

Review Article

Neutrophil Extracellular Traps in Inflammatory and Autoimmune Diseases and Cancer

Kaveh Tari¹ M.Sc., Ayat Abbasi-Shahkouch² B.Sc., Seyedeh Fatemeh Azghadi² B.Sc., Kimiya Rashidan² B.Sc., Zahra-Sadat Mousavi² B.Sc., Mobarakeh Ajam-Hosseini³ M.Sc., Reyhaneh Abriyan² B.Sc., Amir Atashi^{4*} Ph.D.

¹ Department of Hematology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

² Student Research Committee, School of Allied Medical Sciences, Shahroud University of Medical Sciences, Shahroud, Iran

³ Department of Cell and Molecular Biology, Faculty of Basic Sciences, Kharazmi University, Karaj, Iran

⁴ Department of Medical Laboratory Sciences, School of Allied Medical Sciences, Shahroud University of Medical Sciences, Shahroud, Iran

ABSTRACT

Article history

Received: 1 Jul 2022

Accepted: 27 Feb 2023

Available online: 30 Mar 2023

Keywords

Autoimmune

Inflammatory

Neutrophil

Neutrophil extracellular traps

Neutrophils are innate immune system phagocytes that play a central role in immunity defense. They are equipped with effective antimicrobial that is mainly stored in specialized granules. Considering that, it can also damage host tissue. Neutrophil deployment is heavily regulated through various strategies, including phagocytosis, reactive oxygen species, production degranulation, and the formation of neutrophil extracellular traps (NET). This review article will discuss its role in inflammatory, autoimmune diseases, and cancer and place it as a therapeutic target. It depicts that NET formation includes a suicide program morphologically different from other types of cell death, such as apoptosis and necrosis. Besides, NETs have unique DNA and antimicrobial peptide structures, and antimicrobial activity is among the functions of neutrophils as the first response to inflammation. So, it plays a pivotal role in the pathophysiology of various diseases, especially inflammatory and autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis. As a result, it can be effective in the pathogenesis of many diseases, and its pathogenic role can be used as a therapeutic target.

Introduction

Neutrophils are innate immune phagocytes that play a central role in immune defense. They are equipped with effective antimicrobial that is mainly stored in specialized granules. Because it can also damage host tissue, its establishment is strongly regulated by a variety of strategies, including phagocytosis, production of reactive oxygen species (ROS), degranulation, and release of neutrophil extracellular traps (NET) [1, 2]. The discovery of NET in 2004 opened a modern chapter within immune-mediated microbial murdering. Cooper et al. mentioned that when catastrophically invigorated, neutrophils experience an unused shape of a modified cell passing called NETosis, which decompresses all of their chromatin/DNA, and the resulting structure is discharged within the cytoplasm [3]. During the formation of NETs, the plasma membrane ruptures in a programmed manner. It suggests that the NETs formation involves a suicidal plan morphologically different from other types of cell death, such as apoptosis and necrosis [4]. NETs are specific structures of basket-like DNA with antimicrobial peptides [5]. Antimicrobial activity is among the functions of neutrophils as the first response to inflammation [6]. The neutrophil nucleus loses its shape following stimulation, and the chromatin is decompressed by the peptidyl arginine deiminase 4 (PAD4) enzyme [7]. Then the nucleus membrane and granules are destroyed, and the NET components are mixed. Eventually, the core membrane ruptures, and the NET structure is released. This type of cell death differs from necrosis and apoptosis and is called

NETosis [8]. The formation of this structure requires ATPs to organize the microtubule network [9].

One of the most characteristic features of NETosis is the fracture of the nucleus accumbens. This feature distinguishes it from apoptosis and is very reminiscent of the collapse of the nuclear envelope during mitosis in dividing cells [10]. In addition, NET-forming neutrophils lack DNA fragmentation and normal phosphatidylserine exposure to the apoptotic cell death pathway [11]. NET formation and release of mitochondrial DNA do not necessarily lead to neutrophil death or life-shortening [12].

Microorganisms, chemical compounds, and inflammatory mediators, such as Interleukin (IL)-8, complement factor 5a, N-formyl-methionyl-leucyl-phenylalanine, lipopolysaccharide (LPS), and tumor necrosis factor (TNF), as well as pathogens such as *Shigella flexneri*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Streptococcus pneumoniae*, and *Albicans*, can stimulate NET formation, which causes DNA release and neutrophil degranulation [13, 14]. The results show that thick filaments of NET are aggregates of thinner fibers less than 2 nanometers in diameter. In addition, it has been observed that an extensive net is formed in places where thick fibers disintegrate, often attached to spherical structures about 10 nm in diameter. They can combine to form larger masses. The entanglement of these chromatin filaments results in a three-dimensional extracellular structure [15].

NETs are net-like structures composed of nuclear DNA, histones, and proteins such as neutrophil granule proteins, which include myeloperoxidase (MPO), neutrophil elastase (NE), lactoferrin, and cathepsin G [16, 17].

Steps for the NET formation

NET production may occur based on three paths: 1- slow cell death, 2- vesicular secretion leading to the rapid exit of NETs, 3- the mechanism of the formation of NET from mitochondria DNA (mtDNA) [18]. In the first case, nuclear components are scarce, including type B lamin, histones or poly (ADP-ribose) polymer, and other elements such as cytochrome c, b-actin, and cytoplasmic caspase, 3. However, the common feature of these two types of NET is the presence of two proteins -MPO and NE- associated with the mtDNA [15].

