reviews provide a detailed description of the agents that stimulate NEtosis through receptor- and non-receptor-mediated pathways [20-22].

Two main types of NETosis are currently distinguished: suicidal NETosis and vital NETosis (Fig. 1) [23]. They differ in a number of respects. First, the functional capacity of neutrophils after NET expulsion in suicidal and vital NETosis differs. Suicidal NETosis involves both dissolution of the nuclear membrane and rupture of the plasma membrane, culminating in cell death. In vital NETosis, neutrophils retain nuclear and plasma membrane integrity and remain capable of performing functions such as, for example, chemotaxis and phagocytosis. Second, the two types differ in the timing of NET release [24]. Suicidal NETosis takes several hours (1 to 4 h) to complete, whereas vital NETosis is faster, with NET expulsion accomplished within 5 to 60 min after cell stimulation [25]. Third, the signaling mechanisms of their activation and regulation diverge significantly, producing differences in the molecular composition of NETs and, consequently, in their bactericidal activity and contribution to pathology.

In vital NETosis, in which neutrophils remain viable, NETs contain not only nuclear DNA but also mtDNA. This NETosis type, characterized by mitochondrial NET release, has recently been identified as a distinct type termed mitochondrial NETosis (Fig. 1), although it is essentially vital. Mitochondrial NETosis was first observed by Yousefi et al. in 2009, following neutrophil activation by granulocyte-macrophage colony-stimulating factor and subsequent stimulation by lipopolysaccharide (LPS) or complement component C5a [7, 26].

Neutrophils undergo NETosis in response to a stimulus initiating intracellular signal transduction pathways, in which ROS, RHS, Ca^{2+}, various protein kinases, cytokines, transcription factors, and other signaling molecules participate.

The key effector stage of NETosis is chromatin decondensation and loss of nuclear integrity, which leads either to rupture of the nuclear envelope in suicidal NETosis or to vesiculation of the nuclear membrane in vital NETosis (Fig. 1). The mechanisms regulating these processes depend on the NETosis inducer [27, 28]. One of the main steps in NET formation is

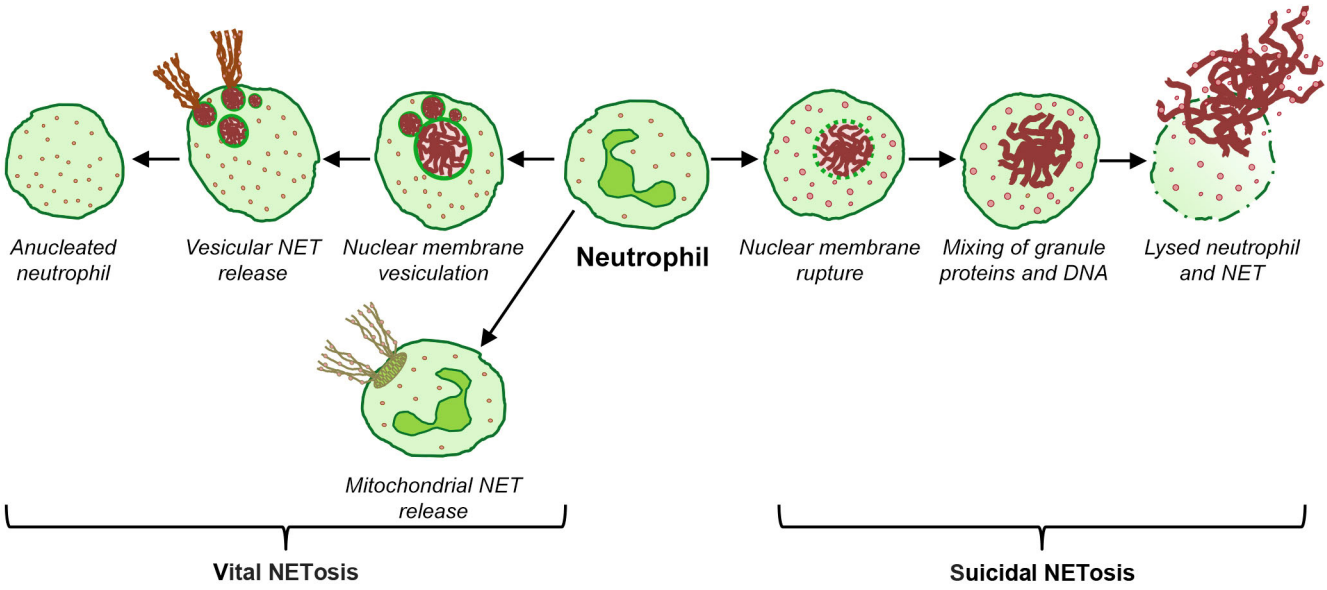

Fig. 1. NETosis types.

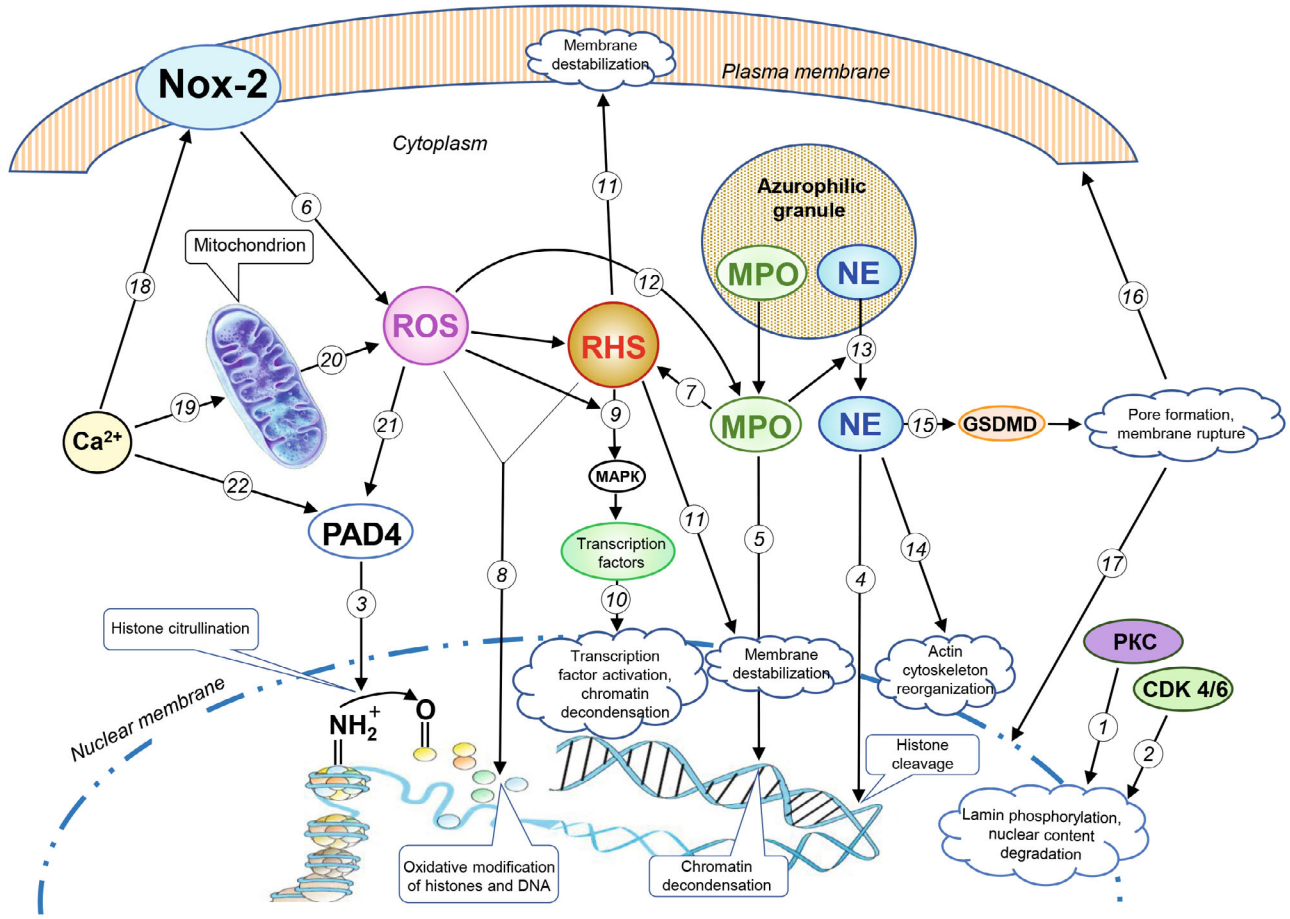

Fig. 2. Intracellular signaling pathways leading to NETosis. See text for explanations.

enzymatic modification of the nuclear molecules, including phosphorylation of lamins, citrullination and proteolytic cleavage of histones, and histone and DNA oxidative modification mediated, among others, by MPO and RHS. These processes prepare the nucleus and cell for subsequent morphological changes during NETosis. The main signaling mechanisms leading to NETosis are shown in Fig. 2.

It is considered that nuclear destruction begins with degradation of the nuclear content, initiated by lamin phosphorylation. Lamin phosphorylation, but not caspase-3-mediated proteolytic cleavage occurring in apoptosis, is responsible for nuclear envelope rupture during NETosis [27, 29, 30]. Protein kinase C (PKC) phosphorylates lamin B [27], while cyclin-dependent protein kinase 4/6 (CDK4/6) phosphorylates lamin A/C [27, 31] (Fig. 2, steps 1 and 2). Mice deficient in CDK4/6 or PKCα show impaired NET formation *in vivo* [27, 31]. CDK4/6 activation is thought to allow neutrophils to utilize cell cycle regulation mechanisms to disrupt the nuclear membrane. S-phase events (DNA synthesis and gene transcription) are absent in NETosis, whereas M-phase events (lamin phosphorylation and centrosome segregation) are required for NET formation [31].

Further nuclear destruction is facilitated by chromatin decondensation achieved in various ways. First of all, of note is post-translational modification of histones by peptidylarginine deiminase 4 (PAD4) (Fig. 2, step 3). PAD4 catalyzes conversion of the positively charged Arg residues in histones to neutral citrulline, leading to a loss of positive charges and weakening of DNA-histone electrostatic interactions, which causes chromatin decondensation [27, 28, 32, 33]. Additionally, histone acetylation also causes chromatin decondensation by reducing the histone's positive charge [34]. Concurrently, NE proteolytically cleaves histones (Fig. 2, step 4) [5, 28]. The histone-DNA interactions are also weakened under the action of MPO (Fig. 2, step 5) [35]. It is assumed that protein kinase-regulated nuclear destruction is crucial for the above-mentioned enzymes to enter the nucleus, since inhibition of PKC or CDK4/6 prevents NE translocation to the nucleus [28].

ROS and RHS generated by NADPH oxidase (Nox-2) and MPO, respectively, make an important contribution to chromatin decondensation and nuclear membrane disintegration (Fig. 2, steps 6 and 7). By reacting in the nucleus with the functional groups of histones and DNA (Fig. 2, step 8), ROS/RHS oxidatively modify them [35], and thereby loosen the protein-DNA interactions. ROS/RHS also activate mitogen-activated protein kinases (MAPKs), including ERK, p38 and JNK (Fig. 2, step 9) [36-39], which phosphorylate transcription factors and thus promote their entry into the nucleus and binding to DNA, which has also recently been considered one of the mechanisms of chromatin decondensation [40] (Fig. 2, step 10). In addition, RHS destabilize the plasma and nuclear membranes through lipid peroxidation [12] and plasmalogen modification [41] (Fig. 2, step 11).

Ultimately, chromatin decondensation and nuclear swelling promote rupture of the nuclear envelope and plasma membrane during suicidal NETosis. Once the nuclear membrane ruptures, decondensed chromatin disperses into the cytoplasm and mixes with the granule proteins to form NETs (Fig. 1).

Thus, a range of effector molecules, including MPO, ROS, NE, PAD4, transcription factors, and protein kinases, whose activity is regulated upon initiation of intracellular signaling in neutrophils in response to a stimulus, disrupt nuclear integrity, as required for NETosis. The main signaling pathways leading to the different NETosis types are outlined below, with emphasis on the roles of MPO and RHS.

Suicidal NETosis. Depending on whether neutrophil NADPH oxidase (Nox-2) is activated, suicidal NETosis is classified as Nox-dependent or Nox-independent.

Neutrophils undergo Nox-dependent NETosis in response to PMA and various pathogens, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* [5, 42]. These stimuli activate signaling enzymes (PKC, MAPK, phosphatidylinositol 3-kinases, non-receptor tyrosine kinase Syk, and others) that initiate assembly of a functionally active NADPH oxidase to produce superoxide anion radical ($^{\bullet}O_2^-$), a precursor of various ROS and RHS [43]. PMA is the most widely used Nox-dependent inducer, and the mechanism of PMA-induced NETosis is, correspondingly, the best characterized. By mimicking diacylglycerol, PMA directly activates PKC [44], which phosphorylates c-Raf MAPK and triggers the Raf/MEK/ERK pathway [37, 45]. PKC, together with other kinases, phosphorylates the cytosolic components of NADPH oxidase, followed by formation, at the plasma membrane, of the active Nox-2 complex generating $^{\bullet}O_2^-$ (Fig. 2, step 6) [46].

ROS/RHS are involved in the regulation of NETosis at several stages. As noted above, they promote chromatin decondensation (Fig. 2, steps 8, 9, 10). ROS also activate MPO-dependent translocation of NE (Fig. 2, steps 12 and 13) and other proteins from azurophilic granules into the cytosol and then into the nucleus, where they decondense chromatin (Fig. 2, steps 4 and 5). Several mechanisms govern this process. First, NE proteolytically cleaves actin filaments [47], blocking phagocytosis and initiating structural changes in the actin cytoskeleton (Fig. 2, step 14), which facilitates entry of NE, MPO, and other granule proteins into the nucleus. Second, NE released from azurophilic granules cleaves gasdermin D (GSDMD) in the cytoplasm (Fig. 2, step 15) to form a pore-forming NH_2-terminal fragment. This fragment integrates into the nuclear, granule, and/or plasma membrane and destabilizes it, ultimately causing membrane rupture (Fig. 2, steps 16 and 17) [48].

Thus, NET formation requires translocation of NE, MPO and other proteins into the nucleus, where,

through mechanisms involving ROS/RHS, they initiate the key effector stage of NETosis, i.e., chromatin decondensation/depolymerization and nuclear swelling.

In Nox-dependent suicidal NETosis, NADPH oxidase activation and ROS/RHS production play a key role, whereas Nox-independent suicidal NETosis is regulated mainly by Ca^{2+} ions. As described below, however, both ROS/RHS and Ca^{2+} ions participate in regulating different NETosis types, underscoring complexity of NET formation.

As a rule, neutrophil activation by various stimuli, including PMA [49], raises cytosolic Ca^{2+} which regulates NETosis through several mechanisms. First, Ca^{2+} contributes to NADPH oxidase activation (Fig. 2, step 18) [50]. Second, rise in Ca^{2+} opens small-conductance Ca^{2+}-activated channels type 3 (SK3) (Fig. 2, step 19), which can modulate mitochondrial ROS (mtROS) production (Fig. 2, step 20) [51]. ROS can thus be generated both through the Ca^{2+}-dependent mitochondrial route and through NADPH oxidase activation, again emphasizing central role of ROS in regulating different NETosis types.

The most important Ca^{2+}-dependent regulator of NETosis, however, is PAD4, which catalyzes conversion of Arg residues to citrulline [52]. The possibility that PAD4 can be activated by ROS has also been proposed (Fig. 2, step 21) [53]. As noted above, PAD4-catalyzed citrullination of Arg and His residues alters the histone-DNA electrostatic interactions, promoting chromatin decondensation. The contribution of PAD4 to NET formation, nonetheless, varies between NETosis types, and its role in Nox-dependent NETosis remains debated. For example, Li et al. [33] reported that about 48.5% of PMA-activated murine neutrophils carried citrullinated histones, whereas Holmes et al. found about 20% [54]. Tatsiy and McDonald [55] showed that GSK484, a specific PAD4 inhibitor, suppresses histone H3 citrullination and inhibits NET formation. Hawez et al. obtained Western blot evidence of increased PAD4 activity and citrullinated histone H3 (CitH3) content in PMA-activated neutrophils [56]. By contrast, Neeli and Radic [57] and Konig and Andrade [58] detected no histone H3 citrullination. Notably, de Bont et al. showed that citrullination in PMA-activated neutrophils is detectable in NETs after inhibition of serine proteases [49], suggesting that serine proteases promote CitH3 degradation. This is one hypothesis that may explain the existing controversy on the role of PAD4 in Nox-dependent suicidal NETosis.

