Neutrophils as emerging protagonists and targets in chronic inflammatory diseases

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Abstract

Introduction Neutrophils are the key cells of our innate immune system with a primary role in host defense. They rapidly arrive at the site of infection and display a range of effector functions including phagocytosis, degranulation, and NETosis to eliminate the invading pathogens. However, in recent years, studies focusing on neutrophil biology have revealed the highly adaptable nature and versatile functions of these cells which extend beyond host defense. Neutrophils are now referred to as powerful mediators of chronic inflammation. In several chronic inflammatory diseases, their untoward actions, such as immense infiltration, hyper-activation, dysregulation of effector functions, and extended survival, eventually contribute to disease pathogenesis. Therefore, a better understanding of neutrophils and their effector functions in prevalent chronic diseases will not only shed light on their role in disease pathogenesis but will also reveal them as novel therapeutic targets.

Methods We performed a computer-based online search using the databases, PubMed.gov and Clinical trials.gov for published research and review articles.

Results and Conclusions This review provides an assessment of neutrophils and their crucial involvement in various chronic inflammatory disorders ranging from respiratory, neurodegenerative, autoimmune, and cardiovascular diseases. In addition, we also discuss the therapeutic approach for targeting neutrophils in disease settings that will pave the way forward for future research.

Keywords Neutrophils · Chronic inflammatory diseases · Hyperactivation · Granular cargoes · NETs · Therapeutic targets

Introduction

Paul Ehrlich in the late nineteenth century first identified heterogeneity in white blood cells and identified ‘neutrophil’: unique cells with polymorphic nucleus and a tendency to retain neutral dyes. Elie Metchnikoff, who is also considered the father of cellular innate immunity, studied neutrophil function and described the recruitment of these phagocytic cells to the site of injury [1]. In human blood, neutrophils constitute about 50–70% of all leukocytes, making them the most abundant immune cells [2]. They constitute the first line of host defense against invading pathogens and are the major ones that initiate an inflammatory response [3]. They are formed during hematopoiesis in the bone marrow and are produced at a rate of $10^{11}$ cells per day, which may further increase at the time of infection [4]. The release of neutrophils from the bone marrow is tightly regulated, wherein chemokine CXCR4 plays a major role [5]. They are the shortest-lived cells in the body with an average life span of 6–8 h in the circulation [1]. Neutrophils leave the circulation and migrate to the site of infection upon sensing an inflammatory stimulus including PAMPs (pathogen-associated molecular patterns), DAMPs (damage-associated molecular patterns), lipid mediators, inflammatory cytokines, and chemokines in a process called neutrophil extravasations [6]. This process includes three major steps: tethering of neutrophils to the activated endothelium which is followed by rolling, neutrophil arrest by tight binding to the endothelium, and finally movement through the endothelial barrier to the site of infection [7]. Once reaching the site of infection, activated neutrophils utilizes diverse mechanisms to eliminate the invading pathogens, including...
phagocytosis, degranulation, oxidative burst, and neutrophil extracellular trap (NETs) formation (Fig. 1). Neutrophils recognize the opsonized target microbes by specific receptors located on the neutrophil surface to initiate the phagocytic process. Immunoglobulins and complement proteins abundantly present in serum play an important role in the opsonization of microbes, thus facilitating the process of phagocytosis [8]. Phagocytic uptake of pathogens increases oxygen consumption and triggers oxidative burst in neutrophils, resulting in the rapid release of reactive oxygen species (ROS). Myeloperoxidase (MPO) and NADPH oxidase present in neutrophils catalyzes the generation of reactive oxygen species (ROS) such as hydrogen peroxide (H2O2), hydroxyl radicals (OH−), and superoxide anions (O2−) [9]. These oxygen derivatives then play a crucial role in the killing of pathogenic microbes. Neutrophils are also equipped with an arsenal of proteases and antimicrobial peptides stored in the distinct neutrophil granules. These granules fuse with phagosomes to facilitate
the killing and digestion of pathogens following phagocytosis [10]. Activated neutrophils also release the granule content in the extracellular spaces in a process called degranulation to mediate extracellular killing of pathogens [11]. Neutrophil granules are divided into three categories, azurophilic/primary granules, specific/secondary granules and gelatinase/tertiary granules. Azurophilic granules are the first one to be synthesized at the promyelocytic stage and store the most toxic and proteolytic mediators, including MPO, neutrophil elastase (NE), cathepsin G, and defensins. Specific or secondary granules are synthesized during the myelocyte stage and include lactoferrin, lipocalin/NGAL, collagenase, gelatinase, lysozyme, and membrane receptors. Similarly, tertiary granules, including gelatinase, acyl transferase, collagenase, and cathepsin are formed during the metamyelocyte stage. Secondary and tertiary granules share some common granule contents that are discriminated against based on their densities [12–14]. In the last few years, neutrophil extracellular traps (NETs) formation has also become an important area of research. In 2004, NETosis was identified as another unique method of pathogen killing adopted by neutrophils. NETs are extracellular fibers or meshes composed of decondensed DNA, histones, and granular proteins of neutrophils such as NE, MPO, and lactoferrin etc. easily visualized upon immunostaining [15]. They entrap and neutralize microbes to promote extracellular killing. According to some reports, NETosis was adopted by neutrophils to encounter large microbes that cannot be phagocyted [16].