In the acute trauma of environmental tissue, mtDNA and oxidized mtDNA forms the NET and sterile inflammation. MtDNA increases the ROS and expresses the rac family small GTPase 2 (Rac 2), PAD4, and the mtDNA oxidized through cyclic GMP-AMP synthase (cGAS), and toll-like receptor 9 (TLR9) activate neutrophils and forms NET. In general, mtDNA forms NET by starting TLR9, ERK1/2, and p38 MAPK signals [19].

Phorbol-12-myristate-13-acetate (PMA) is directly connected to the protein kinase C, and calcium is released from intracellular reserves and activates the Raf-MEK-ERK path. The nicotinamide adenine dinucleotide phosphate (NADPH) complex is accumulated downstream in the phagosomal membrane and produces active oxygen species. Many NETosis inducers stimulate the signaling of MAP kinase, activate NADPH oxidase (NOX2), and further lead to

ROS production, leading to the release of NET and, subsequently, the MPO release of azurophilic granules [20]. It results in the rupture of the granule using a protein complex called "acrosome," which transfers NE to the core [2]. The NE protein serine is mobilized with the MPO and transferred to the core. NE breaks down histones to boost chromatin density [4]. Increasing intracellular calcium levels also activates peptidyl arginine deiminase 4, which reduces the positive charge of histones by the citrullination of these proteins. All of these molecular events reduce chromatin density. As a result, after about 2 hours, the PMA-stimulated neutrophils lose their nucleus heterochromatic regions and the characteristics of nuclear lobules. Then, the cores accumulate and expand. The nuclear cover breaks down into vesicles, granules, and mitochondria. The cytoplasm and the protoplasm are intermixed, and eventually, the cell membrane tears apart and releases cellular content that forms NET in cellular space [21]. This series of events is called suicide.

In contrast, it has been reported that neutrophils can release the whole core or parts of it without breaking the cell membrane. As a result, nuclear cytoplasts will still be able to move phagocyte bacteria, although their crawling pattern will differ from nucleus-containing neutrophils. Since neutrophils survive this form of DNA evacuation, it is called critical NETosis [22].

The formation of the NETs occurs from two general NOX- dependent and NOX- independent routes. NOX2 produces several active oxygen species that activate the NOX-dependent pathway needed to activate ERK, Akt, and P38. In this direction, ERK activation is required (the Raf-

MEK-ERK path) [23], while it is less activated in the independent pathway of NOX [24]. In the NOX-dependent path, the MPO must form and process the ROS to lead to the NET formation [25]. The NOX-independent pathway occurs due to the mitochondrial ROS and the potassium channel activated with calcium. Akt activation is required in both ways, and the amount of p38 activation is the same. PMA is a factor that plays a role in activating the NET from the NOX-dependent path in cell culture [26]. One of the differentiating factors of NET-based NOX is using an antibody against the histone N terminal tail to detect MOP-histone complexes. In this way, NET-based NOX, the neutrophil elastases break the N terminals of the core histones during the NET formation [26]. Neutrophil exposure to PMA or LPS can result in NET formation by the NOX-dependent path [27]. In the NOX-independent pathway, activated platelets are agents of NET formation [28]. Autophagy is one of the factors that trigger the formation of NET without ROS, which acts through the Macrophage-inducible C-type lectin [29] (Fig. 1). ROS is needed to release mtDNA in the NET formation process. Recently, it has been reported that ROS production by NADPH oxidase is essential for the viscosity of neutrophils. Also, it has recently been shown that NADPH oxidase produces ROS in neutrophils and regulates actin polymerization through reversible actin glutathione. Actin polymerization plays a vital role in starting ROS production in neutrophils by strengthening the accumulation and activity of

NADPH oxidase. Actin and tubulin are glutathione proteins in neutrophils that undergo physiological activation and lead to the NET formation. Cysteine, located at the end of C actin (Cys374), is required for the glutathione of actin-induced ROS, actin polymerization, and NET formation [30]. Various stimuli can induce NET formation. Although it may run through different paths, it is accompanied by a transcription induction. MAPK signaling is essential for the NET formation and activating neutrophil transcription programs, and the NET formation is independent of molecular synthesis. Therefore, neutrophil activation involves parallel paths: The path that relies on transcription to produce chemokines and to enhance inflammatory responses, and the latter is independent of transcription, which leads to NET formation and other antimicrobial effects [18, 31].

NET and autoimmune diseases

In addition to NET's role in infectious diseases, such as fungal and parasitic infections, NET formation is involved in autoimmune diseases. To this end, NET's role has been suggested in rheumatoid arthritis (RA) [32], systemic lupus erythematosus (SLE) [33], Antineutrophil cytoplasmic antibody (ANCA) [34], Psoriasis [35], Hashimoto thyroiditis [36], and anti phospholipid syndrome (APS). For instance, one of the clinical manifestations of APS is intravenous and arterial thrombosis; since NETs are implicated in thrombosis, the potential role of NET in APS can be justified.