By contrast, the key role of PAD4 in Nox-independent suicidal NETosis is firmly established. It has been revealed that stimuli such as calcium ionophores (A23187 and ionomycin) [49, 59, 60], *Candida albicans* [61], soluble immune complexes [62], *Staphylococcus aureus*, and monosodium urate crystals [63] induce NETosis independently of the NADPH oxidase activity and the ROS it produces. In this type of NETosis, PAD4 is the principal effector of chromatin decondensation [64]. Indeed, following neutrophil stimulation with ionomycin or other inducers of Nox-independent NETosis, Ca^{2+} rises, PAD4 is activated (Fig. 2, step 22), and Arg residues are citrullinated (Fig. 2, step 3) [65].

Until recently, most studies of protein citrullination during NETosis focused on histone H3 [32, 59, 66]. It is now established that citrullination also occurs in actin cytoskeleton-associated proteins and in the cytosolic components of NADPH oxidase [67, 68]. Upon neutrophil activation, these cytosolic components move to the plasma or phagosomal membrane, where they form a complex with membrane-bound components, resulting in the active enzyme [69, 70]. In resting neutrophils, PAD4 is complexed with the cytosolic NADPH oxidase subunits p47phox (neutrophil cytosolic factor 1, NCF1) and p67phox (neutrophil cytosolic factor 2, NCF2). Upon activation (for example, by ionomycin) PAD4 causes citrullination of p47phox and p67phox, followed by their dissociation from PAD4. This dissociation prevents assembly of the active NADPH oxidase complex and respiratory burst [71]. Unlike ionomycin-activated cells, PMA-treated cells sustain $^{\bullet}O_2^-$ generation with no detectable citrullination of p47phox or p67phox [72].

Although Nox-independent suicidal NETosis depends mainly on the increased Ca^{2+} concentration and PAD4 activation, ROS can, nonetheless, contribute to it. In this case, the ROS originate from mitochondria [60, 73].

Vital NETosis. Numerous recent studies show that NET formation does not always end in cell death [25]. This pathway was identified as a distinct NETosis type, termed vital NETosis (Fig. 1). It is induced when neutrophils are stimulated by, for example, *Staphylococcus aureus*, activated platelets, or LPS, with NETs forming rapidly (within ~30 min) and without cell lysis [48]. Platelets, for instance, can induce NET formation through Toll-like receptor 4 [74]. This is an alternative NET formation pathway that is independent of Nox-2. Activation of membrane receptors can raise intracellular Ca^{2+}, which activates PAD4 [60]. The Ca^{2+} rise can also trigger mtROS production (as described above). PAD4, with or without MPO/NE, then induces chromatin decondensation and formation of DNA complexes with histones and other granule proteins, which are exported from the cell in vesicles. Vital NET formation leaves behind viable cells capable of chemotaxis, phagocytosis, and pathogen clearance (Fig. 1) [74].

ROLE OF MYELOPEROXIDASE AND REACTIVE HALOGEN SPECIES IN NETOSIS

MPO is among the key enzymes driving NETosis and the most important bactericidal agent in NETs. Its role in NETosis rests on two main mechanisms: one associated and the other not associated with its enzymatic activity.

As noted in the previous section, MPO promotes chromatin decondensation and NETosis through at least two routes independent of the enzymatic activity. First, during neutrophil activation, MPO released from azurophilic granules translocates into the nucleus (Fig. 2, pathway 5). As a polycationic dimer, MPO binds to nucleosomal DNA through one protomer, while at the same time it binds with the nucleosome acidic patch through two Arg residues of the other. MPO, thereby, displaces histones from the nucleosome, which culminates in nucleosome unfolding and cleavage and chromatin decondensation. Thus, MPO binding to DNA is mediated by specific molecular interactions that convert chromatin into a non-replicative, non-coding state that endows chromatin with the properties of an immune effector [35], without requiring MPO enzymatic activity.

Second, MPO is required for the ROS-induced release of NE from azurophilic granules into the cytosol without membrane disruption. NE then translocates into the nucleus and participates in chromatin decondensation (Fig. 2, steps 13 and 4). Considering that MPO inhibition did not significantly alter this process, the authors of [47] concluded that MPO activity is not required for it. The precise mechanism by which MPO mediates NE translocation from azurophilic granules into the cytoplasm, nonetheless, remains unclear.

Principal mechanisms by which MPO participates in NETosis, however, depend on its enzymatic activity, specifically on generation of HOCl (see reaction (1) in Introduction section). Inhibition of NADPH oxidase with diphenyleneiodonium chloride has repeatedly been shown to prevent NETosis triggered by diverse activators, including PMA [5, 75], LPS [76], nitric oxide donors [77], and bacteria [42].

NADPH oxidase catalyzes one-electron reduction of oxygen to $^{\bullet}O_2^-$, which is dismutated to H_2O_2 either spontaneously or by superoxide dismutase (SOD). MPO then catalyzes oxidation of chloride by H_2O_2 to produce HOCl (reaction (1), Introduction section). HOCl reacts with H_2O_2 to form a further ROS, singlet oxygen ($^1O_2$). The chain of transformations among the main reactive oxygen-containing metabolites is shown schematically in Fig. 3.

Inhibiting NADPH oxidase should interrupt this chain of metabolite transformations, implying that any of its intermediates could in principle regulate NETosis. Identifying the specific ROS required for NETosis is difficult, because the site of NADPH oxidase activation and the extent of degranulation, which together determine the spectrum and amount of ROS produced, vary with the stimulus.

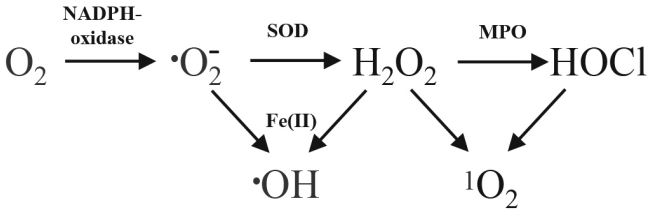

Fig. 3. NADPH oxidase-dependent transformations of reactive oxygen-containing metabolites.

Adding SOD to neutrophils increases NET formation in response to PMA stimulation [75]. Given this, and the fact that $^1O_2$, if formed in activated neutrophils, is present only in trace amounts [78], H_2O_2 and/or HOCl emerge as the more plausible modulators of NETosis. Indeed, exogenous H_2O_2 alone is sufficient to induce NETosis [5, 79-81]. The requirement for this signaling molecule was confirmed using catalase, which eliminates H_2O_2. Neutrophil exposure to catalase reduced NET release, whereas catalase inhibition, by raising H_2O_2, stimulated NETosis [5]. H_2O_2, however, is a substrate of MPO and a precursor of HOCl (reaction (1) (Introduction) and Fig. 3). About 45% of the H_2O_2 consumed by MPO is converted to HOCl [82]. Therefore, all the results obtained for H_2O_2 may suggest involvement of MPO and/or HOCl in the regulation of NETosis.

Indeed, several studies show that addition of HOCl to neutrophils stimulates NETosis [75, 81, 83], whereas addition of the MPO inhibitor 4-aminobenzoic acid hydrazide reduces NET release [81, 84]. Removing extracellular chloride, an MPO substrate in the halogenation cycle, also decreases NETosis. Adding HOCl rescued the NET formation defect caused by pharmacologic MPO inhibition (4-aminobenzoic acid hydrazide) [81]. Moreover, HOCl was capable of stimulating NET release from chronic granulomatous disease neutrophils lacking NADPH oxidase activity and, hence, unable to expel NETs in response to classical stimuli. Finally, exogenously added taurine, abundant in the neutrophil cytosol and able to scavenge HOCl, suppressed PMA- and HOCl-stimulated NET formation, providing a means of cytoprotection against halogenative stress [75]. Collectively, these findings identify HOCl as a necessary and sufficient intermediate in the regulation of NETosis.

Using membrane-permeable MPO inhibitors and ROS/RHS scavengers, the authors of [85] showed that inhibition of intragranular MPO activity or neutralization of intragranular MPO products by luminol

prevented NETosis, whereas complete neutralization of extracellular ROS/RHS did not, as well as addition of exogenous MPO to MPO-deficient neutrophils did not restore their capacity to release NETs. This indicates that MPO generates a NETosis trigger, most likely HOCl, inside the cell. This conclusion is reinforced by the finding that neutrophils with normally functioning MPO cannot rescue NET formation in MPO-deficient neutrophils under co-culture conditions [84].

Histones are the most important NET components. Due to abundant positively charged Arg and Lys residues, these proteins ensure dense packaging of negatively charged DNA into chromatin. Histones are released with NETs into the extracellular space. Because NETs contain HOCl-generating MPO, histones are an evident target for HOCl. HOCl was shown to modify both the linker histone (H1) and the core histones (H2A, H2B, H3 and H4) in a manner dependent on HOCl concentration and incubation time. On exposure to HOCl, histones were shown to lose Lys residues to form unstable Lys N-chloramines, which were converted into Lys nitriles and then into aldehydes [86]. Other oxidation products were also detected, including Met sulfoxide and Met sulfone, as well as chlorination products of Tyr, namely 3-chloro-Tyr and 3,5-dichloro-Tyr, which serve commonly as biomarkers of halogenative stress (see NETosis and Halogenative Stress in Pathology section).

A similar pattern of histone modification was obtained when HOCl was generated by the MPO-catalyzed reaction (1) (Introduction) [87]. Modification of histones by HOCl caused loss of specific residues (Arg, Lys), reduced positive charge, and promoted fragmentation and aggregation. These changes occurred at less than a 20-fold molar excess of HOCl, which is readily attainable under pathophysiological conditions and may contribute to the tissue damage caused by NETs at sites of inflammation [87].

These findings were subsequently confirmed by proteomic analysis of NET proteins from neutrophils and promyelocytic leukemia PLB-985 cells activated with nigericin or PMA [88]. Chlorination of Tyr-88 on histone H4 was more frequent and more extensive in NETs from PMA-activated than from nigericin-activated neutrophils, which is consistent with the non-oxidative pathway of nigericin-induced NETosis. Chlorinated Tyr-88 on histone H4 was also confirmed mass spectrometrically in NETs released by PMA-stimulated neutrophils of patients with systemic lupus erythematosus and rheumatoid arthritis [88].

Owing to its high abundance in blood and interstitial fluid, human serum albumin (HSA) is regarded as the main target for HOCl in plasma under halogenative stress. Adding HOCl to plasma rapidly depleted protein thiols and Met residues, demonstrating capacity of plasma proteins, particularly HSA, to neutralize HOCl effectively [89, 90]. Although reducing agents, such as ascorbate, also react readily with HOCl, their plasma concentrations are too low relative to protein thiols and Met residues to act as major HOCl scavengers [90].

We previously showed that HOCl-modified HSA (HSA-Cl) induces neutrophil degranulation, ROS generation, shape change, actin cytoskeleton reorganization, and NETosis [93, 94]. MPO, NADPH oxidase, and phosphatidylinositol 3-kinase were implicated in HSA-Cl-induced NETosis, which was ROS-dependent, identified as suicidal, and suppressed by monoclonal antibodies against HSA-Cl [94]. HSA-Cl also primed neutrophils and induced NETosis in whole blood *ex vivo*. Using fluorescently labeled anti-MPO antibodies, we found that not all NETs contained MPO, and that NET degradation was accelerated in the presence of HSA-Cl [93].

LF is stored in specific (secondary) neutrophil granules and secreted during degranulation. When modified by HOCl or HOBr, LF loses its ability to mobilize intracellular Ca^{2+}, reorganize actin cytoskeleton, and induce morphological changes in neutrophils. In addition, both halogenated forms, unlike native LF, inhibited the respiratory burst of neutrophils stimulated by N-formyl-L-methionyl-L-leucyl-L-phenylalanine. Native LF inhibited NETosis, and LF modified by HOCl or HOBr retained the ability to inhibit PMA- or ionomycin-induced NETosis [94]. These results confirm a hypothesis that proteins modified in MPO-dependent reactions can regulate NET formation at sites of inflammation, extending the list of compounds capable of modulating NETosis.

HOCl can modify plasmalogens, a class of cellular glycerophospholipids, by reacting with the vinyl ether bond at the sn-1 position [41]. The reaction products are lysophosphatidylcholine and a long-chain 2-Cl-aldehyde, which is readily oxidized to 2-Cl-fatty acid (2-Cl-FA). Neutrophils treated with physiological concentrations of 2-Cl-FA form NETs [95-97]. 2-Cl-FA-induced NETosis is characterized by MPO binding to DNA and redistribution of NE to the perinuclear space. Whereas PMA-induced NETosis depended on NADPH oxidase, MPO, Ca^{2+} mobilization, and PAD4, NETosis elicited by exogenous 2-Cl-FA depended only on Ca^{2+} mobilization and PAD4. Because 2-Cl-FA formation requires NADPH oxidase and MPO activity, exogenous 2-Cl-FA allows bypassing the need for these enzymes in NETosis. Consistent with this, MPO-deficient neutrophils (bone marrow-derived neutrophils from MPO-knockout mice) failed to release NETs in response to PMA [95] but formed NETs in response to 2-Cl-FA. These results are also in agreement with the finding that HOCl treatment restores NETosis in such neutrophils, presumably through 2-Cl-FA formed in the reaction of HOCl with plasmalogens [95].

2-Cl-FA was shown to covalently modify multiple neutrophil proteins, including key NET-forming proteins such as PAD4, defensin alpha 3, and neutrophil collagenase [97]. In addition, 2-Cl-FA increased the PAD4 activity. After NET release, 2-Cl-FA-modified proteins were found across the neutrophil surface and along the DNA strands. The authors suggested that modification and activation of PAD4 by 2-Cl-FA is a key component of 2-Cl-FA-induced NETosis [97].

HOCl is a precursor of free radicals [9], promoting initiation of radical reactions, including lipid peroxidation [12]. The radical scavenger trolox inhibited PMA- or LPS-induced NETosis in murine neutrophils [80]. LPS/platelet co-stimulation of murine neutrophils caused lipid peroxide accumulation inducing NETosis. The alkaloid capsaicin (8-methyl-N-geranyl-6-nonamide) inhibited LPS/platelet-induced lipid peroxide production and suppressed NETosis [98].

On the basis of these studies of ROS/RHS effects on NETosis, ROS/RHS-induced nonspecific oxidation of nuclear components and membrane lipid peroxidation could be proposed to destabilize the nuclear membrane (Fig. 2, step 11), oxidatively modify histones and DNA (Fig. 2, step 8), and promote translocation of the granule proteins into the nucleus (Fig. 2, steps 4 and 5), thereby facilitating NETosis.

HETEROGENEITY OF NEUTROPHIL EXTRACELLULAR TRAPS

It is now evident that NETs constitute a heterogeneous, dynamic population of structures whose abundance and compositional diversity reflect the combined processes of NET formation, degradation, and clearance. As shown in [99], PMA-induced NET production by blood-derived neutrophils varies markedly between healthy subjects. There were variations in neutrophils within a single individual over time in the contents of DNA, total protein, lactate dehydrogenase, NE, proteinase 3, the cytokines IL-8 and IL-1RA, and granulocyte colony-stimulating factor (G-CSF). NET serine protease activity varied substantially within the same individual from day to day, with the main protease activity contributed by NE. Protease susceptibility to inhibitors differed widely across individuals. NET formation changes with age. Neutrophils from neonates form almost no NETs; by two weeks after birth the capacity of neutrophils to produce NETs reaches adult levels and thereafter declines with age [100]. Immature neutrophils are prone to spontaneous NETosis and respond less to stimulation than do mature cells. The capacity to form NETs in response to stimuli increases with neutrophil ageing [101]. The contents of DNA, histone H3, LF, and NE in circulating NETs differ across healthy subjects [102].

NET composition can vary depending on the NETosis type (suicidal, vital, or mitochondrial). From the experiments with blood from healthy subjects, we found that, following induction of suicidal NETosis with PMA, the NETs formed may or may not contain MPO. Moreover, a single neutrophil can form two NETs, one containing MPO and the other lacking detectable MPO [93]. These data indicate that NET composition can vary even when a single NETosis type is initiated.

Notable data were obtained from studies of NET formation induced by 5-fluorouracil. Upon neutrophil exposure to 5-fluorouracil, each segment of the neutrophil nucleus first swells; the nucleus then breaks into separate segments, each of which contacts the plasma membrane and forms its own NET without mixing segment contents with the cytoplasm. A single neutrophil can thereby form up to 4-5 NETs [103]. These results indicate that the resulting NETs contain only the cytoplasmic and granule components that had translocated into the nucleus during the early stages of NETosis and triggered chromatin decondensation and nucleus disintegration. The remaining cytoplasmic components were not incorporated in time.