Neutrophils are the effector cells of the innate immune system and are actively involved in various immunological and inflammatory responses [17]. They are highly efficient in sensing and eradicating invading pathogens. Therefore, individuals with neutrophil deficiency are highly vulnerable to microbial infections [18]. In contrast, overwhelming activation of neutrophils and their inappropriate recruitment can lead to severe inflammation and tissue damage [19] hence considered as a ‘double edge sword’. Chronic inflammation can cause immune paralysis in multiple ways, and emerging reports have now very well established the central role of neutrophils in this process [20]. Altered functioning of neutrophils has been the cause of various chronic diseases and therefore, a better understanding of neutrophils and their effector functions is crucial for the development of novel therapeutics. In the present review, we provide an assessment of neutrophils in respiratory, neurodegenerative, autoimmune and cardiovascular diseases and also summarize potential strategies to regulate neutrophils for therapeutics. Cancer is also one of the major chronic inflammatory diseases wherein neutrophils play an important role but here we do not delve into cancer as we have recently reviewed the role of neutrophils and their granular cargoes in cancer [21].

**Neutrophils in respiratory diseases**

Neutrophils are emerging as crucial players in pathogenesis of various chronic pulmonary diseases [22] (Fig. 2). In lungs, inflammatory processes are characterized by neutrophil influx into the airways. Interestingly, the pulmonary vasculature is the largest reservoir of circulating neutrophils in the human body and their concentration is 35–100 times higher in the pulmonary capillary blood than in the large vessels of systemic circulation [23]. Neutrophil-derived oxidants and proteases cause multiple injuries, including killing of vascular endothelial and pulmonary epithelial cells, their detachment from the matrix support, and deregulation of airway smooth muscle function and lung matrix degradation [24–26]. Surprisingly, neutrophilic inflammation can be initiated even in the absence of infection and can result in severe pathologies. Neutrophil-mediated pulmonary pathologies are also characterized by neutrophil dysfunction, wherein the cells cannot eradicate microbes and thus are unable to perform their normal duties. In genetic disorders like cystic fibrosis and environment linked diseases such as chronic obstructive pulmonary disease (COPD), massive recruitment of neutrophils can cause severe damage by releasing granule contents (NE, MPO and ROS [27, 28]. Similarly, accumulation of neutrophils in the airway space and lung interstitium is a major characteristic of acute respiratory distress syndrome (ARDS). Owing to this, neutrophilic alveolitis is regarded as a histological hallmark of ARDS [29]. Asthma is one of the most common chronic respiratory diseases in adults as well as children. The disease is typically known to be a Th-2 mediated allergy characterized by severe eosinophilic inflammation [30]. However, growing reports now suggest a large sub-group of non-eosinophilic asthma wherein neutrophils are the active players and play a multi-dimensional role in disease pathogenesis [31]. Increase in neutrophil number and over-activation is also associated with the severity of asthma. Neutrophils produce a descent range of inflammatory cytokines and chemokines within the course of asthma including IL-3, IL-6, IL-8, IL-12, GM-CSF, MIP, IFN-γ, oncostatin M (OSM), and leukotriene B4 (LTB4) [32–34]. Bou Ghanem et al. showed that during S. pneumoniae infection, early influx of neutrophils was essential for controlling the infection, but their extended presence in lungs caused significant pulmonary damage with poor outcomes [35]. Bronchiolitis is also one of the common infection of the respiratory tract and is caused due to viral infection, which usually affects children less than two years of age [36]. Respiratory syncytial virus (SRV) is the major pathogen responsible for infection [37]. Respiratory viruses mainly infect bronchial epithelial cells and cause epithelial activation, which in turn accelerate neutrophil recruitment and activation [38]. Neutrophils were found to be the
predominant inflammatory cell population in both upper and lower airways, accounting for 80% of the total cells recovered from the respiratory tracts of RSV patients [39–41].