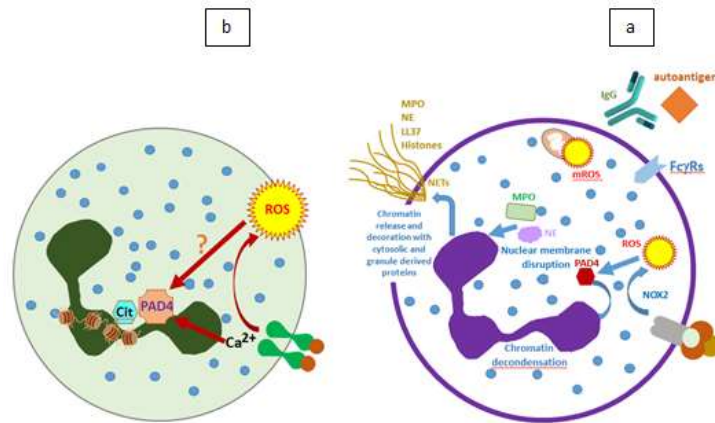


Fig. 1. Mechanisms of NET formation; NOX-dependent path (a) and NOX-independent path (b). PAD4= Peptidyl arginine deiminase 4; NADPH oxidase= Nicotinamide adenine dinucleotide phosphate oxidase; ROS= Reactive oxygen species; MPO= Myeloperoxidase; NETs= Neutrophil extracellular traps; Cit= Citrulline

So far, NET's direct association with Addison's disease, Pernicious anemia, Graves, and Sjogren's syndrome has not been reported [37]. Nonetheless, it is suggested that in patients with Graves treated with Propylthiouracil, this medication affected morphological abnormalities during NET formation and led to ANCA production [38]. In addition, anti-NET antibodies (ANETA) have been detected in Sjogren's syndrome [39]. Many SLE patients have undergone several inactive mutations in DNase I, which indicate the correlation between autoimmune diseases and disruption of NET clearing processes [40]. It should be noted that limited studies have been conducted on the role of NET in the pathogenesis of multiple sclerosis (MS) and Hashimoto thyroiditis, and further studies are required.

NETs and their role in SLE

SLE is an inflammatory and autoimmune disease that affects different body organs and is associated with excessive levels of autoantibodies [41]. Factors such as functional impairment of lymphocytes and dendrites and anti-nuclear antibodies play a role in the

pathogenesis of the disease [42]. The NETs level was studied in skin lesions in various lupus subtypes, such as lupus discoid, acute skin lupus, and lupus panniculitis. It is reported that NETs have contributed to tissue damage in lupus panniculitis. Evidence shows that the NETolytic activity declines in people with lupus. Thus, the level of specific NET indicators, including MPO and cfDNA, rises in the serum of these patients [43, 44]. Hakkim et al. reported that due to DNase1 inhibitors in SLE patients, NETs could not be destroyed, which can exacerbate lupus disease [45]. In patients with lupus, apoptotic particles containing acetylated histones produce accumulation and NET formation, consequently causing lupus nephritis [46].

Role of NETs in RA

RA is a chronic inflammatory disease that is associated with sinusitis inflammation and hyperplasia (swelling), cartilage and bone degradation (deformation), and systemic features, including cardiovascular disorders, pulmonary, Mental and skeletal muscle, which ultimately leads to severe physical disability and early death. Due to the presence of

autoantibodies such as the rheumatoid factor (RF) and anti-protein antibody [47], RA is considered an autoimmune disease. Studies have shown that RA is a multi-factor disease that age, sex, environmental factors, and genetics could play a role in its pathogenesis. However, its pathogenesis is not fully understood [48]. Various elements in the blood of patients with RA, such as antibodies or immunosuppressive molecules, appear to stimulate NET formation [49]. NET over-formation produces amino acids such as citH2B, citH2B, and citH4 histones. In addition, NET-induced citrullinated vimentin is a central autoimmune factor that stimulates the secretion of proinflammatory cytokines (such as TNF- α and IL-1) and the expression of PAD4 along with the Kappa B nuclear factor ligand (RANKL) activation in the Fibroblast-like synoviocyte [50]. Fibroblast-like synoviocyte, the key inflammation agent in RA, produces several cytokines that damage the joints [51]. Furthermore, the proposed citrullinated antigens actuate antigen-based immune system reactions and lead to the generation of anti-NET autoantibodies. Subsequently, unremitting immune system irritation will exist for a long time. Anti-NET RA (rm Abs) monoclonal antibodies delivered from B- synovial CD19+ cells collected from RA patients are always associated with the NET arrangement. NET antigen resistance depends on the physical hypermutation within the variable Heavy chain and variable Light of the B-synovial cell receptor. In expansion, the glycosylation joined to the Fab-N decides the reactivity of antibodies [52]. The RF, anti-citrullinated protein antibodies, and other antibodies in blood

or sinusoidal fluid strongly trigger NET formation in RA [53]. IgG or IgM collected from peripheral blood or synovial fluid in RA patients could trigger more NET antibody production than IgM and IgG collected from healthy people [54].

Hashimoto thyroiditis

Hashimoto thyroiditis, or chronic lymphocyte thyroiditis or autoimmune thyroiditis, is the most common thyroid clinical inflammation, mainly determined by T cells and less by B cells in histology. Among the biochemical characteristics of Hashimoto thyroiditis, antibodies can be found against two major thyroid antigens, including thyroid peroxidase (TPO) and thyroglobulin (TG) [55, 56]. TPO is a membrane protein that synthesizes thyroid hormones, such as thyroxin (T4) and triiodothyronine (T3), in the apical membrane of the follicular cells. TG glycoprotein is inside the follicle and is vital in synthesizing thyroid hormones. There is a correlation between the severity of histological thyroiditis and the level of thyroid antibodies in the serum of Hashimoto thyroiditis patients [57].