Petretto et al. [104] performed a comparative proteomic analysis of NETs produced by isolated neutrophils either spontaneously or under stimulation with *Escherichia coli* LPS, A23187 ionophore, or PMA. Of 330 proteins identified, 74 proteins (22%) were common to NETs obtained in any of the four ways, while 30 (9.1%), 7 (2.1%), 27 (8.2%), and 40 (12.1%) proteins were specific to spontaneous NETs or those obtained with LPS, A23187, or PMA, respectively. NETs formed under different conditions differed in both protein composition and post-translational modifications. The modifications include Met sulfoxidation, thiol alkylation, Asn and Gln deamination, Arg deimination (citrullination), and others. Most modifications were detected in PMA-induced NETs (484), followed by A23187- (442) and LPS- (289) induced and spontaneously formed NETs (297). Met oxidation (sulfoxide formation) was the most numerous modification (82%) across all four conditions of NET formation, with the majority of these modifications (45%) occurring in MPO, on surface-localized Met residues. The position of the oxidized residue depended on the stimulus, indicating that different stimuli direct modification to different MPO sites. Proteomic analysis thus shows that NETs induced by different stimuli are heterogeneous in both protein composition and post-translational modification and may, therefore, exert different biological effects [104].

MPO bound to NETs remains active and generates HOCl to kill pathogens, but it can simultaneously target itself and other proteins. MPO autoinactivation was demonstrated by incubation with HOCl or HOBr,

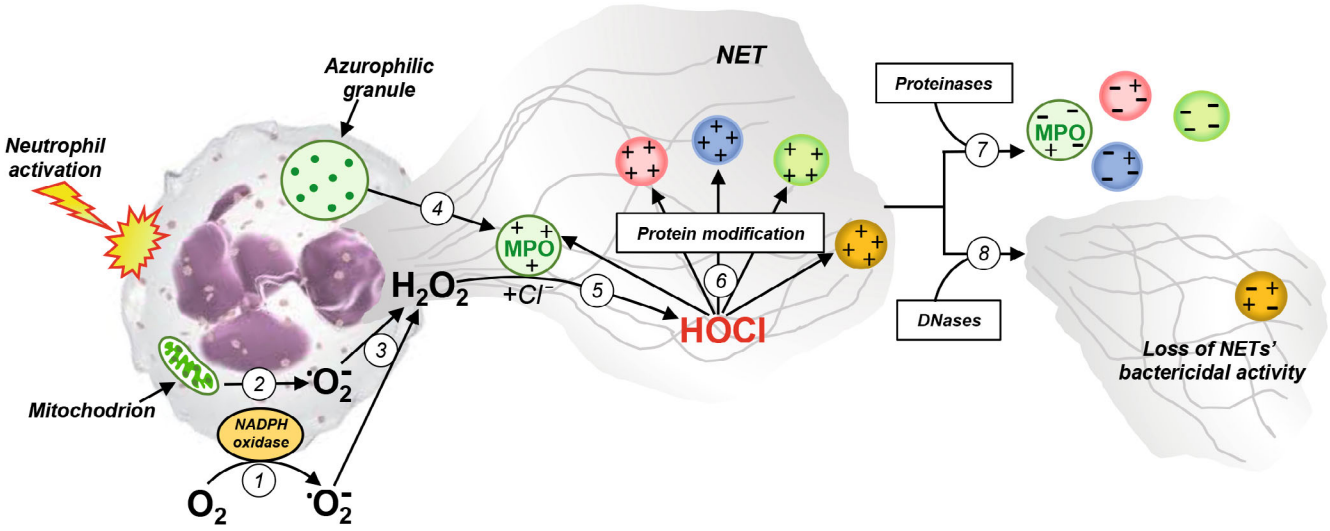

Fig. 4. Scheme illustrating the hypothesis that MPO-mediated damage to proteins occurs in NETs and is accompanied by protein release, leading to loss of NET bactericidal activity. See text for explanations.

or with the substrates H_2O_2 and chloride or bromide at physiological concentrations (140 mM chloride and 100 μM bromide) [105]. The incubation resulted in heme destruction, lowered peroxidase and halogenating activities, and abolished bactericidal activity. Reactions of HOCl/HOBr with proteins frequently reduce their positive charge, as when an H atom in an amino group positively charged at physiological pH is replaced by a halogen to form an uncharged chloramine or bromamine (see reaction (2)):

$$R\text{-}NH_3^+ + HOCl/Br \rightarrow H^+ + R\text{-}NHCl/Br + H_2O. \quad (2)$$

Positive charge on NET-bound proteins can also be reduced by acetylation of amino groups and citrullination of Arg residues. As shown in Fig. 4, stimulation of neutrophils activates NETosis and NADPH oxidase (Nox-dependent NETosis) producing $^{\bullet}O_2^-$ (step 1). In Nox-independent NETosis, mitochondria are the source of $^{\bullet}O_2^-$ (step 2). $^{\bullet}O_2^-$ is dismutated to H_2O_2 by SOD (step 3). MPO released from azurophilic granules and incorporated into NETs (step 4) catalyzes formation of HOCl (step 5), which can modify MPO itself and nearby polycationic proteins (histones, LF, NE, cathepsin G, defensins, lysozyme, etc.) (step 6). This modification makes the proteins less electropositive, weakens their binding to the negatively charged DNA backbone, and causes them to dissociate from the NETs (step 7). NET disintegration is further promoted by proteinases (step 7) and DNases (step 8). Through protein modification, including HOCl-induced modification, and protein loss, the NETs lose their bactericidal activity.

If the hypothesis schematically illustrated in Fig. 4 holds true, variations in the qualitative and quantitative composition of the differently charged molecules within the negatively charged DNA backbone should produce differences in the net charge of NETs. This is supported by experiments using positively and negatively charged microsorbents. It was shown that the blood of healthy subjects and patients with type 2 diabetes mellitus (T2DM) with polyneuropathy contains both positively and negatively charged NETs, and high levels of negatively charged NETs (i.e., those depleted of most of their polycationic bactericidal proteins) are associated with kidney damage [106].

In addition to MPO, NETs contain proteases that degrade proteins, disrupt their binding to DNA, and thereby accelerate NET degradation [35]. Among circulatory enzymes, deoxyribonuclease 1 (DNase1) and DNase1L3 are essential in NET degradation [107-109]. DNase1 preferentially cleaves double-stranded linker DNA, while DNase1L3 efficiently cleaves protein-associated DNA within the chromatin [110-112].

It should be noted that NETs are "struggling" to maintain their stability. For this purpose, they, particularly mitochondrial NETs, contain abundant actin [113]. DNA and actin, two negatively charged biopolymers, bind to positively charged histones to form stable complexes more resistant to degradation. Moreover, actin inhibits DNase1 by complexing directly with the enzyme and thereby preventing DNA degradation in NETs [114, 115].

Extensive evidence thus indicates that, depending on the NETosis stimulus and on modification and degradation of NET components, a heterogeneous population of NETs is formed, differing widely in physicochemical and functional properties, including stability and bactericidal activity, which may predispose to various processes, in particular, pathological ones.

NETOSIS AND HALOGENATIVE STRESS IN PATHOLOGY

Excessive NET formation and/or impaired NET degradation and clearance contribute to inflammation-associated pathologies. Antimicrobial components of NETs are directly cytotoxic. NETs can modulate cellular processes and, by interacting with immune cells, amplify inflammation and promote autoimmunity. NETs are a source of circulating cell-free DNA fragments and histones, which can act as distress-associated molecular patterns that, by binding to receptors on immune cells, elicit a proinflammatory response and promote non-infectious systemic inflammation.

Upon NETosis, MPO is released into the extracellular space, where HOCl and HOBr, the primary products of MPO halogenating activity, undergo further reactions to produce a wide range of halogenated/oxidized compounds of protein, lipid, and other origin. Thus, excessive NET levels and concomitant rise in MPO levels and activity can cause halogenative stress.

NETs can be visualized by several methods, including fluorescence microscopy and flow cytometry, with immunochemical detection of NET components, such as MPO, NE, CitH3 and/or proteinase 3, combined with DNA staining. The presence and extent of NETosis in circulation is assessed by measuring NET degradation products in plasma or serum samples, and in other fluids, such as synovial fluid, using the same approach. It should be noted, however, that DNA, MPO, and NE are released from neutrophils not only via NETosis, but also by degranulation and necrosis, and extracellular DNA can also arise from the necrosis of other cells. More specific markers for NET quantification are extracellular CitH3 and DNA complexes with CitH3, MPO or NE, which can be detected by ELISA.

At physiological pH and halide concentrations, MPO is the only source of HOCl in the human body, so biomolecules modified by this hypohalous acid are specific markers of MPO activity and halogenative stress. 3-Chlorotyrosine, a chemically stable compound, is widely used as a marker of MPO-dependent protein modification. Chlorinated derivatives of DNA, RNA, and lipids are also measured in clinical studies. Stable chlorinated derivatives of nucleotides found at sites of inflammation include 5-chlorouracil, 8-chloro-(2′-deoxy)guanosine, and 5-chloro-(2′-deoxy) cytidine. In lipids, HOCl targets alkenes and vinyl ethers to form chlorinated products such as chlorohydrins of phospholipid acyl chains, α-chloroaldehydes of fatty acids, etc. Halogenative stress markers are discussed in several reviews [9, 12].

Excessive levels of chlorinated derivatives of proteins, lipids, and nucleic acids clearly reflect a rise in extracellular MPO concentration and its activity and are signs of halogenative stress. MPO is expressed most highly in neutrophils and is released extracellularly mainly through NETosis [10], giving reason to assume a link between NETosis and halogenative stress. This link is supported by findings in patients with diseases associated with chronic sterile inflammation. Presented below are some of these findings indicating increased levels of chlorinated products (i.e., halogenative stress), increased NETosis, and increased MPO levels, which together point to MPO as an interlink between NETosis and halogenative stress.

Cardiovascular diseases. Numerous data are available, which indicate interrelationship between NETosis, MPO and halogenative stress in various cardiovascular diseases, including atherosclerosis, chronic heart failure, coronary heart disease, acute coronary syndrome, myocardial infarction, atrial fibrillation, and thrombosis. The patients show elevated levels of circulating NET markers [116, 117], presence of NETs in atherosclerotic lesions [118], and coronary artery thrombi [119, 120]. These patients also have elevated blood levels of MPO [121, 122], which correlate positively with disease severity and risk of further cardiovascular complications [14, 123-125]. MPO content is higher in atherosclerotic lesions than in normal arterial tissue [126-128].

The same holds for chlorinated proteins, whose levels have been found increased in the blood in acute myocardial infarction [121], in atria in atrial fibrillation [122], and in aortic atherosclerotic lesions [129]. Regarding atherosclerosis, chlorination of apolipoproteins in low- and high-density lipoproteins (LDL and HDL) is of particular interest, since HOCl modification renders these lipoproteins atherogenic, thus promoting disease development [41, 130]. LDL and HDL from the arterial lesions and blood of atherosclerosis patients showed elevated 3-chlorotyrosine content compared to that in the lipoproteins from normal aortic tissue and healthy blood [129-131].

Of particular note are studies that assessed MPO and chlorinated proteins concurrently [121, 122, 126, 127]. In the blood of cardiovascular patients and in diseased arterial and cardiac tissues, both were increased, and the study [127] provided immunohistochemical evidence for co-localization of MPO and HOCl-modified protein in atherosclerotic lesions. The levels of 5-chlorouracil were considerably higher in atherosclerotic than in normal aortic tissue [132], and the levels of 2-chlorohexadecanal and lysophosphatidylcholine-chlorohydrin were also markedly elevated in atherosclerotic lesions [133, 134].

Autoimmune diseases. Circulating NET markers are increased in rheumatoid arthritis and correlate positively with disease activity [135]. NET-forming neutrophils have been detected in synovial tissue and rheumatoid nodules [136]. Neutrophils from the blood

and synovial fluid of rheumatoid arthritis patients display higher capacity to form NETs, spontaneously or under LPS stimulation, than neutrophils from the blood of healthy individuals or from the synovial fluid of osteoarthritis patients [136]. In rheumatoid arthritis, blood shows elevated MPO levels and activity [137-139] together with markedly increased 3-chlorotyrosine content, particularly in HDL [140]. The MPO content/activity and the content of 3-chlorotyrosine are also elevated in synovial fluid in rheumatoid arthritis compared to osteoarthritis, and their levels correlate positively [138, 141]. Enhanced NETosis as well as halogenative stress in systemic lupus erythematosus are indicated by elevated blood levels of NET markers [142] and MPO and by increased 3-chlorotyrosine content in HDL [143, 144].

Oncological diseases. The study [145] examined NET production in patients with malignancies at different sites, including mammary gland, gastrointestinal tract, pancreas, bile ducts, peritoneum, prostate, female genital organs, lungs, etc. Elevated blood levels of NET markers were detected in all cases, especially in metastatic diseases, and the same was shown for ovarian cancer [146], bladder cancer [147], and hepatocellular carcinoma [148]. In patients with lung and upper esophagogastric adenocarcinoma, blood levels of NET markers were not only elevated but correlated positively with tumor stage [149].

Elevated MPO levels have been found in the diseased tissue [150], feces [151] and blood [152] of colorectal cancer patients, in the blood of gastric cancer patients [153], and in the bronchoalveolar lavage fluid and blood of lung cancer patients [154]. Increased MPO expression has been detected in patients with ovarian cancer [155]. Halogenative stress hallmarks such as elevated blood levels of 3-chlorotyrosine or elevated extents of tyrosine chlorination in hemoglobin have been shown to be present in patients with colorectal cancer [156] and with gastric and breast cancers [157, 158], respectively.

Neurodegenerative diseases. Blood from Alzheimer's disease (AD) patients with dementia shows neutrophil hyperactivation associated with increased NET formation [159]. Neutrophils normally do not penetrate blood-brain barrier and enter brain parenchyma or cerebrospinal fluid, but they do so in neurological disorders. In AD, neutrophil adhesion to and spreading along the cerebral venule endothelium, and presence of neutrophils and NETs in the brain parenchyma have been observed [160]. MPO content and activity were significantly higher in extracts from post-mortem frontal, parietal, and temporal cortex brain tissues from AD patients than in extracts from control tissues [161]. 3-Chlorotyrosine in proteins isolated from the hippocampus (one of the first brain areas affected by AD) of AD patients was elevated compared to proteins from normal hippocampus [161]. MPO content in the midbrain of Parkinson's disease (PD) patients is heightened compared to control tissue [162]. In both PD and AD, the blood levels of MPO and its activity are elevated and correlate positively with the extent of the pathological process [163, 164].

Type 2 diabetes mellitus. Circulating NET markers are elevated in T2DM patients compared to healthy subjects [165-168]. Moreover, their levels in T2DM patients with retinopathy or foot ulceration exceed those in uncomplicated T2DM [166-168]. In diabetic retinopathy, they are also raised in the vitreous bodies [167]. Diabetic neutrophils are more prone to spontaneous and PMA-induced NET formation than those of healthy subjects [165, 167]. MPO blood levels are higher in T2DM patients than in controls [13, 169, 170]. HOCl production and MPO activity in isolated neutrophils increase in the order healthy < T2DM without dyslipidemia < T2DM with dyslipidemia [171]. MPO and HSA-Cl blood levels are both increased in T2DM patients [165]. Rise in MPO chlorinating activity in T2DM is confirmed by elevated levels of chlorinated hemoglobin in blood [172] and of 8-chloro-(2′-deoxy)guanosine in urine [173].

Bowel diseases. Significant NET accumulation was observed in colonic biopsies from the inflamed regions of patients with active Crohn's disease (CD) and active ulcerative colitis [174]. Intestinal biopsies also demonstrated higher NET formation in CD patients, and especially in patients with ulcerative colitis, than in controls [175]. NET-associated proteins were found over-expressed in the inflamed colon of ulcerative colitis patients [176]. A comparison of biopsies from different intestinal regions within a single CD or ulcerative colitis patient showed decrease in NET formation when moving from the diseased tissue with evidence of severe inflammation to the adjacent tissue with less features of inflammation, and further to the unaffected area, where NETosis levels were the same as in healthy subjects [175, 177]. Greater blood levels of NET markers are observed in patients with CD and ulcerative colitis in the active phase than in the remission phase and in healthy persons [178]. Neutrophils from the blood of ulcerative colitis patients exhibit an enhanced propensity to form NETs upon PMA stimulation when compared to healthy neutrophils [175]. Fecal MPO levels and activity are higher in patients with ulcerative colitis or CD than in healthy individuals and correlate positively with endoscopic disease activity scores [179, 180]. 5-Chloro-(2′-deoxy)cytidine and 3-chlorotyrosine are present in the inflamed colon of ulcerative colitis and CD patients [181]. 3-Chlorotyrosine blood levels are most elevated when the diseases are in the active phase, and decrease during remission, with 3-chlorotyrosine even in inactive ulcerative colitis remaining higher than in healthy individuals [181].