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was responsible for the pandemic COVID-19 and primarily target the respiratory system leading to respiratory tract infections [42]. COVID-19 patients showed moderate to severe pneumonia, ARDS, and rapid multiple organ failure ultimately resulting in death [43]. Reports on the involvement of neutrophils in initiation and progression of COVID-19 are rapidly emerging [44]. Barnes et al. in their report highlighted the role of NETs in COVID-19 pathology and introduced them as potential drivers of COVID-19 [45]. In a study by Zhou et al., 99 cases in Wuhan were analyzed showing an increase in total neutrophil count (38%) and a reduction in total lymphocytes (35%) [46]. Another study from Wuhan revealed that ICU patients had a similar pattern of high neutrophils and reduced lymphocytes count. On the contrary, the non-ICU patients showed a statistically different pattern. This neutrophil to lymphocyte ratio was also correlated with disease extremity [47]. Wang et al. showed that massive neutrophil presence predicts poor outcomes in COVID-19 patients [48]. Similarly, Lie et al. suggested neutrophil to lymphocyte ratio, an independent risk factor for mortality in patients with severe disease [49]. Besides respiratory complications, neurological and thrombotic complications are also observed in COVID-19 patients. Genchi et al. recently showed the predominant role of neutrophils in cerebral thrombi in COVID-19 stroke patients [50].

Neutrophils in neurodegeneration

Over the past few decades, the view of neurodegenerative diseases was only limited to neurons. But recently it became evident that immunological processes can actively...
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Neuroinflammation is now regarded as a major hallmark of neurodegenerative diseases wherein immune cells such as NK cells, dendritic cells, T-cells and neutrophils are known to be the major players. These cells can infiltrate the brain during injury or inflammation and exert their direct toxic effects which gradually results in neuroinflammation. These cells via releasing inflammatory mediators can damage neuronal structure and further disrupt neuronal function. Among the various immune cells, neutrophils have emerged as key players in neuroinflammatory disorders, wherein they infiltrate the central nervous system and their untoward actions further contribute to neuronal damage. Alzheimer’s disease (AD) is a complex neurodegenerative disorder of older age characterized by gradual deterioration of cognitive function. It is a most prevalent form of dementia and known to affect more than 35 million people worldwide. Various clinical and animal studies have shown that neutrophils may translocate to the cerebral blood vessels and co-localize with amyloid plaques within the brain parenchyma. Neutrophils can elevate AD pathogenesis via NETosis and release of granular cargoes like NE, MPO and NGAL. Similarly, multiple sclerosis (MS) is a most frequent, chronic inflammatory disease of the central nervous system affecting around 2 million people worldwide. The disease is characterized by demyelination, axonal loss and systemic inflammation leading to neurological disability. In MS patients, high NLR has been proposed as a disease activity marker and the ratio shows a gradual increase with worsening of symptoms.