Furthermore, studies show a significant positive relationship between the concentration of NETs (with the help of the NE and PR3 indicator) and the TG Ab and TPO Ab header in the plasma, which can prove the role of NETs in Hashimoto thyroiditis pathogenesis. One of the most important cytokines in the inflammation process is IL-6. Researchers have considered IL-6 an inducing factor of NETosis in Hashimoto thyroiditis pathogenesis, stimulating and promoting inflammation in the thyroid gland tissue [58]. Although further

investigation of NETosis's role in Hashimoto thyroiditis pathogenesis is required, it can be expected that in the future, NET can be one of the potential therapeutic mechanisms in patients with Hashimoto thyroiditis.

Multiple Sclerosis (MS)

MS is a chronic inflammatory disorder and central nervous system demyelination. In this disease, an autoimmune response to axon's myelin sheathes leads to axon atrophy and the death of neurons. MS is a heterogeneous disease that is found in two major forms, the relapsing and remitting MS (RRMS) and the primary progressive MS, which affects about 85-90% and 10-15% of patients, respectively [59, 60]. PAD4, a member of the PAD family, is a cytoplasmic enzyme involved in histone citrullination in NETosis. PAD4 contains a nuclear localization signal (PPAKKST) that does not exist in other PAD enzymes. It has been reported in several studies that the concentration of PAD4 in white central nervous system of MS patients was higher than that of healthy people (control group). These findings highlight the important role of nuclear PAD4 in MS pathogenesis [61-63]. In addition, a high level of IL-8, circulating neutrophils, and NETs in the serum of RRMS patients has been observed; Some researchers mention that IL-8 is involved in stimulating the NET formation and prolonging the survival of neutrophils in MS. On the other hand, the increase in neutrophils in these patients indicates effective mechanisms such as degranulation, oxidative explosion, and NET diffusion, which can ultimately exacerbate inflammation and tissue damage [64].

Sjogren's syndrome

Sjogren's syndrome is a systemic autoimmune disease that mainly affects the exocrine glands (mostly salivary and lacrimal glands) and leads to severe dryness of the mucosal surfaces [69]. About 76% of ANETA were observed in the serum of patients with Sjogren's syndrome, indicating the importance of NETs in Sjogren's syndrome [70].

NET and diabetes

Type 1 diabetes (T1D)

T1D is an autoimmune disease that destroys β cells that produce insulin [65]. In patients with T1D, an average decrease in the neutrophil count has been reported with increased circulating protein levels and enzymatic activities of NE and proteinase 3 (PR3). These changes are partly attributed to the strengthening of the NETosis process and increasing NET formation, leading to the release of NE and PR3 into the bloodstream, where NE and PR3 are used as sensitive biomarkers to diagnose type 1 diabetes [66]. Based on experimental findings, physiological beta cell death induces the uptake and activation of B-1a cells, neutrophils, and plasmacytoid dendritic cells (pDCs) into the pancreas. Activated B-1a cells secrete dsDNA-specific IgGs (dsDNA-specific IgGs) that, by stimulating neutrophils, release the Cathepsin-associated antimicrobial peptide to bind to their DNA. Eventually, interferon- α is produced in the pancreatic islets. These studies confirm the association between the existence of NETs and the release of cathepsin-associated antimicrobial peptide. NETs are key in delaying wound healing [67, 68].

In the mouse model, the NET formation was observed in the islets of Langerhans in the early second week after delivery, while clinical studies in patients with T1D showed an increase in the rate of NETosis and a positive association with circulating NE. It may indicate a key role for neutrophils and NETosis in initiating autoimmunity in the pancreas [67].

Type 2 diabetes mellitus (T2DM)

T2DM could be an unremitting illness characterized by tall blood glucose levels, impeded affront discharge, and affront resistance due to impeded carbohydrate, fat, and protein digestion system. Affront resistance happens for a long time; recently, the onset of T2DM is due to weight, the need for physical inertia, and hereditary inclination [72]. NETosis occurs during inflammation, and patients with T2DM have a low-level chronic inflammation condition [73]. Neutrophil activation is increased in obese and T2DM patients compared with lean individuals. Obesity surgery reduces neutrophil activation in patients to some extent [74]. Insulin signaling is disturbed by nitration of the affront receptor β subunit by MPO and debasement of affront receptor substrate 1 by NE [75]. Neutrophil effects can also be caused by direct interaction with fat cells [76].

NET and inflammatory diseases

Under normal circumstances, neutrophils help tissue degradation by phagocytosing necrotic cells to prevent the uptake of more immune cells, releasing mediators to promote growth and angiogenesis, and producing resolvins and proteins [68]. Neutrophils also play an important role in chronic inflammation. Neutrophils are continuously recruited to the site of chronic

inflammation and help by releasing serine proteases, forming NETs, and activating other immune cells during the inflammatory process [71].

NET and nonalcoholic fatty liver (NAFLD)

Chronic liver illness is predominant worldwide and is the foremost common cause of passing and complications related to liver infection. NAFLD incorporates various liver conditions, counting nonalcoholic greasy liver maladies characterized by steatosis and nonalcoholic steatohepatitis (NASH) with irritation, liver cell harm, and fibrosis [77]. Several studies in humans and mice have suggested the role of neutrophils in NAFLD. Infiltration of neutrophils into the liver promotes NASH, and circulating neutrophils are associated with the severity of NASH disease [78, 79]. ROS produced by adsorbed neutrophils activate the hepatic stress apoptosis signal, which regulates kinase 1 and p38, and promotes progression to NASH [80, 81].