Table 1. Factors whose elevated levels in diseases associated with chronic non-infectious inflammation indicate NETosis activation and halogenative stress

Disease nosology and types	NETs	MPO	Products of MPO chlorinating activity
Cardiovascular diseases (atherosclerosis, chronic heart failure, coronary heart disease, acute coronary syndrome, myocardial infarction, atrial fibrillation, thrombosis)	NETs in blood [116, 117, 119], in atherosclerotic lesions [118], in coronary artery thrombi [119, 120]	MPO in blood [14, 121-125], in atrium tissue [122], in atherosclerotic lesions [126-128]	3-chlorotyrosine in blood [121], in atrium tissue [122], in atherosclerotic lesions [129], in LDL and HDL from atherosclerotic lesions and in circulating LDL and HDL [129-131]; 5-chlorouracil [132], 2-chlorohexadecanal [133] and lysophosphatidylcholine-chlorohydrin [134] in atherosclerotic lesions
Autoimmune diseases (rheumatoid arthritis (RA), systemic lupus erythematosus (SLE))	NETs in blood (RA and SLE) [135, 142], in synovial tissue and rheumatoid nodules (RA) [136]	MPO in blood (RA and SLE) [137-140, 143, 144], in synovial fluid (RA) [138, 141]	3-chlorotyrosine in synovial fluid (RA) [138, 141], in circulating HDL (RA and SLE) [140, 144]
Oncological diseases (malignancies of various localizations)	NETs in blood [145-149]	MPO in blood [152-154], in diseased tissue (colorectal cancer [150]), in feces (colorectal cancer [151]), in bronchoalveolar fluid (lung cancer [154])	3-chlorotyrosine in blood (colorectal cancer [156]), in hemoglobin (stomach cancer [157], breast cancer [158])
Neurodegenerative diseases (Alzheimer's disease (AD), Parkinson's disease (PD))	NETs in blood (AD) [159], in brain tissue (AD) [160]	MPO in blood (AD and PD) [163, 164], in brain tissue (AD and PD) [161, 162]	3-chlorotyrosine in brain tissue (AD) [161]
Type 2 diabetes mellitus	NETs in blood [165-168], in vitreous body in retinopathy [167]	MPO in blood [13, 165, 169, 170]	HSA-Cl in blood [165], 3-chlorotyrosine in hemoglobin [172], 8-chloro-(2′-deoxy)guanosine in urine [173]
Bowel diseases (ulcerative colitis (UC), Crohn's disease (CD))	NETs in intestinal biopsy specimens (UC and CD) [174-177], in blood (UC and CD) [174]	MPO in feces (UC and CD) [179, 180]	3-chlorotyrosine in blood (UC and CD) [181], 3-chlorotyrosine and 5-chloro-(2′-deoxy)cytidine in intestinal biopsy specimens (UC and CD) [181]
Lung diseases (chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF))	NETs in sputum (COPD and CF) [182-185]	MPO in sputum (COPD and CF) [186-188], in bronchoalveolar fluid (CF) [189]	3-chlorotyrosine in sputum (COPD and CF) [187, 188], in bronchoalveolar fluid (CF) [189]

Lung diseases. Atomic force, scanning electron and immunofluorescence microscopies showed that the sputum of cystic fibrosis patients, unlike that of healthy subjects, contains high amounts of NETs [182, 183]. Elevated extracellular DNA levels in cystic fibrosis sputum were shown to associate with reduced pulmonary function [183]. Excessive NETosis and correlation between NET formation and disease severity are also observed in the sputum of patients with chronic obstructive pulmonary disease [184, 185]. Sputum MPO levels are also elevated in these patients, especially during exacerbations [186]. In parallel with the rise in MPO levels, 3-chlorotyrosine levels increase, correlating positively with MPO activity [187]. The same is observed for the sputum of cystic fibrosis patients. Compared to normal sputum, this sputum exhibits elevations in both active MPO and 3-chlorotyrosine levels, with a positive correlation between them [188]. MPO and 3-chlorotyrosine contents were also shown to be elevated in cystic fibrosis bronchoalveolar lavage fluid, displaying a strong positive correlation [189].

The above-described data on NETosis and halogenative stress in diseases are summarized in Table 1.

CONCLUSIONS

It is evident from the above that non-infectious inflammatory pathologies are characterized by activation of NETosis and by halogenative stress, and that the two are interlinked in a “vicious circle”. On the one hand, halogenative stress stimulates NETosis, as shown by experiments in which not only HOCl but also other molecules (proteins, lipids) modified by it activated neutrophils and induced NET formation (see The Role of Myeloperoxidase and Reactive Halogen Species in NETosis section). On the other hand, active MPO bound to NETs or released during their degradation generates HOCl and so contributes to halogenative stress (see Heterogeneity of Neutrophil Extracellular Traps section). This brings several strategies to the fore: (i) timely elimination of “aging” NETs driving inflammation and tissue damage, with concomitant increase in the proportion of newly formed, highly bactericidal NETs; (ii) early diagnosis of NETosis implicated in the development and progression of diseases including those discussed above (see Netosis and Halogenative Stress in Pathology section); and (iii) development of therapeutic approaches to suppress NETosis, promote timely NET degradation, and prevent excessive formation of the products of MPO halogenating activity.

Importantly, steps have already been taken toward these therapeutic goals. Sensitive methods are being developed for real-time imaging of MPO halogenating activity (RHS production) in neutrophils during NETosis [190]. Translationally, therapies that inhibit NET production, facilitate NET degradation, or regulate NET release and activity of NET-bound enzymes by using PAD4, MPO, and NE inhibitors are under active investigation [43]. These interventions aim to counteract pathological consequences of NET formation, while preserving protective role of NETs in innate immunity.

Abbreviations

2-Cl-FA	2-Cl-fatty acid
AD	Alzheimer’s disease
CD	Crohn’s disease
CDK	cyclin-dependent kinase
CitH3	citrullinated histone H3
HDL	high-density lipoproteins
HSA	human serum albumin
HSA-Cl	HOCl-modified human serum albumin
LDL	low-density lipoproteins
LF	lactoferrin
LPS	lipopolysaccharide
MPO	myeloperoxidase
mtDNA	mitochondrial DNA
mtROS	mitochondrial ROS
NE	neutrophil elastase
NET	neutrophil extracellular trap
PAD4	peptidylarginine deiminase 4
PKC	protein kinase C
PMA	phorbol 12-myristate 13-acetate
RHS	reactive halogen species
ROS	reactive oxygen species
SOD	superoxide dismutase
T2DM	type 2 diabetes mellitus

Contributions

O.M.P., I.V.G., and S.A.G. – conceptualization; O.M.P., I.V.G., T.V.V., and S.A.G. – writing; D.V.G., T.V.V., and A.V.S. – editing; O.M.P., D.V.G., and A.V.S. – visualization; O.M.P. – supervision.

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Ethics approval and consent to participate

This work does not contain any studies involving human and animal subjects.

Conflict of interest

The authors of this work declare that they have no conflicts of interest.

REFERENCES

1. Mantovani, A., Cassatella, M. A., Costantini, C., and Jaillon, S. (2011) Neutrophils in the activation and regulation of innate and adaptive immunity, *Nat. Rev. Immunol.*, **11**, 519-531, https://doi.org/10.1038/nri3024.
2. Takei, H., Araki, A., Watanabe, H., Ichinose, A., and Sendo, F. (1996) Rapid killing of human neutrophils by the potent activator phorbol 12-myristate 13-acetate (PMA) accompanied by changes different from typical apoptosis or necrosis, *J. Leukoc. Biol.*, **59**, 229-240, https://doi.org/10.1002/jlb.59.2.229.
3. Brinkmann, V., Reichard, U., Goosmann, C., Fauler, D., Uhlemann, Y., Weiss, D. S., Weinrauch, Y., and Zychlinsky, A. (2004) Neutrophil extracellular traps kill bacteria, *Science*, **303**, 1532-1535, https://doi.org/10.1126/science.1092385.
4. Steinberg, B. E., and Grinstein, S. (2007) Unconventional roles of the NADPH oxidase: signaling, ion homeostasis, and cell death, *Sci. STKE*, **200**7, pe11, https://doi.org/10.1126/stke.3792007pe11.
5. Fuchs, T. A., Abed, U., Goosmann, C., Hurwitz, R., Schulze, I., Wahn, V., Weinrauch, Y., Brinkmann, V., and Zychlinsky, A. (2007) Novel cell death program leads to neutrophil extracellular traps, *J. Cell Biol.*, **176**, 231-241, https://doi.org/10.1083/jcb.200606027.
6. Pilsczek, F. H., Salina, D., Poon, K. K., Fahey, C., Yipp, B. G., Sibley, C. D., Robbins, S. M., Green, F. H., Surette, M. G., Sugai, M., Bowden, M. G., Hussain, M., Zhang, K., and Kubes, P. (2010) A novel mechanism of rapid nuclear neutrophil extracellular trap formation in response to *Staphylococcus aureus*, *J. Immunol.*, **185**, 7413-7425, https://doi.org/10.4049/jimmunol.1000675.
7. Yousefi, S., Mihalache, C., Kozlowski, E., Schmid, I., and Simon, H. U. (2009) Viable neutrophils release mitochondrial DNA to form neutrophil extracellular traps, *Cell Death Differ.*, **16**, 1438-1444, https://doi.org/10.1038/cdd.2009.96.
8. Galluzzi, L., Vitale, I., Aaronson, S. A., Abrams, J. M., Adam, D., Agostinis, P., Alnemri, E. S., Altucci, L., Amelio, I., Andrews, D. W., Annicchiarico-Petruzzelli, M., Antonov, A. V., Arama, E., Baehrecke, E. H., Barlev, N. A., Bazan, N. G., Bernassola, F., Bertrand, M. J. M., Bianchi, K., Blagosklonny, M. V., Blomgren, K., Borner, C., Boya, P., Brenner, C., Campanella, M., Candi, E., Carmona-Gutierrez, D., Cecconi, F., Chan, F. K., Chandel, N. S., Cheng, E. H., Chipuk, J. E., Cidlowski, J. A., Ciechanover, A., Cohen, G. M., Conrad, M., Cubillos-Ruiz, J. R., Czabotar, P. E., D'Angiolella, V., Dawson, T. M., Dawson, V. L., De Laurenzi, V., De Maria, R., Debatin, K. M., DeBerardinis, R. J., Deshmukh, M., Di Daniele, N., Di Virgilio, F., Dixit, V. M., Dixon, S. J., Duckett, C. S., Dynlacht, B. D., El-Deiry, W. S., Elrod, J. W., Fimia, G. M., Fulda, S., García-Sáez, A. J., Garg, A. D., Garrido, C., Gavathiotis, E., Golstein, P., Gottlieb, E., Green, D. R., Greene, L. A., Gronemeyer, H., Gross, A., Hajnoczky, G., Hardwick, J. M., Harris, I. S., Hengartner, M. O., Hetz, C., Ichijo, H., Jäättelä, M., Joseph, B., Jost, P. J., Juin, P. P., Kaiser, W. J., Karin, M., Kaufmann, T., Kepp, O., Kimchi, A., Kitsis, R. N., Klionsky, D. J., Knight, R. A., Kumar, S., Lee, S. W., Lemasters, J. J., Levine, B., Linkermann, A., Lipton, S. A., Lockshin, R. A., López-Otín, C., Lowe, S. W., Luedde, T., Lugli, E., MacFarlane, M., Madeo, F., Malewicz, M., Malorni, W., Manic, G., Marine, J. C., Martin, S. J., Martinou, J. C., Medema, J. P., Mehlen, P., Meier, P., Melino, S., Miao, E. A., Molkentin, J. D., Moll, U. M., Muñoz-Pinedo, C., Nagata, S., Nuñez, G., Oberst, A., Oren, M., Overholtzer, M., Pagano, M., Panaretakis, T., Pasparakis, M., Penninger, J. M., Pereira, D. M., Pervaiz, S., Peter, M. E., Piacentini, M., Pinton, P., Prehn, J. H. M., Puthalakath, H., Rabinovich, G. A., Rehm, M., Rizzuto, R., Rodrigues, C. M. P., Rubinsztein, D. C., Rudel, T., Ryan, K. M., Sayan, E., Scorrano, L., Shao, F., Shi, Y., Silke, J., Simon, H. U., Sistigu, A., Stockwell, B. R., Strasser, A., Szabadkai, G., Tait, S. W. G., Tang, D., Tavernarakis, N., Thorburn, A., Tsujimoto, Y., Turk, B., Vanden Berghe, T., Vandenabeele, P., Vander Heiden, M. G., Villunger, A., Virgin, H. W., Vousden, K. H., Vucic, D., Wagner, E. F., Walczak, H., Wallach, D., Wang, Y., Wells, J. A., Wood, W., Yuan, J., Zakeri, Z., Zhivotovsky, B., Zitvogel, L., Melino, G., and Kroemer, G. (2018) Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death, *Cell Death. Differ.*, **25**, 486-541, https://doi.org/10.1038/s41418-017-0012-4.
9. Panasenko, O. M., Gorudko, I. V., and Sokolov, A. V. (2013) Hypochlorous acid as a precursor of free radicals in living systems, *Biochemistry (Moscow)*, **78**, 1466-1489, https://doi.org/10.1134/S0006297913130075.
10. Parker, H., Albrett, A. M., Kettle, A. J., and Winterbourn, C. C. (2012) Myeloperoxidase associated with neutrophil extracellular traps is active and mediates bacterial killing in the presence of hydrogen peroxide, *J. Leukoc. Biol.*, **91**, 369-376, https://doi.org/10.1189/jlb.0711387.
11. Panasenko, O. M., and Sergienko, V. I. (2010) Halogenative stress and its biomarkers [in Russian], *Vestnik Ros. Akad. Med. Nauk*, **65**, 27-39.
12. Panasenko, O. M., Vladimirov, Yu. A., and Sergienko, V. I. (2024) Free radical lipid peroxidation induced by reactive halogen species, *Biochemistry (Moscow)*, **89**, S148-S179, https://doi.org/10.1134/S0006297924140098.
13. Gorudko, I. V., Kostevich, V. A., Sokolov, F. V., Buko, I. V., Konstantinova, E. E., Tsapaeva, N. L., Mironova, E. V., Zakharova, E. T., Vasilyev, V. B., Cherenkevich, S. N., and Panasenko, O. M. (2011) Increased myeloperoxidase activity is a risk factor for ischemic heart disease in patients with diabetes mellitus, *Biochem. (Mosc.). Suppl. B Biomed. Chem.*, **5**, 307-312, https://doi.org/10.1134/S199075081103005X.