In neurodegeneration, neutrophils infiltrate the central nervous system and their untoward actions further contribute to neuronal damage. In cardiovascular diseases, neutrophils contribute to plaque instability, activate coagulation cascade and are involved in thrombotic processes. Similarly, in Rheumatoid Arthritis, neutrophils are the most abundant infiltrating cell type found in the synovial fluid and synovial tissue of RA patients. In Psoriasis, neutrophils contribute to sustained inflammation and keratinocyte proliferation through their granular components, oxidative stress and release of NETs.
Neutrophil infiltration was observed in the cerebrospinal fluid of MS patients at the initial stage of disease progression and also during the disease relapse [61]. Similarly, high infiltration of neutrophils was observed in the cerebrospinal fluid (CSF) of children suffering from early-onset MS as compared to the one with later-onset MS suggesting the crucial role of neutrophils in promoting disease severity [62]. In MS, neutrophils also actively contribute to breakdown of blood brain barrier (BBB) whereas depletion of neutrophils enhances BBB integrity [63]. It is suggested that neutrophils can mediate BBB damage through the action of toxic granular cargoes including MPO, MMPs and ROS [64].

Neutrophils in autoimmune diseases

Autoimmune diseases are organ-specific or systemic disorders wherein our immune system acts against the body’s own tissue [65]. Approximately 3–10% of the world’s population is suffering from some form of autoimmune disorder and this percentage is increasing continuously [66]. Neutrophils have emerged as crucial players in the majority of autoimmune disorders ranging from psoriasis, rheumatoid arthritis to type I diabetes. Psoriasis patients show high NLR as compared to the healthy controls [67]. In view of this, abundance of neutrophils in psoriatic skin lesions is adopted as a histopathological hallmark of the disease [68]. Neutrophils contribute to sustained inflammation in psoriasis through their granular components (NE, MPO, proteinase3), oxidative stress and release of NETs [69] which is further correlated with disease severity in psoriasis patients [70]. Interestingly, sera obtained from psoriasis patients can also promote NETosis in the neutrophils of the healthy individuals [71]. Rheumatoid Arthritis (RA) is another complex autoimmune and inflammatory disorder characterized by chronic synovial dysplasia, bone erosion and deformity. Besides affecting synovial joints, RA can target other organs such as lungs, vasculature and skin, thus also considered as a systemic disease [72]. Emerging reports suggest the crucial involvement of neutrophils in the initiation and perpetuation of RA through directly affecting the synovium or modulation of systemic innate and adaptive immune responses [73]. Indeed, neutrophils are the most abundant infiltrating cell type found in the synovial fluid and synovial tissue of RA patients [74]. High concentrations of neutrophil granular proteins have been observed in the synovial fluid which is responsible for cartilage damage, synoviocyte proliferation and activating cytokines and their receptors [75]. Similarly, type I diabetes is another highly prevalent autoimmune disease and by the year 2035, more than 500 million people will be affected by the disease [76]. Various clinical and experimental studies have shown an alteration in neutrophil function in case of Type I diabetes [77, 78]. An increase in the levels of plasma and total NE was observed in the type 1 diabetes patients as compared with the control [79]. This increase in the elastase levels was further attributed to coronary artery disease, diabetic angiopathy and other vascular diseases [80]. In Type I diabetes patients with nephropathy, neutrophils can actively migrate to the kidney and contribute to disease pathogenesis. Apart from producing toxic mediators, neutrophils also contribute to Type I diabetes pathogenesis via NETosis [78].