NET and inflammatory bowel diseases (IBD)

IBD includes Crohn's disease and ulcerative colitis, which are chronic and recurrent inflammations that damage the structure of the gastrointestinal tract. Clinically, inflammatory bowel disease is characterized by severe diarrhea, bleeding, abdominal pain, and fluid loss due to the underlying inflammatory process [82, 83]. In this disease, neutrophils can act as beneficial agents that promote pathogen clearance and wound healing through IL-22 and NETs or as harmful agents that increase inflammation through PAD4, proteases, and ROS secretion [84]. Neutrophils are the first cells to infiltrate and appear involved in

epithelial barrier damage, tissue destruction by oxidative and proteolytic damage, and the continuation of inflammation with the release of cytokines and chemokines associated with proinflammatory effects. The dominance of the inflammatory and oxidative environment in IBD provides potential NETosis stimuli such as DAMPs, PAMPs, and cytokines [85]. The role of NET-related compounds in the pathophysiology of IBD includes promoting inflammatory responses by modulating cytokine production and maturation and changes in intestinal epithelial barrier function that lead to increased intestinal permeability. Extracellular matrix (ECM) degradation and potential loss of therapeutic effect of monoclonal antibodies are associated with increased proteolytic activity [86]. NETs are a major cause of initiation and progression of colitis that causes intestinal damage by activating macrophages to secrete proinflammatory cytokines IL-6, TNF- α , and monocyte chemoattractant protein-1, activating platelets and endothelial cells [87].

NET and asthma

Asthma may be an inveterate fiery malady of the aviation route that influences individuals of any race, ethnicity, and age. Patients were partitioned into mild, moderate, and extreme asthma based on the recurrence of side effects, the impact on action and rest, and the alteration in lung work [88]. Due to the lack of established golden standards, asthma diagnosis is mainly based on frequent respiratory symptoms. Asthma has various symptoms, including wheezing, shortness of breath, chest, coughing, and different respiratory airflow restrictions [89]. Studies have shown that asthma is mainly caused

by the T helper 2 (Th2) cells, which secrete IL-4, IL-5, and IL-13, leading to airway eosinophilia [90]. Asthma can also be caused by airway neutrophil inflammation, especially in acute, severe, and insensitive to glucocorticoid asthma. According to studies, neutrophils play a role in early asthma and eosinophils in late asthma responses. Pulmonary neutrophil penetration in asthma may reflect a "natural" reaction to pneumonic aggravation, which is activated, for case, by aviation route contaminants or the nearness of possibly pathogenic microbes. On the other hand, inalienable variables related to the patient's socioeconomics (counting age-related changes in neutrophil work) and comorbidities, counting weight, body mass index, and affront resistance, can too influence aviation route neutrophilia [91].

NET and dermatomyositis

Dermatomyositis is an idiopathic provocative myopathy characterized by proximal muscle shortcomings and a typical rash. Interstitial lung disease (ILD) is another distinct clinical feature of dermatomyositis that affects the prognosis of the disease [92, 93]. In addition to causing significant cytotoxicity to endothelial cells and disrupting the differentiation of endothelial progenitor cells into adult endothelial cells, Low-density granulocytes (LDGs) can also increase secretion of type I interferons (IFNs), TNF- α and IFN- γ , which are likely to induce large numbers of neutrophils to produce NET in vivo. Studies have shown a significant increase in the percentage of LDG in peripheral blood mononuclear cells in patients with SLE and diabetic patients with ILD. On the other hand, a

positive correlation between the percentage of LDG and plasma NET-related markers in diabetic patients with ILD could indicate the important role of LDG in the development of DM-related ILD and NET formation [94, 95].

NET and psoriasis

Psoriasis is an inveterate fiery dermatosis characterized by over-the-top expansion of keratinocytes and silver-white plaques due to expanded cell turnover. Neutrophils separated from the patients' blood appeared more noteworthy helpless to ROS-dependent NETosis than neutrophils separated from healthy individuals [96]. Assist ponders appeared that neutrophil take-up and NETs are vital in psoriasis skin injuries. Centrally pathogenic IL-17, discharged by neutrophils, can increment the neutrophil expression of defensins and LL-37, an antimicrobial peptide of the cathelicidin bunch. These molecules have appeared to advance NETosis and irritation in psoriasis plaques and other dermatopathological illnesses [97, 98].

NET and tumors

NETs were found in different tests of human and creature tumors such as pancreatic, breast, liver, and stomach cancers and around metastatic tumors [99, 100]. The part of NETs in tumor improvement progressively includes altering cancer insusceptibility and the interaction between the resistant framework and cancer cells. NETs play a key administrative part within the tumor microenvironment, such as shaping far-off metastases through the discharge of proteases, specifically lattice metalloproteinases and proinflammatory cytokines [101]. NETs increment the accumulation and expansion of single cancer cells and discharge tumor matrix