14. Baldus, S., Heeschen, C., Meinertz, T., Zeiher, A. M., Eiserich, J. P., Münzel, T., Simoons, M. L., Hamm, C. W., and CAPTURE Investigators (2003) Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes, *Circulation*, **108**, 1440-1445, https://doi.org/10.1161/01.CIR.0000090690.67322.51.
15. Ulfig, A., and Leichert, L. I. (2021) The effects of neutrophil-generated hypochlorous acid and other hypohalous acids on host and pathogens, *Cell. Mol. Life Sci*, **78**, 385-414, https://doi.org/10.1007/s00018-020-03591-y.
16. Demkow, U. (2023) Molecular mechanisms of neutrophil extracellular trap (NETs) degradation, *Int. J. Mol. Sci.*, **24**, 4896, https://doi.org/10.3390/ijms24054896.
17. Wang, Y., Du, C., Zhang, Y., and Zhu, L. (2024) Composition and function of neutrophil extracellular traps, *Biomolecules*, **14**, 416, https://doi.org/10.3390/biom14040416.
18. Papayannopoulos, V. (2018) Neutrophil extracellular traps in immunity and disease, *Nat. Rev. Immunol.*, **18**, 134-147, https://doi.org/10.1038/nri.2017.105.
19. Ríos-López, A. L., González, G. M., Hernández-Bello, R., and Sánchez-González, A. (2021) Avoiding the trap: mechanisms developed by pathogens to escape neutrophil extracellular traps, *Microbiol. Res.*, **243**, 126644, https://doi.org/10.1016/j.micres.2020.126644.
20. Remijsen, Q., Kuijpers, T. W., Wirawan, E., Lippens, S., Vandenabeele, P., and Vanden Berghe, T. (2011) Dying for a cause: NETosis, mechanisms behind an antimicrobial cell death modality, *Cell Death Differ.*, **18**, 581-588, https://doi.org/10.1038/cdd.2011.1.
21. Hoppenbrouwers, T., Autar, A. S. A., Sultan, A. R., Abraham, T. E., van Cappellen, W. A., Houtsmuller, A. B., van Wamel, W. J. B., van Beusekom, H. M. M., van Neck, J. W., and de Maat, M. P. M. (2017) In vitro induction of NETosis: comprehensive live imaging comparison and systematic review, *PLoS One*, **12**, e0176472, https://doi.org/10.1371/journal.pone.0176472.
22. Laukova, L., and Konecna, B. (2018) NETosis – Dr. Jekyll and Mr. Hyde in inflammation, *J. Appl. Biomed.*, **16**, 1-9, https://doi.org/10.1016/j.jab.2017.10.002.
23. Burgener, S. S., and Schroder, K. (2020) Neutrophil extracellular traps in host defense, *Cold Spring Harb. Perspect. Biol.*, **12**, a037028, https://doi.org/10.1101/cshperspect.a037028.
24. Wright, H. L., Lyon, M., Chapman, E. A., Moots, R. J., and Edwards, S. W. (2020) Rheumatoid arthritis synovial fluid neutrophils drive inflammation through production of chemokines, reactive oxygen species, and neutrophil extracellular traps, *Front. Immunol.*, **11**, 584116, https://doi.org/10.3389/fimmu.2020.584116.
25. Clark, S. R., Ma, A. C., Tavener, S. A., McDonald, B., Goodarzi, Z., Kelly, M. M., Patel, K. D., Chakrabarti, S., McAvoy, E., Sinclair, G. D., Keys, E. M., Allen-Vercoe, E., Devinney, R., Doig, C. J., Green, F. H., and Kubes, P. (2007) Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood, *Nat. Med.*, **13**, 463-469, https://doi.org/10.1038/nm1565.
26. Tan, C., Aziz, M., and Wang, P. (2021) The vitals of NETs, *J. Leukoc. Biol.*, **110**, 797-808, https://doi.org/10.1002/JLB.3RU0620-375R.
27. Li, Y., Li, M., Weigel, B., Werth, V. P., Mall, M., and Liu, M. L. (2020) Nuclear envelope rupture and neutrophil extracellular trap formation is driven by PKCα-mediated lamin B disassembly, *EMBO Rep.*, **21**, e48779, https://doi.org/10.15252/embr.201948779.
28. Thiam, H. R., Wong, S. L., Qiu, R., Kittisopikul, M., Vahabikashi, A., Goldman, A. E., Wagner, D. D., and Waterman, C. M. (2020) NETosis proceeds by cytoskeleton and endomembrane disassembly and PAD4-mediated chromatin decondensation and nuclear envelope rupture, *Proc. Natl. Acad. Sci. USA*, **117**, 7326-7337, https://doi.org/10.1073/pnas.1909546117.
29. Li, M., Lyu, X., Liao, J., Werth, V. P., and Liu, M. L. (2022) Rho Kinase regulates neutrophil NET formation that is involved in UVB-induced skin inflammation, *Theranostics*, **12**, 2133-2149, https://doi.org/10.7150/thno.66457.
30. Liu, M. L. (2020) Functional actin cytoskeleton is required in early stage of NETosis induction, *Proc. Natl. Acad. Sci. USA*, **117**, 22653-22654, https://doi.org/10.1073/pnas.2013790117.
31. Amulic, B., Knackstedt, S. L., Abu Abed, U., Deigendesch, N., Harbort, C. J., Caffrey, B. E., Brinkmann, V., Heppner, F. L., Hinds, P. W., and Zychlinsky, A. (2017) Cell-cycle proteins control production of neutrophil extracellular traps, *Dev. Cell*, **43**, 449-462, https://doi.org/10.1016/j.devcel.2017.10.013.
32. Wang, Y., Li, M., Stadler, S., Correll, S., Li, P., Wang, D., Hayama, R., Leonelli, L., Han, H., Grigoryev, S. A., Allis, C. D., and Coonrod, S. A. (2009) Histone hypercitrullination mediates chromatin decondensation and neutrophil extracellular trap formation, *J. Cell Biol.*, **184**, 205-213, https://doi.org/10.1083/jcb.200806072.
33. Li, P., Li, M., Lindberg, M. R., Kennett, M. J., Xiong, N., and Wang, Y. (2010) PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps, *J. Exp. Med.*, **207**, 1853-1862, https://doi.org/10.1084/jem.20100239.
34. Hamam, H. J., Khan, M. A., and Palaniyar, N. (2019) Histone acetylation promotes neutrophil extracellular trap formation, *Biomolecules*, **9**, 32, https://doi.org/10.3390/biom9010032.

35. Burn, G. L., Raisch, T., Tacke, S., Winkler, M., Prumbaum, D., Thee, S., Gimber, N., Raunser, S., and Zychlinsky, A. (2025) Myeloperoxidase transforms chromatin into neutrophil extracellular traps, *Nature*, **647**, 747-756, https://doi.org/10.1038/s41586-025-09523-9.
36. Khan, M. A., Farahvash, A., Douda, D. N., Licht, J.-C., Grasemann, H., Sweezey, N., and Palaniyar, N. (2017) JNK activation turns on LPS-and Gram-negative bacteria-induced NADPH oxidase-dependent suicidal NETosis, *Sci. Rep.*, **7**, 3409, https://doi.org/10.1038/s41598-017-03257-z.
37. Hakkim, A., Fuchs, T. A., Martinez, N. E., Hess, S., Prinz, H., Zychlinsky, A., and Waldmann, H. (2011) Activation of the Raf-MEK-ERK pathway is required for neutrophil extracellular trap formation, *Nat. Chem. Biol.*, **7**, 75-77, https://doi.org/10.1038/nchembio.496.
38. Fonseca, Z., Díaz-Godínez, C., Mora, N., Alemán, O. R., Uribe-Querol, E., Carrero, J. C., and Rosales, C. (2018) *Entamoeba histolytica* induce signaling via Raf/MEK/ERK for neutrophil extracellular trap (NET) formation, *Front. Cell. Infect. Microbiol.*, **8**, 226, https://doi.org/10.3389/fcimb.2018.00226.
39. Gorudko, I. V., Grigorieva, D. V., Reut, V. E., Martinovich, G. G., Sokolov, A. V., and Panasenko, O. M. (2026) Myeloperoxidase as a regulator of cell signaling under oxidative/halogenative stress, *Biochemistry (Moscow)*, **91**, S51-S83, https://doi.org/10.1134/S0006297925603788.
40. Khan, M. A., and Palaniyar, N. (2017) Transcriptional firing helps to drive NETosis, *Sci. Rep.*, **7**, 41749, https://doi.org/10.1038/srep41749.
41. Panasenko, O. M., Torkhovskaya, T. I., Gorudko, I. V., and Sokolov, A. V. (2020) The role of halogenative stress in atherogenic modification of low-density lipoproteins, *Biochemistry (Moscow)*, **85**, S34-S55, https://doi.org/10.1134/S0006297920140035.
42. Parker, H., Dragunow, M., Hampton, M. B., Kettle, A. J., and Winterbourn, C. C. (2012) Requirements for NADPH oxidase and myeloperoxidase in neutrophil extracellular trap formation differ depending on the stimulus, *J. Leukoc. Biol.*, **92**, 841-849, https://doi.org/10.1189/jlb.1211601.
43. Shahzad, A., Ni, Y., Yang, Y., Liu, W., Teng, Z., Bai, H., Liu, X., Sun, Y., Xia, J., Cui, K., Duan, Q., Xu, Z., Zhang, J., Yang, Z., and Zhang, Q. (2025) Neutrophil extracellular traps (NETs) in health and disease, *Mol. Biomed.*, **6**, 130, https://doi.org/10.1186/s43556-025-00337-9.
44. Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U., and Nishizuka, Y. (1982) Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters, *J. Biol. Chem.*, **257**, 7847-7851, https://doi.org/10.1016/S0021-9258(18)34459-4.
45. Keshari, R. S., Verma, A., Barthwal, M. K., and Dikshit, M. (2013) Reactive oxygen species-induced activation of ERK and p38 MAPK mediates PMA-induced NETs release from human neutrophils, *J. Cell. Biochem.*, **114**, 532-540, https://doi.org/10.1002/jcb.24391.
46. Chessa, T. A., Anderson, K. E., Hu, Y., Xu, Q., Rausch, O., Stephens, L. R., and Hawkins, P. T. (2010) Phosphorylation of threonine 154 in p40phox is an important physiological signal for activation of the neutrophil NADPH oxidase, *Blood*, **116**, 6027-6036, https://doi.org/10.1182/blood-2010-08-300889.
47. Metzler, K. D., Goosmann, C., Lubojemska, A., Zychlinsky, A., and Papayannopoulos, V. (2014) A myeloperoxidase-containing complex regulates neutrophil elastase release and actin dynamics during NETosis, *Cell Rep.*, **8**, 883-896, https://doi.org/10.1016/j.celrep.2014.06.044.
48. Huang, J., Hong, W., Wan, M., and Zheng, L. (2020) Molecular mechanisms and therapeutic target of NETosis in diseases, *MedComm (2020)*, **3**, e162, https://doi.org/10.1002/mco2.162.
49. De Bont, C. M., Koopman, W. J. H., Boelens, W. C., and Pruijn, G. J. M. (2018) Stimulus-dependent chromatin dynamics, citrullination, calcium signalling and ROS production during NET formation, *Biochim. Biophys. Acta Mol. Cell Res.*, **1865**, 1621-1629, https://doi.org/10.1016/j.bbamcr.2018.08.014.
50. Inoue, M., Enomoto, M., Yoshimura, M., and Mizowaki, T. (2021) Pharmacological inhibition of sodium-calcium exchange activates NADPH oxidase and induces infection-independent NETotic cell death, *Redox Biol.*, **43**, 101983, https://doi.org/10.1016/j.redox.2021.101983.
51. Adelman, J. P., Maylie, J., and Sah, P. (2012) Small-conductance Ca^{2+}-activated K^+ channels: form and function, *Annu. Rev. Physiol.*, **74**, 245-269, https://doi.org/10.1146/annurev-physiol-020911-153336.
52. Wigerblad, G., and Kaplan, M. J. (2023) Neutrophil extracellular traps in systemic autoimmune and autoinflammatory diseases, *Nat. Rev. Immunol.*, **23**, 274-288, https://doi.org/10.1038/s41577-022-00787-0.
53. Guo, W., Gong, Q., Zong, X., Wu, D., Li, Y., Xiao, H., Song, J., Zhang, S., Fu, S., Feng, Z., and Zhuang, L. (2023) GPR109A controls neutrophil extracellular traps formation and improve early sepsis by regulating ROS/PAD4/Cit-H3 signal axis, *Exp. Hematol. Oncol.*, **12**, 15, https://doi.org/10.1186/s40164-023-00376-4.
54. Holmes, C. L., Shim, D., Kernien, J., Johnson, C. J., Nett, J. E., and Shelef, M. A. (2019) Insight into neutrophil extracellular traps through systematic evaluation of citrullination and peptidylarginine deiminases, *J. Immunol. Res.*, **2019**, 2160192, https://doi.org/10.1155/2019/2160192.

55. Tatsiy, O., and McDonald, P. P. (2018) Physiological stimuli induce PAD4-dependent, ROS-independent NETosis, with early and late events controlled by discrete signaling pathways, *Front. Immunol.*, **9**, 2036, https://doi.org/10.3389/fimmu.2018.02036.
56. Hawez, A., Al-Haidari, A., Madhi, R., Rahman, M., and Thorlacius, H. (2019) MiR-155 regulates PAD4-dependent formation of neutrophil extracellular traps, *Front. Immunol.*, **10**, 2462, https://doi.org/10.3389/fimmu.2019.02462.
57. Neeli, I., and Radic, M. (2013) Opposition between PKC isoforms regulates histone deimination and neutrophil extracellular chromatin release, *Front. Immunol.*, **4**, 38, https://doi.org/10.3389/fimmu.2013.00038.
58. Konig, M. F., and Andrade, F. (2016) A critical reappraisal of neutrophil extracellular traps and NETosis mimics based on differential requirements for protein citrullination, *Front. Immunol.*, **7**, 461, https://doi.org/10.3389/fimmu.2016.00461.
59. Kenny, E. F., Herzig, A., Krüger, R., Muth, A., Mondal, S., Thompson, P. R., Brinkmann, V., Bernuth, H. V., and Zychlinsky, A. (2017) Diverse stimuli engage different neutrophil extracellular trap pathways, *Elife*, **6**, e24437, https://doi.org/10.7554/eLife.24437.
60. Douda, D. N., Khan, M. A., Grasemann, H., and Palaniyar, N. (2015) SK3 channel and mitochondrial ROS mediate NADPH oxidase-independent NETosis induced by calcium influx, *Proc. Natl. Acad. Sci. USA*, **112**, 2817-2822, https://doi.org/10.1073/pnas.1414055112.
61. Byrd, A. S., O'Brien, X. M., Johnson, C. M., Lavigne, L. M., and Reichner, J. S. (2013) An extracellular matrix-based mechanism of rapid neutrophil extracellular trap formation in response to *Candida albicans*, *J. Immunol.*, **190**, 4136-4148, https://doi.org/10.4049/jimmunol.1202671.
62. Chen, K., Nishi, H., Travers, R., Tsuboi, N., Martinod, K., Wagner, D. D., Stan, R., Croce, K., and Mayadas, T. N. (2012) Endocytosis of soluble immune complexes leads to their clearance by FcγRIIIB but induces neutrophil extracellular traps via FcγRIIA *in vivo*, *Blood*, **120**, 4421-4431, https://doi.org/10.1182/blood-2011-12-401133.
63. Van der Linden, M., Westerlaken, G. H. A., van der Vlist, M., van Montfrans, J., and Meyaard, L. (2017) Differential signalling and kinetics of neutrophil extracellular trap release revealed by quantitative live imaging, *Sci. Rep.*, **7**, 6529, https://doi.org/10.1038/s41598-017-06901-w.
64. Morales-Primo, A. U., Becker, I., and Zamora-Chimal, J. (2022) Neutrophil extracellular trap-associated molecules: a review on their immunophysiological and inflammatory roles, *Int. Rev. Immunol.*, **41**, 253-274, https://doi.org/10.1080/08830185.2021.1921174.
65. Donis-Maturano, L., Sánchez-Torres, L. E., Cerbulo-Vázquez, A., Chacón-Salinas, R., García-Romo, G. S., Orozco-Uribe, M. C., Yam-Puc, J. C., González-Jiménez, M. A., Paredes-Vivas, Y. L., Calderón-Amador, J., Estrada-Parra, S., Estrada-García, I., and Flores-Romo, L. (2015) Prolonged exposure to neutrophil extracellular traps can induce mitochondrial damage in macrophages and dendritic cells, *Springerplus*, **4**, 161, https://doi.org/10.1186/s40064-015-0932-8.
66. Lewis, H. D., Liddle, J., Coote, J. E., Atkinson, S. J., Barker, M. D., Bax, B. D., Bicker, K. L., Bingham, R. P., Campbell, M., Chen, Y. H., Chung, C. W., Craggs, P. D., Davis, R. P., Eberhard, D., Joberty, G., Lind, K. E., Locke, K., Maller, C., Martinod, K., Patten, C., Polyakova, O., Rise, C. E., Rüdiger, M., Sheppard, R. J., Slade, D. J., Thomas, P., Thorpe, J., Yao, G., Drewes, G., Wagner, D. D., Thompson, P. R., Prinjha, R. K., and Wilson, D. M. (2015) Inhibition of PAD4 activity is sufficient to disrupt mouse and human NET formation, *Nat. Chem. Biol.*, **11**, 189-191, https://doi.org/10.1038/nchembio.1735.
67. Reis, L. R., Souza Junior, D. R., Tomasin, R., Bruni-Cardoso, A., Di Mascio, P., and Ronsein, G. E. (2023) Citrullination of actin-ligand and nuclear structural proteins, cytoskeleton reorganization and protein redistribution across cellular fractions are early events in ionomycin-induced NETosis, *Redox Biol.*, **64**, 102784, https://doi.org/10.1016/j.redox.2023.102784.
68. Chaerkady, R., Zhou, Y., Delmar, J. A., Weng, S. H. S., Wang, J., Awasthi, S., Sims, D., Bowen, M. A., Yu, W., Cazares, L. H., Sims, G. P., and Hess, S. (2021) Characterization of citrullination sites in neutrophils and mast cells activated by ionomycin via integration of mass spectrometry and machine learning, *J. Proteome Res.*, **20**, 3150-3164, https://doi.org/10.1021/acs.jproteome.1c00028.
69. Belambri, S. A., Rolas, L., Raad, H., Hurtado-Nedelec, M., Dang, P. M., and El-Benna, J. (2018) NADPH oxidase activation in neutrophils: role of the phosphorylation of its subunits, *Eur. J. Clin. Invest.*, **48**, e12951, https://doi.org/10.1111/eci.12951.
70. Panday, A., Sahoo, M. K., Osorio, D., and Batra, S. (2015) NADPH oxidases: an overview from structure to innate immunity-associated pathologies, *Cell. Mol. Immunol.*, **12**, 5-23, https://doi.org/10.1038/cmi.2014.89.
71. Zhou, Y., An, L. L., Chaerkady, R., Mittereder, N., Clarke, L., Cohen, T. S., Chen, B., Hess, S., Sims, G. P., and Mustelin, T. (2018) Evidence for a direct link between PAD4-mediated citrullination and the oxidative burst in human neutrophils, *Sci. Rep.*, **8**, 15228, https://doi.org/10.1038/s41598-018-33385-z.