Neutrophils in cardiovascular diseases

Cardiovascular diseases (CVDs) are chronic inflammatory states of the blood vessels and represent the major critical threat to public health throughout the world, since they contribute to one-third of the global morbidity. Some common CVDs include atherosclerosis, acute coronary syndrome thrombosis and ischemic myocardium [81]. Various studies have reported a crucial involvement of neutrophils in pathogenesis of cardiovascular diseases [82]. Of note, an elevated neutrophil-to-lymphocyte ratio (NLR) has been associated with adverse outcomes in cardiovascular diseases including mortality, stroke and heart failure [83]. Atherosclerosis is a progressive chronic cardiovascular disorder wherein the major targets are large and medium-sized arteries. Neutrophils and their granular cargoes have been detected in the early-stage and late-stage atherosclerotic lesions [84]. Studies suggest a positive correlation between plasma MPO levels and disease severity in patients with established atherosclerosis [85]. Similarly, MMP-2, MMP-8 and MMP-9, which are abundant in secondary and tertiary granules of neutrophils, were found to be elevated in patients with acute coronary syndrome [86]. These matrix-degrading proteases actively participate in the degradation of type IV collagen leading to plaque instability [87]. Recent clinical studies have also shown the presence of NETs in atherosclerotic lesions, which is further correlated with disease severity [88]. Neutrophils are also actively involved in the thrombotic processes. Neutrophil-derived granular cargoes including neutrophil elastase and cathepsin G have been shown to enhance coagulation via degradation of tissue factor pathway inhibitor (TFPI) which is the main inhibitor of tissue factor (TF) whereas inhibition of neutrophils regulated the coagulation cascade. Similarly, NETs formation in circulation also impacts thrombosis. NETs can bind to active coagulation factor XII and trigger coagulation cascade [89]. Neutrophils also infiltrate ischemic myocardium within a few hours following myocardial infarction (MI). These recruited neutrophils are initially aimed to perform phagocytosis and clear dead cell debris caused by ischemia. Instead their huge infiltration results in collateral cardiac injury by releasing toxic granular proteases, reactive oxygen species (ROS) and other inflammatory mediators [90].
Neutrophils as therapeutic targets

Neutrophils are now not-so-neutral in chronic inflammatory diseases and their crucial involvement in these diseases provides a rationale for development of neutrophil-targeted therapeutic interventions. Therapeutic strategies that can be adopted include reducing neutrophil production, recruitment, activation, or lowering down the effects of neutrophil-derived granular cargoes. The important strategy that has shown efficacy in preclinical models includes inhibition of G-CSF [91]. It is a key regulator of granulopoiesis and inducer of neutrophil production. G-CSF binds to G-CSF receptors, which is predominantly expressed on mature neutrophils [92]. Campbell et al. showed that targeting the G-CSFR receptor with a neutralizing monoclonal antibody reduced neutrophil mobilization to the joints which further halted the progression of established arthritis in mice [93]. Also, therapeutic inhibition of G-CSF or genetic deletion of G-CSF showed a significant reduction in the severity of experimental autoimmune uveoretinitis via regulating neutrophil trafficking [94]. IL-17 and IL-23 act as upstream regulators of G-CSF, therefore targeting IL-17 and IL-23 is another therapeutic approach to regulate neutrophil production [95]. Interestingly, the blockade of IL-17 activity with anti-IL-17 antibody showed a reduction in the numbers of neutrophils in BAL fluids in a mice model of allergic asthma [96]. Importantly, the clinical trials evaluating the safety, efficacy and tolerability of a monoclonal antibody against IL-17 are underway for various chronic diseases including rheumatoid arthritis, Crohn’s disease and psoriasis [97, 98] (Table 1).

Neutrophils are highly motile cells and their migration to the site of inflammation or injury is mediated by chemokines such as CXCL1 (Gro-α) and CXCL8 (IL-8). They act as potent chemoattractants for neutrophils which further activate GPCRs, particularly, CXCR1 and CXCR2. Activation of CXCR2 by IL-8 triggers neutrophil migration to the site of infection [99], whereas receptor-ligand interaction of CXCR1 is responsible for neutrophil degranulation [100]. Currently, chemokine receptor antagonists are in different stages of clinical trials. For instance, SB-656933, a CXCR2 antagonist has been found effective in cystic fibrosis wherein it reduces the levels of inflammatory biomarkers in the sputum of patients [101]. Similarly, MK-7123 (navarixin) is another CXCR2 antagonist which can improve lung function in COPD patients [102]. In a study by Nair et al. the effect of SCH527123, a dual CXCR1/CXCR2 receptor antagonist was tested in severe asthma patients with increased sputum neutrophils. The drug showed a significant reduction in sputum neutrophils with mild exacerbations [103]. Ladarixin is an allosteric non-competitive antagonist which inhibits the biological activity of CXCL8 through inhibition of the activation of CXCL8 receptors (CXCR1 and CXCR2). It is orally available and is currently tested in Phase 2 clinical trials (NCT02814838, NCT01571895). Mattos et al. showed