metalloproteinases and NE, contributing to tumor metastasis, which, through ECM debasement, tumor cells can take off their unique location and relocate to other organs [102]. NETs wake torpid cancer cells. The concept of tumor cell torpidity was included for most common strong cancers, counting breast, prostate, lung, colon, and kidney cancers, as well as melanoma and hematologic malignancies, such as different myeloma, lymphoma, and leukemia. Slow-cycle cancer cells can spread within the early stages, causing cancer to repeat [103]. Different mediators, counting granulocyte colony stimulating factor, granulocyte-macrophage colony stimulating factor, CXC, and CCL3, delivered by tumor or stroke cells, fortify granulopoiesis, discharging neutrophils from the bone marrow and their relocation. In expansion, NETs initiate epithelial transmission to mesenchymal cells in tumor cells. NET proteinases can annihilate the ECM and increment the number of cancer cells. NETs can trap circulating cancer cells in expansion, encouraging metastasis [104]. The affiliation of NETs with coagulation changes in tumor patients has highlighted the significance of NETs in cancer. NETs have appeared to actuate cancer-associated thrombosis in tumors, and a marvel went with by a destitute guess in patients. In expansion, higher levels of plasma citrullinated histone H3 (H3Cit) were watched in progressed cancer patients compared with solid people, and neutrophils in cancer patients appeared higher H3Cit substance than others. In expansion, H3Cit within the plasma of cancer patients is related to NE, MPO, IL-6, and IL-8, which are all NETosis activators [105-107].

NET recognition laboratory methods

Several methods to detect and then quantify NETs, including immunofluorescence microscopy, enzyme-linked immunosorbent assay (ELISA), flow cytometry, and microfluidic assay, have various advantages and disadvantages. The standard gold method for detecting NETs is immunofluorescence microscopy, which works on co-localizing the most specific NET markers, including complexes of extracellular DNA and neutrophil granular enzymes. This technique employs different fluorescent-labeled antibodies against various components of the NETs, specifically anti-DNA, anti-MPO, anti-NE, and anti-citrullinated histones antibodies. The protein-DNA as mentioned above complexes, such as MPO-DNA and NE-DNA complexes, can also be determined in fluid samples through ELISA that uses a capture antibody specific for the protein component and a dsDNA detection antibody [108, 109]. Nowadays, the application of flow cytometry techniques to detect NETs is expanding. This approach allows for the rapid screening of a large number of samples, it is objective, and the results are not biased by the observer [110]. Similar to what we do in immunofluorescence microscopy, we can use fluorescent-labeled antibodies against key NETs constituents in this assay to identify and quantify NETs. Another approach that is used less commonly in research is the quantifying circulating neutrophil extracellular traps, using a microfluidic device that traps cell-free DNA, in addition to an immuno-

fluorescence method that locates neutrophil-specific proteins [111].

The most popular method for detecting NET *in vitro* is microscopic observation, which shows the presence of NETs based on the simultaneous staining and co-localization of neutrophil-derived proteins and extracellular DNA. Since the main base of the NET is DNA, Different colors against DNA, such as Dapi [112, 113], Propidium iodide [114], SYTOX [8], and Hoechst [115], are widely used to visualize NET in immunofluorescence microscopy. However, since histones and several granules, such as myeloperoxidase or elastase, also exist in high NET values, the immunization coloring of these enzymes with the relevant antibodies can help better visualize NET. However, the fluorescence microscope has two main limitations: the observer may bias the results and not allow rapid screening of more cells or samples [116].

In outline agreeing to the substance specified, it can be said that NET plays a part in numerous diseases and can be utilized as one of the restorative objectives, even though nowadays, in a few infections, it is utilized as a helpful objective. In others, it is within the early stages of clinical trials, but there are still more illnesses related to its pathogenesis.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgment

The authors would like to thank the National Elite Foundation of Iran for supporting the project No 62301 (November 2021).