72. Reis, L. R., Nascimento, R. O., Massafera, M. P., Di Mascio, P., and Ronsein, G. E. (2025) Investigating neutrophil responses to stimuli: comparative analysis of reactive species-dependent and independent mechanisms, *Redox Biol.*, **81**, 103540, https://doi.org/10.1016/j.redox.2025.103540.
73. Schönrich, G., and Raftery, M. J. (2016) Neutrophil extracellular traps go viral, *Front. Immunol.*, **7**, 366, https://doi.org/10.3389/fimmu.2016.00366.
74. Herre, M., Cedervall, J., Mackman, N., and Olsson, A. K. (2023) Neutrophil extracellular traps in the pathology of cancer and other inflammatory diseases, *Physiol. Rev.*, **103**, 277-312, https://doi.org/10.1152/physrev.00062.2021.
75. Palmer, L. J., Cooper, P. R., Ling, M. R., Wright, H. J., Huissoon, A., and Chapple, I. L. (2012) Hypochlorous acid regulates neutrophil extracellular trap release in humans, *Clin. Exp. Immunol.*, **167**, 261-268, https://doi.org/10.1111/j.1365-2249.2011.04518.x.
76. Yost, C. C., Cody, M. J., Harris, E. S., Thornton, N. L., McInturff, A. M., Martinez, M. L., Chandler, N. B., Rodesch, C. K., Albertine, K. H., Petti, C. A., Weyrich, A. S., and Zimmerman, G. A. (2009) Impaired neutrophil extracellular trap (NET) formation: a novel innate immune deficiency of human neonates, *Blood*, **113**, 6419-6427, https://doi.org/10.1182/blood-2008-07-171629.
77. Keshari, R. S., Jyoti, A., Kumar, S., Dubey, M., Verma, A., Srinag, B. S., Krishnamurthy, H., Barthwal, M. K., and Dikshit, M. (2012) Neutrophil extracellular traps contain mitochondrial as well as nuclear DNA and exhibit inflammatory potential, *Cytometry A*, **81**, 238-247, https://doi.org/10.1002/cyto.a.21178.
78. Hurst, J. K. (2012) What really happens in the neutrophil phagosome? *Free Radic. Biol. Med.*, **53**, 508-520, https://doi.org/10.1016/j.freeradbiomed.2012.05.008.
79. Neeli, I., Dwivedi, N., Khan, S., and Radic, M. (2009) Regulation of extracellular chromatin release from neutrophils, *J. Innate. Immun.*, **1**, 194-201, https://doi.org/10.1159/000206974.
80. Lim, M. B., Kuiper, J. W., Katchky, A., Goldberg, H., and Glogauer, M. (2011) Rac2 is required for the formation of neutrophil extracellular traps, *J. Leukoc. Biol.*, **90**, 771-776, https://doi.org/10.1189/jlb.1010549.
81. Akong-Moore, K., Chow, O. A., von Köckritz-Blickwede, M., and Nizet, V. (2012) Influences of chloride and hypochlorite on neutrophil extracellular trap formation, *PLoS One*, **7**, e42984, https://doi.org/10.1371/journal.pone.0042984.
82. Prokopowicz, Z., Marcinkiewicz, J., Katz, D. R., and Chain, B. M. (2012) Neutrophil myeloperoxidase: soldier and statesman, *Arch. Immunol. Ther. Exp. (Warsz)*, **60**, 43-54, https://doi.org/10.1007/s00005-011-0156-8.
83. Hazeldine, J., Harris, P., Chapple, I. L., Grant, M., Greenwood, H., Livesey, A., Sapey, E., and Lord, J. M. (2014) Impaired neutrophil extracellular trap formation: a novel defect in the innate immune system of aged individuals, *Aging Cell*, **13**, 690-698, https://doi.org/10.1111/acel.12222.
84. Metzler, K. D., Fuchs, T. A., Nauseef, W. M., Reumaux, D., Roesler, J., Schulze, I., Wahn, V., Papayannopoulos, V., and Zychlinsky, A. (2011) Myeloperoxidase is required for neutrophil extracellular trap formation: implications for innate immunity, *Blood*, **117**, 953-959, https://doi.org/10.1182/blood-2010-06-290171.
85. Björnsdottir, H., Welin, A., Michaëlsson, E., Osla, V., Berg, S., Christenson, K., Sundqvist, M., Dahlgren, C., Karlsson, A., and Bylund, J. (2015) Neutrophil NET formation is regulated from the inside by myeloperoxidase-processed reactive oxygen species, *Free Radic. Biol. Med.*, **89**, 1024-1035, https://doi.org/10.1016/j.freeradbiomed.2015.10.398.
86. Lin, H., Levison, B. S., Buffa, J. A., Huang, Y., Fu, X., Wang, Z., Gogonea, V., DiDonato, J. A., and Hazen, S. L. (2017) Myeloperoxidase-mediated protein lysine oxidation generates 2-aminoadipic acid and lysine nitrile *in vivo*, *Free Radic. Biol. Med.*, **104**, 20-31, https://doi.org/10.1016/j.freeradbiomed.2017.01.006.
87. Hallberg, L. A. E., Thorsen, N. W., Hartsema, E. A., Hägglund, P. M., and Hawkins, C. L. (2022) Mapping the modification of histones by the myeloperoxidase-derived oxidant hypochlorous acid (HOCl), *Free Radic. Biol. Med.*, **192**, 152-164, https://doi.org/10.1016/j.freeradbiomed.2022.09.016.
88. Hemmling, H., Hallberg, L. A. E., Hägglund, P., and Hawkins, C. L. (2025) Histones in neutrophil extracellular traps (NETs) contain oxidative post-translational modifications induced by the myeloperoxidase oxidant hypochlorous acid, *Redox Biol.*, **84**, 103696, https://doi.org/10.1016/j.redox.2025.103696.
89. Arnhold, J., Hammerschmidt, S., and Arnold, K. (1991) Role of functional groups of human plasma and luminol in scavenging of NaOCl and neutrophil-derived hypochlorous acid, *Biochim. Biophys. Acta*, **1097**, 145-151, https://doi.org/10.1016/0925-4439(91)90099-U.
90. Hu, M. L., Louie, S., Cross, C. E., Motchnik, P., and Halliwell, B. (1993) Antioxidant protection against hypochlorous acid in human plasma, *J. Lab. Clin. Med.*, **121**, 257-262.
91. Gorudko, I. V., Grigorieva, D. V., Shamova, E. V., Kostevich, V. A., Sokolov, A. V., Mikhalchik, E. V., Cherenkevich, S. N., Arnhold, J., and Panasenko, O. M. (2014) Hypohalous acid-modified human serum albumin induces neutrophil NADPH oxidase activation, degranulation, and shape change, *Free Radic. Biol. Med.*, **68**, 326-334, https://doi.org/10.1016/j.freeradbiomed.2013.12.023.

92. Grigorieva, D. V., Gorbunov, N. P., Kostevich, V. A., Sokolov, A. V., Basyreva, L. Yu., Shmeleva, E. V., Vakhrusheva, T. V., Gusev, S. A., Gorudko, I. V., and Panasenko, O. M. (2026) Human serum albumin modified in myeloperoxidase-dependent reactions is a mediator of neutrophil extracellular trap formation, *J. Biomed. Res.*, **40**, 147-158, https://doi.org/10.7555/JBR.39.20250069.
93. Basyreva, L. Y., Shmeleva, E. V., Vakhrusheva, T. V., Gorudko, I. V., Grigorieva, D. V., Kostevich, V. A., Gorbunov, N. P., Sokolov, A. V., Gusev, S. A., Panasenko, O. M., and Sergienko, V. I. (2024) Hypochlorous acid-modified serum albumin causes NETosis in the whole blood *ex vivo* and in isolated neutrophils, *Bull. Exp. Biol. Med.*, **177**, 197-202, https://doi.org/10.1007/s10517-024-06155-3.
94. Grigorieva, D. V., Gorudko, I. V., Grudinina, N. A., Panasenko, O. M., Semak, I. V., Sokolov, A. V., and Timoshenko, A. V. (2022) Lactoferrin modified by hypohalous acids: partial loss in activation of human neutrophils, *Int. J. Biol. Macromol.*, **195**, 30-40, https://doi.org/10.1016/j.ijbiomac.2021.11.165.
95. Palladino, E. N. D., Katunga, L. A., Kolar, G. R., and Ford, D. A. (2018) 2-Chlorofatty acids: lipid mediators of neutrophil extracellular trap formation, *J. Lipid Res.*, **59**, 1424-1432, https://doi.org/10.1194/jlr.M084731.
96. Amunugama, K., Kolar, G. R., and Ford, D. A. (2021) Neutrophil myeloperoxidase derived chlorolipid production during bacteria exposure, *Front. Immunol.*, **12**, 701227, https://doi.org/10.3389/fimmu.2021.701227.
97. Carlson, H. L., McGuffee, R. M., Patel, R. P., and Ford, D. A. (2025) 2-chlorofatty acid modification of neutrophil proteins: identification, localization and role in NETosis, *Cell Commun. Signal.*, **23**, 461, https://doi.org/10.1186/s12964-025-02435-1.
98. Ono, M., Toyomoto, M., Yamauchi, M., and Hagiwara, M. (2024) Platelets accelerate lipid peroxidation and induce pathogenic neutrophil extracellular trap release, *Cell Chem. Biol.*, **31**, 2085-2095.e4, https://doi.org/10.1016/j.chembiol.2024.11.003.
99. Collins, M. S., Imbrogno, M. A., Kopras, E. J., Howard, J. A., Zhang, N., Kramer, E. L., and Hudock, K. M. (2024) Heterogeneity in neutrophil extracellular traps from healthy human subjects, *Int. J. Mol. Sci.*, **25**, 525, https://doi.org/10.3390/ijms25010525.
100. Ortmann, W., and Kolaczkowska, E. (2018) Age is the work of art? Impact of neutrophil and organism age on neutrophil extracellular trap formation, *Cell Tissue Res.*, **371**, 473-488, https://doi.org/10.1007/s00441-017-2751-4.
101. Zhang, D., Chen, G., Manwani, D., Mortha, A., Xu, C., Faith, J. J., Burk, R. D., Kunisaki, Y., Jang, J. E., Scheiermann, C., Merad, M., and Frenette, P. S. (2015) Neutrophil ageing is regulated by the microbiome, *Nature*, **525**, 528-532, https://doi.org/10.1038/nature15367.
102. Barrientos, L., Marin-Esteban, V., de Chaisemartin, L., Le-Moal, V. L., Sandré, C., Bianchini, E., Nicolas, V., Pallardy, M., and Chollet-Martin, S. (2013) An improved strategy to recover large fragments of functional human neutrophil extracellular traps, *Front. Immunol.*, **4**, 166, https://doi.org/10.3389/fimmu.2013.00166.
103. Basyreva, L. Y., Voinova, E. V., Gusev, A. A., Mikhalchik, E. V., Kuskov, A. N., Goryachaya, A. V., Gusev, S. A., Shtilman, M. I., Velonia, K., and Tsatsakis, A. M. (2020) Fluorouracil neutrophil extracellular traps formation inhibited by polymer nanoparticle shielding, *Mater. Sci. Eng. C Mater. Biol. Appl.*, **108**, 110382, https://doi.org/10.1016/j.msec.2019.110382.
104. Petretto, A., Bruschi, M., Pratesi, F., Croia, C., Candiano, G., Ghiggeri, G., and Migliorini, P. (2019) Neutrophil extracellular traps (NET) induced by different stimuli: a comparative proteomic analysis, *PLoS One*, **14**, e0218946, https://doi.org/10.1371/journal.pone.0218946.
105. Vakhrusheva, T. V., Grigorieva, D. V., Gorudko, I. V., Sokolov, A. V., Kostevich, V. A., Lazarev, V. N., Vasilyev, V. B., Cherenkevich, S. N., and Panasenko, O. M. (2018) Enzymatic and bactericidal activity of myeloperoxidase in conditions of halogenative stress, *Biochem. Cell Biol.*, **96**, 580-591, https://doi.org/10.1139/bcb-2017-0292.
106. Basyreva, L. Yu., Shmeleva, E. V., Zubko, V. S., Filatova, E. S., Galkina, N. V., Khoroshilova, Ya. B., Rusakova, T. A., Lazov, M. A., Skopintsev, I. V., Lvovsky, A. I., Sivaev, A. A., Gritskova, I. A., Sokolov, A. V., Gusev, S. A., Chvalun, S. N., and Panasenko, O. M. (2025) On the application of polymer microspheres to identify positively and negatively charged extracellular traps of blood cells, *Nanobiotechnol. Rep.*, **20**, 190-199, https://doi.org/10.1134/S2635167625600415.
107. Marques, P. E., Oliveira, A. G., Pereira, R. V., David, B. A., Gomides, L. F., Saraiva, A. M., Pires, D. A., Novaes, J. T., Patricio, D. O., Cisalpino, D., Menezes-Garcia, Z., Leevy, W. M., Chapman, S. E., Mahecha, G., Marques, R. E., Guabiraba, R., Martins, V. P., Souza, D. G., Mansur, D. S., Teixeira, M. M., Leite, M. F., and Menezes, G. B. (2015) Hepatic DNA deposition drives drug-induced liver injury and inflammation in mice, *Hepatology*, **61**, 348-360, https://doi.org/10.1002/hep.27216.
108. Napirei, M., Ludwig, S., Mezrhab, J., Klöckl, T., and Mannherz, H. G. (2009) Murine serum nucleases – contrasting effects of plasmin and heparin on the activities of DNase1 and DNase1-like 3 (DNase1l3), *FEBS J.*, **276**, 1059-1073, https://doi.org/10.1111/j.1742-4658.2008.06849.x.