| Table 1 | List of drugs under clinical trials for targeting neutrophils in various chronic inflammatory diseases |
|-----------------|-------------------------------------------------|-----------------|-----------------|-----------------|
| Drug            | Mode of action                                  | Diseases        | Clinical trial phase | Clinical Trials.gov identifier |
| BAY85-8501      | Human neutrophil elastase (NE) inhibitor         | Non-CF bronchiectasis | Phase 2          | NCT01818544     |
| POL6014         |                                                  | Cystic fibrosis | Phase 1          | NCT03748199     |
| CHF 6333        |                                                  | Cystic fibrosis | Phase 1          | NCT04010799     |
| Elafin           |                                                  | Pulmonary arterial hypertension | Phase 1 | NCT03522935     |
| Crisaborole ointment 2% | Phosphodiesterase 4 (PDE4) inhibitor | Atopic dermatitis | Phase 4          | NCT03356977     |
| Ensifentrine     |                                                  | COPD            | Phase 3          | NCT04535986     |
| Rolflumilast     |                                                  | COPD            | Phase 4          | NCT01329029     |
| Apremilast       |                                                  | Psoriatic arthritis | Phase 4         | NCT02425826     |
| Disulfiram       |                                                  | COVID-19        | Phase 2          | NCT04594343     |
| Pulmozyme        |                                                  | COVID-19        | Phase 2          | NCT04402944     |
| AZD5069          | CXCR2 antagonist                                 | Bronchiectasis  | Phase 2          | NCT01255592     |
| Canakinumab      | IL-1b (mAb)                                     | COVID-19-pneumonia | Phase 3     | NCT04362813     |
| Tocilizumab      | IL-6 (mAb)                                      | COVID-19-pneumonia | Phase 2   | NCT04317092     |
| Brensocatib      | Dipeptidyl peptidase 1 (DPP-1) Inhibitor        | Non-cystic fibrosis bronchiectasis | Phase 3 | NCT04594369     |
| N-acetylcysteine |                                                  | COVID-19        | Phase 2          | NCT04419025     |
| Ixekizumab       | IL-17A (mAb)                                    | Psoriatic, arthritis | Phase 3     | NCT01695239     |
| Risankizumab     | IL-23 (mAb)                                     | Psoriasis       | Phase 3          | NCT02684370     |
| Colchicine       | Microtubule polymerization inhibitor             | COVID-19        | Phase 3          | NCT04322682     |
Another study conducted by Lapponi et al. showed that neutrophil NETs in endotoxin-triggered acute lung injury [116]. Mice with aspirin showed decreased formation of intravascular NETs and anti-thrombotic effects [115]. In a study, pretreatment of acid (Aspirin) is a non-steroidal drug with anti-inflammatory and neutrophil-mediated endothelial cell damage in patients with ANCA-associated vasculitis [112]. In addition, there are several other NE inhibitors which are under different stages of clinical trials such as AZD9668 [108], lonodelestat [109] and elafin [45]. Dipeptidyl peptidase 1 (DPP1) also known as cathepsin C controls the activation of the neutrophil serine proteases (NSPs) including NE, cathepsin G, proteinase 3 [110]. Brensocatib (INS1007 or AZD7986) is a DPP1 inhibitor which was found to reduce NE activity in healthy humans [111]. It is now in phase 2 of clinical trials for bronchiectasis (NCT03218917) and in phase 3 for COVID-19 (NCT04817332). MPO inhibitors, such as PF-1355 have been shown to reduce atherosclerotic burden, stabilize atherosclerotic plaque, and prevent cardiac complications in in vitro and in vivo studies [111]. Similarly, MPO inhibition with AZM198 reduced neutrophil degranulation and neutrophil-mediated endothelial cell damage in patients with ANCA-associated vasculitis [112]. In addition, a variety of MPO inhibitors have been developed and investigated in preclinical studies, including triazolopyrimidines, acetalinophen, flavonoids, benzoic acid hydrazides, thiouracil derivatives, and ferulic acid derivative [113]. Furthermore, various studies have also reported the active compounds from medicinal herbs with potential as MPO inhibitors [114].