References

- [1]. Mulay SR, Anders HJ. Neutrophils and neutrophil extracellular traps regulate immune responses in health and disease. Multidisciplinary Digital Publishing Institute; 2020, p. 2130.
- [2]. Sollberger G, Amulic B, Zychlinsky A. Neutrophil extracellular trap formation is independent of de novo gene expression. *PLoS One* 2016; 11(6): 157454.
- [3]. Cooper PR, Palmer LJ, Chapple IL. Neutrophil extracellular traps as a new paradigm in innate immunity: friend or foe? *Periodontol.* 2013; 63(1): 165-97.
- [4]. Van Avondt K, Hartl D. Mechanisms and disease relevance of neutrophil extracellular trap formation. *European Journal of Clinical Investigation* 2018; 48: 12919.
- [5]. Ravindran M, Khan MA, Palaniyar N. Neutrophil extracellular trap formation: physiology, pathology, and pharmacology. *Biomolecules* 2019; 9(8): 365.
- [6]. Estúa-Acosta GA, Zamora-Ortiz R, Buentello-Volante B, García-Mejía M, Garfias Y. Neutrophil extracellular traps: current perspectives in the eye. *Cells* 2019; 8(9): 979.
- [7]. Leshner M, Wang S, Lewis C, Zheng H, Chen XA, Santy L, et al. PAD4 mediated histone hypercitullination induces heterochromatin decondensation and chromatin unfolding to form neutrophil extracellular trap-like structures. *Frontiers Immunol.* 2012; 3: 307.
- [8]. Fuchs TA, Abed U, Goosmann C, Hurwitz R, Schulze I, Wahn V, et al. Novel cell death program leads to neutrophil extracellular traps. *J Cell Biol.* 2007; 176(2): 231-41.
- [9]. Amini P, Stojkov D, Felser A, Jackson CB, Courage C, Schaller A, et al. Neutrophil extracellular trap formation requires OPA1-dependent glycolytic ATP production. *Nature Communica.* 2018; 9(1): 1-16.
- [10]. Amulic B, Knackstedt SL, Abed UA, Deigendesch N, Harbort CJ, Caffrey BE, et al. Cell-cycle proteins control production of neutrophil extracellular traps. *Developmental cell.* 2017; 43(4): 449-62.
- [11]. Medina E. Neutrophil extracellular traps: a strategic tactic to defeat pathogens with potential consequences for the host. *Journal of Innate Immunity* 2009; 1(3): 176-80.
- [12]. Yousefi S, Mihalache C, Kozlowski E, Schmid I, Simon HU. Viable neutrophils release mitochondrial DNA to form neutrophil extracellular traps. *Cell Death & Differentiation* 2009; 16(11): 1438-444.
- [13]. de Bont CM, Boelens WC, Pruijn GJ. NETosis, complement, and coagulation: a triangular relationship. *Cellular Molecular Immunol.* 2019; 16(1): 19-27.
- [14]. Zou Y, Chen X, Xiao J, Zhou DB, Lu XX, Li W, et al. Neutrophil extracellular traps promote lipopolysaccharide-induced airway inflammation and mucus hypersecretion in mice. *Oncotarget* 2018; 9(17): 13276.
- [15]. Dąbrowska D, Jabłońska E, Garley M, Ratajczak-Wrona W, Iwaniuk A. New aspects of the biology of neutrophil extracellular traps. *Scandinavian Journal of Immunology* 2016; 84(6): 317-22.
- [16]. Nauseef WM, Kubes P. Pondering neutrophil extracellular traps with healthy skepticism. *Cellular microbiology* 2016; 18(10): 1349-357.
- [17]. Urban CF, Ermert D, Schmid M, Abu-Abed U, Goosmann C, Nacken W, et al. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against *Candida albicans*. *PLoS Pathogens* 2009; 5(10): 1000639.
- [18]. Rosazza T, Warner J, Sollberger G. NET formation—mechanisms and how they relate to other cell death pathways. *The FEBS Journal* 2021; 288(11): 3334-350.
- [19]. Liu L, Mao Y, Xu B, Zhang X, Fang C, Ma Y, et al. Induction of neutrophil extracellular traps during tissue injury: Involvement of STING and Toll-like receptor 9 pathways. *Cell Proliferation* 2019; 52(3): 12579.
- [20]. Papayannopoulos V, Metzler KD, Hakkim A, Zychlinsky A. Neutrophil elastase and myeloperoxidase regulate the formation of neutrophil extracellular traps. *Journal of Cell Biology* 2010; 191(3): 677-91.
- [21]. Desai J, Kumar SV, Mulay SR, Konrad L, Romoli S, Schauer C, et al. PMA and crystal-induced neutrophil extracellular trap formation involves RIPK1-RIPK3-MLKL signaling. *European Journal of Immunology* 2016; 46(1): 223-29.
- [22]. Brinkmann V. Neutrophil extracellular traps in the second decade. *Journal of Innate Immunity* 2018; 10(5-6): 414-21.
- [23]. Hakkim A, Fuchs TA, Martinez NE, Hess S, Prinz H, Zychlinsky A, et al. Activation of the Raf-MEK-ERK pathway is required for neutrophil extracellular trap formation. *Nature Chemical Biology* 2011; 7(2): 75-7.
- [24]. Douda DN, Khan MA, Grasemann H, Palaniyar N. SK3 channel and mitochondrial ROS mediate NADPH oxidase-independent NETosis induced by calcium influx. *Proceedings of the National Academy of Sciences* 2015; 112(9): 2817-822.
- [25]. Björnsdóttir H, Welin A, Michaëlsson E, Osla V, Berg S, Christenson K, et al. Neutrophil NET formation is regulated from the inside by myeloperoxidase-processed reactive oxygen species. *Free Radical Biology and Medicine* 2015; 89(12): 1024-1035.
- [26]. Pieterse E, Rother N, Yanginlar C, Gerretsen J, Boeltz S, Munoz LE, et al. Cleaved N-terminal histone tails distinguish between NADPH oxidase (NOX)-dependent and NOX-independent pathways of neutrophil extracellular trap formation. *Annals of the Rheumatic Diseases* 2018; 77(12): 1790-798.
- [27]. Yipp BG, Kubes P. NETosis: how vital is it? *Blood, The Journal of the American Society of Hematology* 2013; 122(16): 2784-794.
- [28]. Pieterse E, Rother N, Yanginlar C, Hilbrands LB, Van der Vlag J. Neutrophils discriminate between