109. Jiménez-Alcázar, M., Rangaswamy, C., Panda, R., Bitterling, J., Simsek, Y. J., Long, A. T., Bilyy, R., Krenn, V., Renné, C., Renné, T., Kluge, S., Panzer, U., Mizuta, R., Mannherz, H. G., Kitamura, D., Herrmann, M., Napirei, M., and Fuchs, T. A. (2017) Host DNases prevent vascular occlusion by neutrophil extracellular traps, *Science*, **358**, 1202-1206, https://doi.org/10.1126/science.aam8897.
110. Napirei, M., Wulf, S., Eulitz, D., Mannherz, H. G., and Kloeckl, T. (2005) Comparative characterization of rat deoxyribonuclease 1 (Dnase1) and murine deoxyribonuclease 1-like 3 (Dnase1l3), *Biochem. J.*, **389**, 355-364, https://doi.org/10.1042/BJ20042124.
111. Mizuta, R., Araki, S., Furukawa, M., Furukawa, Y., Ebara, S., Shiokawa, D., Hayashi, K., Tanuma, S., and Kitamura, D. (2013) DNase γ is the effector endonuclease for internucleosomal DNA fragmentation in necrosis, *PLoS One*, **8**, e80223, https://doi.org/10.1371/journal.pone.0080223.
112. Englert, H., Göbel, J., Khong, D., Omidi, M., Wolska, N., Konrath, S., Frye, M., Mailer, R. K., Beerens, M., Gerwers, J. C., Preston, R. J. S., Odeberg, J., Butler, L. M., Maas, C., Stavrou, E. X., Fuchs, T. A., and Renné, T. (2023) Targeting NETs using dual-active DNase1 variants, *Front. Immunol.*, **14**, 1181761, https://doi.org/10.3389/fimmu.2023.1181761.
113. Yousefi, S., Stojkov, D., Germic, N., Simon, D., Wang, X., Benarafa, C., and Simon, H. U. (2019) Untangling "NETosis" from NETs, *Eur. J. Immunol.*, **49**, 221-227, https://doi.org/10.1002/eji.201747053.
114. Hitchcock, S. E. (1980) Actin deoxyroboncuclease I interaction. Depolymerization and nucleotide exchange, *J. Biol. Chem.*, **255**, 5668-5673, https://doi.org/10.1016/S0021-9258(19)70681-4.
115. Mannherz, H. G., Goody, R. S., Konrad, M., and Nowak, E. (1980) The interaction of bovine pancreatic deoxyribonuclease I and skeletal muscle actin, *Eur. J. Biochem.*, **104**, 367-379, https://doi.org/10.1111/j.1432-1033.1980.tb04437.x.
116. Borissoff, J. I., Joosen, I. A., Versteylen, M. O., Brill, A., Fuchs, T. A., Savchenko, A. S., Gallant, M., Martinod, K., Ten Cate, H., Hofstra, L., Crijns, H. J., Wagner, D. D., and Kietselaer, B. L. J. H. (2013) Elevated levels of circulating DNA and chromatin are independently associated with severe coronary atherosclerosis and a prothrombotic state, *Arterioscler. Thromb. Vasc. Biol.*, **33**, 2032-2040, https://doi.org/10.1161/ATVBAHA.113.301627.
117. Dumont, B. L., Neagoe, P. E., Charles, E., Villeneuve, L., Ninni, S., Tardif, J. C., Räkel, A., White, M., and Sirois, M. G. (2024) Low-density neutrophils and neutrophil extracellular traps (NETs) are new inflammatory players in heart failure, *Can. J. Cardiol.*, **40**, 1524-1535, https://doi.org/10.1016/j.cjca.2024.03.018.
118. Megens, R. T., Vijayan, S., Lievens, D., Döring, Y., van Zandvoort, M. A., Grommes, J., Weber, C., and Soehnlein, O. (2012) Presence of luminal neutrophil extracellular traps in atherosclerosis, *Thromb. Haemost.*, **107**, 597-598, https://doi.org/10.1160/TH11-09-0650.
119. Maugeri, N., Campana, L., Gavina, M., Covino, C., De Metrio, M., Panciroli, C., Maiuri, L., Maseri, A., D'Angelo, A., Bianchi, M. E., Rovere-Querini, P., and Manfredi, A. A. (2014) Activated platelets present high mobility group box 1 to neutrophils, inducing autophagy and promoting the extrusion of neutrophil extracellular traps, *J. Thromb. Haemost.*, **12**, 2074-2088, https://doi.org/10.1111/jth.12710.
120. Mangold, A., Alias, S., Scherz, T., Hofbauer, M., Jakowitsch, J., Panzenböck, A., Simon, D., Laimer, D., Bangert, C., Kammerlander, A., Mascherbauer, J., Winter, M. P., Distelmaier, K., Adlbrecht, C., Preissner, K. T., and Lang, I. M. (2015) Coronary neutrophil extracellular trap burden and deoxyribonuclease activity in ST-elevation acute coronary syndrome are predictors of ST-segment resolution and infarct size, *Circ. Res.*, **116**, 1182-1192, https://doi.org/10.1161/CIRCRESAHA.116.304944.
121. Cheng, M. L., Chen, C. M., Gu, P. W., Ho, H. Y., and Chiu, D. T. (2008) Elevated levels of myeloperoxidase, white blood cell count and 3-chlorotyrosine in Taiwanese patients with acute myocardial infarction, *Clin. Biochem.*, **41**, 554-560, https://doi.org/10.1016/j.clinbiochem.2008.02.006.
122. Rudolph, V., Andrié, R. P., Rudolph, T. K., Friedrichs, K., Klinke, A., Hirsch-Hoffmann, B., Schwoerer, A. P., Lau, D., Fu, X., Klingel, K., Sydow, K., Didié, M., Seniuk, A., von Leitner, E. C., Szoecs, K., Schrickel, J. W., Treede, H., Wenzel, U., Lewalter, T., Nickenig, G., Zimmermann, W. H., Meinertz, T., Böger, R. H., Reichenspurner, H., Freeman, B. A., Eschenhagen, T., Ehmke, H., Hazen, S. L., Willems, S., and Baldus, S. (2010) Myeloperoxidase acts as a profibrotic mediator of atrial fibrillation, *Nat. Med.*, **16**, 470-474, https://doi.org/10.1038/nm.2124.
123. Tang, W. H., Tong, W., Troughton, R. W., Martin, M. G., Shrestha, K., Borowski, A., Jasper, S., Hazen, S. L., and Klein, A. L. (2007) Prognostic value and echocardiographic determinants of plasma myeloperoxidase levels in chronic heart failure, *J. Am. Coll. Cardiol.*, **49**, 2364-2370, https://doi.org/10.1016/j.jacc.2007.02.053.
124. Wainstein, R. V., Wainstein, M. V., Ribeiro, J. P., Dornelles, L. V., Tozzati, P., Ashton-Prolla, P., Ewald, I. P., Vietta, G., and Polanczyk, C. A. (2010) Association between myeloperoxidase polymorphisms and its plasma levels with severity of coronary artery disease, *Clin. Biochem.*, **43**, 57-62, https://doi.org/10.1016/j.clinbiochem.2009.07.022.
125. Grigorieva, D. V., Gorudko, I. V., Kostevich, V. A., Sokolov, A. V., Buko, I. V., Vasilyev, V. B., Polonetsky, L. Z., Panasenko, O. M., and Cherenkevich, S. N. (2016) Plasma myeloperoxidase activity as a criterion of therapeutic

effectiveness for patients with cardiovascular diseases, *Biochem. (Mosc.). Suppl. B Biomed. Chem.*, **10**, 173-179, https://doi.org/10.1134/S1990750816020050.

126. Hazell, L. J., Baernthaler, G., and Stocker, R. (2001) Correlation between intima-to-media ratio, apolipoprotein B-100, myeloperoxidase, and hypochlorite-oxidized proteins in human atherosclerosis, *Free Radic. Biol. Med.*, **31**, 1254-1262, https://doi.org/10.1016/s0891-5849(01)00717-1.
127. Malle, E., Waeg, G., Schreiber, R., Gröne, E. F., Sattler, W., and Gröne, H. J. (2000) Immunohistochemical evidence for the myeloperoxidase/H_2O_2/halide system in human atherosclerotic lesions: colocalization of myeloperoxidase and hypochlorite-modified proteins, *Eur. J. Biochem.*, **267**, 4495-4503, https://doi.org/10.1046/j.1432-1327.2000.01498.x.
128. Daugherty, A., Dunn, J. L., Rateri, D. L., and Heinecke, J. W. (1994) Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions, *J. Clin. Invest.*, **94**, 437-444, https://doi.org/10.1172/JCI117342.
129. Hazen, S. L., and Heinecke, J. W. (1997) 3-Chlorotyrosine, a specific marker of myeloperoxidase-catalyzed oxidation, is markedly elevated in low density lipoprotein isolated from human atherosclerotic intima, *J. Clin. Invest.*, **99**, 2075-2081, https://doi.org/10.1172/JCI119379.
130. Bergt, C., Pennathur, S., Fu, X., Byun, J., O'Brien, K., McDonald, T. O., Singh, P., Anantharamaiah, G. M., Chait, A., Brunzell, J., Geary, R. L., Oram, J. F., and Heinecke, J. W. (2004) The myeloperoxidase product hypochlorous acid oxidizes HDL in the human artery wall and impairs ABCA1-dependent cholesterol transport, *Proc. Natl. Acad. Sci. USA*, **101**, 13032-13037, https://doi.org/10.1073/pnas.0405292101.
131. Hazell, L. J., Arnold, L., Flowers, D., Waeg, G., Malle, E., and Stocker, R. (1996) Presence of hypochlorite-modified proteins in human atherosclerotic lesions, *J. Clin. Invest.*, **97**, 1535-1544, https://doi.org/10.1172/JCI118576.
132. Takeshita, J., Byun, J., Nhan, T. Q., Pritchard, D. K., Pennathur, S., Schwartz, S. M., Chait, A., and Heinecke, J. W. (2006) Myeloperoxidase generates 5-chlorouracil in human atherosclerotic tissue: a potential pathway for somatic mutagenesis by macrophages, *J. Biol. Chem.*, **281**, 3096-3104, https://doi.org/10.1074/jbc.M509236200.
133. Thukkani, A. K., McHowat, J., Hsu, F. F., Brennan, M. L., Hazen, S. L., and Ford, D. A. (2003) Identification of alpha-chloro fatty aldehydes and unsaturated lysophosphatidylcholine molecular species in human atherosclerotic lesions, *Circulation*, **108**, 3128-3133, https://doi.org/10.1161/01.CIR.0000104564.01539.6A.
134. Messner, M. C., Albert, C. J., McHowat, J., and Ford, D. A. (2008) Identification of lysophosphatidylcholine-chlorohydrin in human atherosclerotic lesions, *Lipids*, **43**, 243-249, https://doi.org/10.1007/s11745-008-3151-z.
135. Pérez-Sánchez, C., Ruiz-Limón, P., Aguirre, M. A., Jiménez-Gómez, Y., Arias-de la Rosa, I., Ábalos-Aguilera, M. C., Rodriguez-Ariza, A., Castro-Villegas, M. C., Ortega-Castro, R., Segui, P., Martinez, C., Gonzalez-Conejero, R., Rodríguez-López, S., Gonzalez-Reyes, J. A., Villalba, J. M., Collantes-Estévez, E., Escudero, A., Barbarroja, N., and López-Pedrera, C. (2017) Diagnostic potential of NETosis-derived products for disease activity, atherosclerosis and therapeutic effectiveness in rheumatoid arthritis patients, *J. Autoimmun.*, **82**, 31-40, https://doi.org/10.1016/j.jaut.2017.04.007.
136. Khandpur, R., Carmona-Rivera, C., Vivekanandan-Giri, A., Gizinski, A., Yalavarthi, S., Knight, J. S., Friday, S., Li, S., Patel, R. M., Subramanian, V., Thompson, P., Chen, P., Fox, D. A., Pennathur, S., and Kaplan, M. J. (2013) NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis, *Sci. Transl. Med.*, **5**, 178ra40, https://doi.org/10.1126/scitranslmed.3005580.
137. Fernandes, R. M., da Silva, N. P., and Sato, E. I. (2012) Increased myeloperoxidase plasma levels in rheumatoid arthritis, *Rheumatol. Int.*, **32**, 1605-1609, https://doi.org/10.1007/s00296-011-1810-5.
138. Stamp, L. K., Khalilova, I., Tarr, J. M., Senthilmohan, R., Turner, R., Haigh, R. C., Winyard, P. G., and Kettle, A. J. (2012) Myeloperoxidase and oxidative stress in rheumatoid arthritis, *Rheumatology (Oxford)*, **51**, 1796-1803, https://doi.org/10.1093/rheumatology/kes193.
139. Baskol, G., Demir, H., Baskol, M., Kilic, E., Ates, F., Karakukcu, C., and Ustdal, M. (2006) Investigation of protein oxidation and lipid peroxidation in patients with rheumatoid arthritis, *Cell Biochem. Funct.*, **24**, 307-311, https://doi.org/10.1002/cbf.1257.
140. Vivekanandan-Giri, A., Slocum, J. L., Byun, J., Tang, C., Sands, R. L., Gillespie, B. W., Heinecke, J. W., Saran, R., Kaplan, M. J., and Pennathur, S. (2013) High density lipoprotein is targeted for oxidation by myeloperoxidase in rheumatoid arthritis, *Ann. Rheum. Dis.*, **72**, 1725-1731, https://doi.org/10.1136/annrheumdis-2012-202033.
141. Nzeusseu Toukap, A., Delporte, C., Noyon, C., Franck, T., Rousseau, A., Serteyn, D., Raes, M., Vanhaeverbeek, M., Moguilevsky, N., Nève, J., Vanhamme, L., Durez, P., Van Antwerpen, P., and Zouaoui Boudjeltia, K. (2014) Myeloperoxidase and its products in synovial fluid of patients with treated or untreated rheumatoid arthritis, *Free Radic. Res.*, **48**, 461-465, https://doi.org/10.3109/10715762.2014.886327.
142. Liu, L., de Leeuw, K., van Goor, H., Doornbos-van der Meer, B., Arends, S., and Westra, J. (2024) Neutrophil extracellular traps and oxidative stress in systemic lupus erythematosus patients with and without renal involvement, *Arthritis Res. Ther.*, **26**, 220, https://doi.org/10.1186/s13075-024-03454-y.