NETs also play a role in enhancement of the inflammation in various chronic inflammatory diseases; hence targeting NETs can also be an easy and applicable strategy. There are numerous studies evaluating the possible effects of certain compounds against NETs. For instance, Acetylsalicylic acid (Aspirin) is a non-steroidal drug with anti-inflammatory and anti-thrombotic effects [115]. In a study, pretreatment of mice with aspirin showed decreased formation of intravascular NETs in endotoxin-triggered acute lung injury [116]. Another study conducted by Lapponi et al. showed that neutrophils treatment with aspirin prevented NET formation via inhibiting NF-κB, an inflammatory transcriptional regulator that promotes NETosis [117]. In a mouse model of lupus, treatment with BB-C1-amidine which is a PAD4 inhibitor, disrupts NET formation and protect the mice from NET-mediated endothelial dysfunction, vascular damage and kidney injury [118]. Similarly, PA-dPEG24 is a peptide inhibitor of complement C1 (PIC1) which is known to mitigate the peroxidase activity of MPO. Hair et al. demonstrated that PA-dPEG24 inhibits NETs formation by blocking the MPO pathway of NET formation [119]. Metformin is a widely prescribed antidiabetic drug that suppresses immune responses via regulation of ROS [120]. Menegazzo et al. examined the effect of Metformin on NETosis and found a significant decline in the concentrations of NETs as well as reduction in elastase, proteinase-3 and histones [121]. Furthermore, antibiotics used in the management of bacterial infections, have immunomodulating properties by influencing various immune cells, including neutrophils [122]. Manda-Handzlik et al. examined the effects of gentamicin and cefotaxime on NETs and found that gentamicin was able to inhibit NET release by human neutrophils, while no such effect was found upon cefotaxime treatment [122]. In summary, neutrophils, their granular cargoes and NETs demonstrate as potential therapeutic targets for the treatment of various chronic inflammatory diseases.

**Conclusion**

Neutrophils are now regarded as central players in various chronic inflammatory diseases. Irrespective of their defined task to eliminate infection, pro-survival and hyperactivation of neutrophils usually aggravate tissue injury. Neutrophil-derived proteases, ROS, and NETs are known to exert a double-sided consequence on the severity of various chronic inflammatory disorders. While there have been significant advances in understanding the role of neutrophils in diseases, still there remains much to learn about what underlies the influx of neutrophils to various tissues in disease condition. Regulating their recruitment through precise mechanisms has the highest possibility to limit the tissue-destructive potential of neutrophils. Also, delineating signaling pathways that are specifically activated to induce the release of toxic mediators and NETs can also aid in the development of drugs that will prevent neutrophil hyperactivation in various inflammatory disorders. Furthermore, several plant-derived compounds have been identified over the years for their potent immunomodulatory characteristics. Such natural immunomodulators can be screened and tested for the effective regulation of neutrophil hyperactivation. All together, these points could be important for understanding both the neutrophil-mediated pathogenesis and possible
neutrophil-directed therapeutic interventions for various chronic inflammatory diseases and a due consideration must be given to this predictive view.

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Declarations
Conflict of interest To the best of our knowledge, the named authors have no conflict of interest, financial or otherwise.

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