143. Telles, R. W., Ferreira, G. A., da Silva, N. P., and Sato, E. I. (2010) Increased plasma myeloperoxidase levels in systemic lupus erythematosus, *Rheumatol. Int.*, **30**, 779-784, https://doi.org/10.1007/s00296-009-1067-4.
144. Smith, C. K., Vivekanandan-Giri, A., Tang, C., Knight, J. S., Mathew, A., Padilla, R. L., Gillespie, B. W., Carmona-Rivera, C., Liu, X., Subramanian, V., Hasni, S., Thompson, P. R., Heinecke, J. W., Saran, R., Pennathur, S., and Kaplan, M. J. (2014) Neutrophil extracellular trap-derived enzymes oxidize high-density lipoprotein: an additional proatherogenic mechanism in systemic lupus erythematosus, *Arthritis Rheumatol.*, **66**, 2532-2544, https://doi.org/10.1002/art.38703.
145. Thålin, C., Lundström, S., Seignez, C., Daleskog, M., Lundström, A., Henriksson, P., Helleday, T., Phillipson, M., Wallén, H., and Demers, M. (2018) Citrullinated histone H3 as a novel prognostic blood marker in patients with advanced cancer, *PLoS One*, **13**, e0191231, https://doi.org/10.1371/journal.pone.0191231.
146. Tomás-Pérez, S., Oto, J., Aghababyan, C., Herranz, R., Cuadros-Lozano, A., González-Cantó, E., Mc Cormack, B., Arrés, J., Castaño, M., Cana, F., Martínez-Fernández, L., Santonja, N., Ramírez, R., Herreros-Pomares, A., Cañete-Mota, S., Llueca, A., Marí-Alexandre, J., Medina, P., and Gilabert-Estellés, J. (2023) Increased levels of NETosis biomarkers in high-grade serous ovarian cancer patients' biofluids: potential role in disease diagnosis and management, *Front. Immunol.*, **14**, 1111344, https://doi.org/10.3389/fimmu.2023.1111344.
147. Herranz, R., Oto, J., Hueso, M., Plana, E., Cana, F., Castaño, M., Cordón, L., Ramos-Soler, D., Bonanad, S., Vera-Donoso, C. D., Martínez-Sarmiento, M., and Medina, P. (2023) Bladder cancer patients have increased NETosis and impaired DNaseI-mediated NET degradation that can be therapeutically restored *in vitro*, *Front. Immunol.*, **14**, 1171065, https://doi.org/10.3389/fimmu.2023.1171065.
148. Zenlander, R., Havervall, S., Magnusson, M., Engstrand, J., Ågren, A., Thålin, C., and Stål, P. (2021) Neutrophil extracellular traps in patients with liver cirrhosis and hepatocellular carcinoma, *Sci. Rep.*, **11**, 18025, https://doi.org/10.1038/s41598-021-97233-3.
149. Rayes, R. F., Mouhanna, J. G., Nicolau, I., Bourdeau, F., Giannias, B., Rousseau, S., Quail, D., Walsh, L., Sangwan, V., Bertos, N., Cools-Lartigue, J., Ferri, L. E., and Spicer, J. D. (2019) Primary tumors induce neutrophil extracellular traps with targetable metastasis promoting effects, *JCI Insight.*, **5**, e128008, https://doi.org/10.1172/jci.insight.128008.
150. Weng, M., Yue, Y., Wu, D., Zhou, C., Guo, M., Sun, C., Liao, Q., Sun, M., Zhou, D., and Miao, C. (2022) Increased MPO in colorectal cancer is associated with high peripheral neutrophil counts and a poor prognosis: a TCGA with propensity score-matched analysis, *Front. Oncol.*, **12**, 940706, https://doi.org/10.3389/fonc.2022.940706.
151. Chalkias, A., Nikotian, G., Koutsovasilis, A., Bramis, J., Manouras, A., Mystrioti, D., and Katergiannakis, V. (2011) Patients with colorectal cancer are characterized by increased concentration of fecal hb-hp complex, myeloperoxidase, and secretory IgA, *Am. J. Clin. Oncol.*, **34**, 561-566, https://doi.org/10.1097/COC.0b013e3181f9457e.
152. Paridar, Y., Dabirian, M. M., Ahmadi, F., Shayanpour, M. H., Mard-Soltani, M., Shakerian, N., and Mobarakeh, A. (2024) Analysis of myeloperoxidase levels in serum of patients with colorectal cancer, *J. Adv. Immunopharmacol.*, **4**, e156620, https://doi.org/10.5812/jai-156620.
153. Czygier, M., Kamocki, Z., Ławicki, S., and Szmitkowski, M. (2010) The plasma level of myeloperoxidase (MPO) and total antioxidant status (TAS) in gastric cancer patients after surgery [in Polish], *Przegl. Lek.*, **67**, 443-445.
154. Vaguliene, N., Zemaitis, M., Lavinskiene, S., Miliauskas, S., and Sakalauskas, R. (2013) Local and systemic neutrophilic inflammation in patients with lung cancer and chronic obstructive pulmonary disease, *BMC Immunol.*, **14**, 36, https://doi.org/10.1186/1471-2172-14-36.
155. Castillo-Tong, D. C., Pils, D., Heinze, G., Braicu, I., Sehouli, J., Reinthaller, A., Schuster, E., Wolf, A., Watrowski, R., Maki, R. A., Zeillinger, R., and Reynolds, W. F. (2014) Association of myeloperoxidase with ovarian cancer, *Tumour Biol.*, **35**, 141-148, https://doi.org/10.1007/s13277-013-1017-3.
156. Fleszar, M. G., Fortuna, P., Zawadzki, M., Kosyk, B., and Krzystek-Korpacka, M. (2020) Simultaneous LC-MS/MS-based quantification of free 3-nitro-l-tyrosine, 3-chloro-l-tyrosine, and 3-bromo-l-tyrosine in plasma of colorectal cancer patients during early postoperative period, *Molecules*, **25**, 5158, https://doi.org/10.3390/molecules25215158.
157. Wu, D. C., Yang, T. C., Hu, S. X., and Candy Chen, H. J. (2022) Multiple oxidative and advanced oxidative modifications of hemoglobin in gastric cancer patients measured by nanoflow LC-MS/MS, *Clin. Chim. Acta.*, **531**, 137-144, https://doi.org/10.1016/j.cca.2022.03.023.
158. Pan, W.-R., Lee, R.-J., Tu, C.-W., and Candy Chen, H.-J. (2026) Chlorotyrosine and dichlorotyrosine in hemoglobin of breast cancer patients analyzed by liquid chromatography tandem mass spectrometry, *Chem. Res. Toxicol.*, **39**, 178-188, https://doi.org/10.1021/acs.chemrestox.5c00436.
159. Dong, Y., Lagarde, J., Xicota, L., Corne, H., Chantran, Y., Chaigneau, T., Crestani, B., Bottlaender, M., Potier, M. C., Aucouturier, P., Dorothée, G., Sarazin, M., and Elbim, C. (2018) Neutrophil hyperactivation correlates with Alzheimer's disease progression, *Ann. Neurol.*, **83**, 387-405, https://doi.org/10.1002/ana.25159.

160. Zenaro, E., Pietronigro, E., Della Bianca, V., Piacentino, G., Marongiu, L., Budui, S., Turano, E., Rossi, B., Angiari, S., Dusi, S., Montresor, A., Carlucci, T., Nanì, S., Tosadori, G., Calciano, L., Catalucci, D., Berton, G., Bonetti, B., and Constantin, G. (2015) Neutrophils promote Alzheimer's disease-like pathology and cognitive decline via LFA-1 integrin, *Nat. Med.*, **21**, 880-886, https://doi.org/10.1038/nm.3913.
161. Green, P. S., Mendez, A. J., Jacob, J. S., Crowley, J. R., Growdon, W., Hyman, B. T., and Heinecke, J. W. (2004) Neuronal expression of myeloperoxidase is increased in Alzheimer's disease, *J. Neurochem.*, **90**, 724-733, https://doi.org/10.1111/j.1471-4159.2004.02527.x.
162. Choi, D. K., Pennathur, S., Perier, C., Tieu, K., Teismann, P., Wu, D. C., Jackson-Lewis, V., Vila, M., Vonsattel, J. P., Heinecke, J. W., and Przedborski, S. (2005) Ablation of the inflammatory enzyme myeloperoxidase mitigates features of Parkinson's disease in mice, *J. Neurosci.*, **25**, 6594-6600, https://doi.org/10.1523/JNEUROSCI.0970-05.2005.
163. Tzikas, S., Schlak, D., Sopova, K., Gatsiou, A., Stakos, D., Stamatelopoulos, K., Stellos, K., and Laske, C. (2014) Increased myeloperoxidase plasma levels in patients with Alzheimer's disease, *J. Alzheimers Dis.*, **39**, 557-564, https://doi.org/10.3233/JAD-131469.
164. Espejo, E. F., Guerra, M. D., and Castellano, S. (2025) Association between serum myeloperoxidase enzyme activity and Parkinson's disease status, *NPJ Parkinsons Dis.*, **11**, 94, https://doi.org/10.1038/s41531-025-00941-0.
165. Ivanov, V. A., Sokolov, A. V., Gorbunov, N. P., Mikhalchik, E. V., Basyreva, L. Y., Galkina, N. V., Galkina, A. P., Khoroshilova, Y. B., Rusakova, T. A., Gusev, S. A., and Panasenko, O. M. (2025) Markers of halogenating stress and netosis in patients with type 2 diabetes mellitus, *Med. Acad. J.*, **25**, 68-75, https://doi.org/10.17816/MAJ642420.
166. Park, J. H., Kim, J. E., Gu, J. Y., Yoo, H. J., Park, S. H., Kim, Y. I., Nam-Goong, I. S., Kim, E. S., and Kim, H. K. (2016) Evaluation of circulating markers of neutrophil extracellular trap (NET) formation as risk factors for diabetic retinopathy in a case-control association study, *Exp. Clin. Endocrinol. Diabetes*, **124**, 557-561, https://doi.org/10.1055/s-0042-101792.
167. Wang, L., Zhou, X., Yin, Y., Mai, Y., Wang, D., and Zhang, X. (2019) Hyperglycemia induces neutrophil extracellular traps formation through an NADPH oxidase-dependent pathway in diabetic retinopathy, *Front. Immunol.*, **9**, 3076, https://doi.org/10.3389/fimmu.2018.03076.
168. Ibrahim, I., Nuermaimaiti, Y., Maimaituxun, G., Luo, X., Maimaituxun, M., Akbar, A., Tuerxun, K., and Wu, Y. (2024) Neutrophil extracellular traps (NETs) are associated with type 2 diabetes and diabetic foot ulcer related amputation: a prospective cohort study, *Diabetes Ther.*, **15**, 1333-1348, https://doi.org/10.1007/s13300-024-01579-6.
169. Begum, M., AshokKumar, J., and D'Souzaetal, H. P. (2015) Myeloperoxidase, malondialdehyde and serum lipids in type 2 diabetes mellitus, *J. Invest. Biochem.*, **4**, 13-17, https://doi.org/10.5455/jib.20150331115356.
170. Gorudko, I. V., Kostevich, V. A., Sokolov, A. V., Shamova, E. V., Buko, I. V., Konstantinova, E. E., Vasiliev, V. B., Cherenkevich, S. N., and Panasenko, O. M. (2012) Functional activity of neutrophils in diabetes mellitus and coronary heart disease: role of myeloperoxidase in the development of oxidative stress, *Bull. Exp. Biol. Med.*, **154**, 23-26, https://doi.org/10.1007/s10517-012-1865-7.
171. Ghoshal, K., Das, S., Aich, K., Goswami, S., Chowdhury, S., and Bhattacharyya, M. (2016) A novel sensor to estimate the prevalence of hypochlorous (HOCl) toxicity in individuals with type 2 diabetes and dyslipidemia, *Clin. Chim. Acta*, **458**, 144-153, https://doi.org/10.1016/j.cca.2016.05.006.
172. Chen, H. J., Yang, Y. F., Lai, P. Y., and Chen, P. F. (2016) Analysis of chlorination, nitration, and nitrosylation of tyrosine and oxidation of methionine and cysteine in hemoglobin from type 2 diabetes mellitus patients by nanoflow liquid chromatography tandem mass spectrometry, *Anal. Chem.*, **88**, 9276-9284, https://doi.org/10.1021/acs.analchem.6b02663.
173. Asahi, T., Kondo, H., Masuda, M., Nishino, H., Aratani, Y., Naito, Y., Yoshikawa, T., Hisaka, S., Kato, Y., and Osawa, T. (2010) Chemical and immunochemical detection of 8-halogenated deoxyguanosines at early stage inflammation, *J. Biol. Chem.*, **285**, 9282-9291, https://doi.org/10.1074/jbc.M109.054213.
174. Li, T., Wang, C., Liu, Y., Li, B., Zhang, W., Wang, L., Yu, M., Zhao, X., Du, J., Zhang, J., Dong, Z., Jiang, T., Xie, R., Ma, R., Fang, S., Zhou, J., and Shi, J. (2020) Neutrophil extracellular traps induce intestinal damage and thrombotic tendency in inflammatory bowel disease, *J. Crohns Colitis*, **14**, 240-253, https://doi.org/10.1093/ecco-jcc/jjz132.
175. Shukrun, R., Fidel, V., Baron, S., Unger, N., Ben-Shahar, Y., Cohen, S., Elhasid, R., and Yerushalmy-Feler, A. (2024) Neutrophil extracellular traps in pediatric inflammatory bowel disease: a potential role in ulcerative colitis, *Int. J. Mol. Sci.*, **25**, 11126, https://doi.org/10.3390/ijms252011126.
176. Dinallo, V., Marafini, I., Di Fusco, D., Laudisi, F., Franzè, E., Di Grazia, A., Figliuzzi, M. M., Caprioli, F., Stolfi, C., Monteleone, I., and Monteleone, G. (2019) Neutrophil extracellular traps sustain inflammatory signals in ulcerative colitis, *J. Crohns Colitis*, **13**, 772-784, https://doi.org/10.1093/ecco-jcc/jjy215.

177. Schroder, A. L., Chami, B., Liu, Y., Doyle, C. M., El Kazzi, M., Ahlenstiel, G., Ahmad, G., Pathma-Nathan, N., Collins, G., Toh, J., Harman, A., Byrne, S., Ctercteko, G., and Witting, P. K. (2022) Neutrophil extracellular trap density increases with increasing histopathological severity of Crohn's disease, *Inflamm. Bowel Dis.*, **28**, 586-598, https://doi.org/10.1093/ibd/izab239.
178. Lu, F.-Y., Huang, X., Zhang, K., Yin, X., Lv, Y., Du, Y.-C., Zhou, Q., Min, J.-Y., and Cheng, C.-E. (2025) Circulating neutrophil extracellular traps as diagnostic and prognostic markers for inflammatory bowel disease: a case-control study, *J. Inflamm. Res.*, **18**, 7867-7877, https://doi.org/10.2147/JIR.S519545.
179. Masoodi, I., Kochhar, R., Dutta, U., Vaishnavi, C., Prasad, K. K., Vaiphei, K., Hussain, S., and Singh, K. (2012) Evaluation of fecal myeloperoxidase as a biomarker of disease activity and severity in ulcerative colitis, *Dig. Dis. Sci.*, **57**, 1336-1340, https://doi.org/10.1007/s10620-012-2027-5.
180. Borichevsky, G. M., Swaminathan, A., Smith, B. R., Edwards, T. S., Ashby, L. V., Frampton, C. M. A., Day, A. S., Gearry, R. B., and Kettle, A. J. (2025) Myeloperoxidase enzyme activity in feces reflects endoscopic severity in inflammatory bowel disease, *Inflamm. Bowel Dis.*, **31**, 2254-2268, https://doi.org/10.1093/ibd/izaf109.
181. Knutson, C. G., Mangerich, A., Zeng, Y., Raczynski, A. R., Liberman, R. G., Kang, P., Ye, W., Prestwich, E. G., Lu, K., Wishnok, J. S., Korzenik, J. R., Wogan, G. N., Fox, J. G., Dedon, P. C., and Tannenbaum, S. R. (2013) Chemical and cytokine features of innate immunity characterize serum and tissue profiles in inflammatory bowel disease, *Proc. Natl. Acad. Sci. USA*, **110**, E2332-E2341, https://doi.org/10.1073/pnas.1222669110.
182. Manzenreiter, R., Kienberger, F., Marcos, V., Schilcher, K., Krautgartner, W. D., Obermayer, A., Huml, M., Stoiber, W., Hector, A., Griese, M., Hannig, M., Studnicka, M., Vitkov, L., and Hartl, D. (2012) Ultrastructural characterization of cystic fibrosis sputum using atomic force and scanning electron microscopy, *J. Cyst. Fibros.*, **11**, 84-92, https://doi.org/10.1016/j.jcf.2011.09.008.
183. Piva, T. C., Luft, C., Antunes, K. H., Marostica, P. J. C., Pinto, L. A., and Donadio, M. V. F. (2020) Extracellular DNA in sputum is associated with pulmonary function and hospitalization in patients with cystic fibrosis, *Respir. Med.*, **172**, 106144, https://doi.org/10.1016/j.rmed.2020.106144.
184. Wright, T. K., Gibson, P. G., Simpson, J. L., McDonald, V. M., Wood, L. G., and Baines, K. J. (2016) Neutrophil extracellular traps are associated with inflammation in chronic airway disease, *Respirology*, **21**, 467-475, https://doi.org/10.1111/resp.12730.
185. Grabcanovic-Musija, F., Obermayer, A., Stoiber, W., Krautgartner, W. D., Steinbacher, P., Winterberg, N., Bathke, A. C., Klappacher, M., and Studnicka, M. (2015) Neutrophil extracellular trap (NET) formation characterises stable and exacerbated COPD and correlates with airflow limitation, *Respir. Res.*, **16**, 59, https://doi.org/10.1186/s12931-015-0221-7.
186. Zhu, A., Ge, D., Zhang, J., Teng, Y., Yuan, C., Huang, M., Adcock, I. M., Barnes, P. J., and Yao, X. (2014) Sputum myeloperoxidase in chronic obstructive pulmonary disease, *Eur. J. Med. Res.*, **19**, 12, https://doi.org/10.1186/2047-783X-19-12.
187. O'Donnell, C., Newbold, P., White, P., Thong, B., Stone, H., and Stockley, R. A. (2010) 3-Chlorotyrosine in sputum of COPD patients: relationship with airway inflammation, *COPD*, **7**, 411-417, https://doi.org/10.3109/15412555.2010.528086.
188. Van Der Vliet, A., Nguyen, M. N., Shigenaga, M. K., Eiserich, J. P., Marelich, G. P., and Cross, C. E. (2000) Myeloperoxidase and protein oxidation in cystic fibrosis, *Am. J. Physiol. Lung Cell. Mol. Physiol.*, **279**, L537-L546, https://doi.org/10.1152/ajplung.2000.279.3.L537.
189. Kettle, A. J., Chan, T., Osberg, I., Senthilmohan, R., Chapman, A. L., Mocatta, T. J., and Wagener, J. S. (2004) Myeloperoxidase and protein oxidation in the airways of young children with cystic fibrosis, *Am. J. Respir. Crit. Care Med.*, **170**, 1317-1323, https://doi.org/10.1164/rccm.200311-1516OC.
190. Ramos Cáceres, E., Kemperman, L., and Bonger, K. M. (2024) Environment-sensitive turn-on fluorescent probe enables live cell imaging of myeloperoxidase activity during NETosis, *Commun. Chem.*, **7**, 262, https://doi.org/10.1038/s42004-024-01338-5.

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