- lipopolysaccharides of different bacterial sources and selectively release neutrophil extracellular traps. *Frontiers in Immunology* 2016; 7(11):484.
- [29]. Sharma A, Simonson TJ, Jondle CN, Mishra BB, Sharma J. Mincle-mediated neutrophil extracellular trap formation by regulation of autophagy. *The Journal of Infectious Diseases* 2017; 215(7): 1040-1048.
- [30]. Stojkov D, Amini P, Oberson K, Sokollik C, Duppenhaler A, Simon HU, et al. ROS and glutathionylation balance cytoskeletal dynamics in neutrophil extracellular trap formation. *Journal of Cell Biology* 2017; 216(12): 4073-90.
- [31]. Kolaczowska E, Jenne CN, Surewaard BG, Thanabalasuriar A, Lee WY, Sanz MJ, et al. Molecular mechanisms of NET formation and degradation revealed by intravital imaging in the liver vasculature. *Nature Communications* 2015; 6(1): 1-13.
- [32]. Chapman EA, Lyon M, Simpson D, Mason D, Beynon RJ, Moots RJ, et al. Caught in a trap? Proteomic analysis of neutrophil extracellular traps in rheumatoid arthritis and systemic lupus erythematosus. *Frontiers in immunology* 2019:423.
- [33]. Yu Y, Su K. Neutrophil extracellular traps and systemic lupus erythematosus. *J Clin Cell Immunol.* 2013; 4(4): 139.
- [34]. Kambas K, Chrysanthopoulou A, Vassilopoulos D, Apostolidou E, Skendros P, Girod A, et al. Tissue factor expression in neutrophil extracellular traps and neutrophil derived microparticles in antineutrophil cytoplasmic antibody associated vasculitis may promote thromboinflammation and the thrombophilic state associated with the disease. *Annals of the Rheumatic Diseases* 2014; 73(10): 1854-863.
- [35]. Watanabe S, Iwata Y, Fukushima H, Saito K, Tanaka Y, Hasegawa Y, et al. Neutrophil extracellular traps are induced in a psoriasis model of interleukin-36 receptor antagonist-deficient mice. *Scientific Reports* 2020; 10(1): 1-11.
- [36]. Cristinziano L, Modestino L, Loffredo S, Varricchi G, Braile M, Ferrara AL, et al. Anaplastic thyroid cancer cells induce the release of mitochondrial extracellular DNA traps by viable neutrophils. *The Journal of Immunology* 2020; 204(5): 1362-372.
- [37]. Leffler J, Stojanovich L, Shoenfeld Y, Bogdanovic G, Hesselstrand R, Blom AM. Degradation of neutrophil extracellular traps is decreased in patients with antiphospholipid syndrome. *Clin Exp Rheumatol* 2014; 32(1): 66-70.
- [38]. Watanabe-Kusunoki K, Abe N, Nakazawa D, Karino K, Hattanda F, Fujieda Y, et al. A case report dysregulated neutrophil extracellular traps in a patient with propylthiouracil-induced antineutrophil cytoplasmic antibody-associated vasculitis. *Medicine* 2019; 98(17): 15328.
- [39]. Monsalve DM, Acosta-Ampudia Y, Ramírez-Santana C, Polo JF, Anaya JM. Neutrophil extracellular traps in autoimmune diseases. *Revista Colombiana de Reumatología* 2020; 27(10): 4-14.
- [40]. Leffler J, Martin M, Gullstrand B, Tydén H, Lood C, Truedsson L, et al. Neutrophil extracellular traps that are not degraded in systemic lupus erythematosus activate complement exacerbating the disease. *The Journal of Immunology* 2012; 188(7): 3522-531.
- [41]. Basta F, Fasola F, Triantafyllias K, Schwarting A. Systemic lupus erythematosus (SLE) therapy: the old and the new. *Rheumatology and Therapy* 2020; 7(3): 433-46.
- [42]. Song L, Wang Y, Zhang J, Song N, Xu X, Lu Y. The risks of cancer development in systemic lupus erythematosus (SLE) patients: a systematic review and meta-analysis. *Arthritis research & therapy* 2018; 20(1):1-13.
- [43]. Lande R, Ganguly D, Facchinetti V, Frasca L, Conrad C, Gregorio J, et al. Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. *Science Translational Medicine* 2011; 3(73): 1-9.
- [44]. Jeremic I, Djuric O, Nikolic M, Vljajnic M, Nikolic A, Radojkovic D, et al. Neutrophil extracellular traps-associated markers are elevated in patients with systemic lupus erythematosus. *Rheumatology International* 2019; 39(11): 1849-857.
- [45]. Hakkim A, Fürnrohr BG, Amann K, Laube B, Abed UA, Brinkmann V, et al. Impairment of neutrophil extracellular trap degradation is associated with lupus nephritis. *Proceedings of the National Academy of Sciences* 2010; 107(21): 9813-818.
- [46]. Kraaij T, Kamerling SW, de Rooij EN, van Daele PL, Bredewold OW, Bakker JA, et al. The NET-effect of combining rituximab with belimumab in severe systemic lupus erythematosus. *Journal of Autoimmunity* 2018; 91: 45-54.
- [47]. McInnes IB, Schett G. La patogenia de la artritis reumatoide. *N Engl J Med* 2011; 365(23): 2205-219.
- [48]. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham III CO, et al. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/ European League Against Rheumatism collaborative initiative. *Arthritis & Rheumatism* 2010; 62(9): 2569-5681.
- [49]. Song W, Ye J, Pan N, Tan C, Herrmann M. Neutrophil extracellular traps tied to rheumatoid arthritis: points to ponder. *Frontiers in Immunology* 2021; 11: 578129.
- [50]. Fan L, He D, Wang Q, Zong M, Zhang H, Yang L, et al. Citrullinated vimentin stimulates proliferation, proinflammatory cytokine secretion, and PADI4 and RANKL expression of fibroblast-like synoviocytes in rheumatoid arthritis. *Scandinavian Journal of Rheumatology* 2012; 41(5): 354-58.
- [51]. Bartok B, Firestein GS. Fibroblast-like synoviocytes: key effector cells in rheumatoid arthritis. *Immunological Reviews* 2010; 233(1): 233-55.
- [52]. Corsiero E, Carlotti E, Jagemann L, Perretti M, Pitzalis C, Bombardieri M. H and L chain affinity maturation and/or Fab N-glycosylation influence immunoreactivity toward neutrophil extracellular

- trap antigens in rheumatoid arthritis synovial B cell clones. *The Journal of Immunology* 2020; 204(9): 2374-379.
- [53]. Lundberg K, Nijenhuis S, Vossenaar ER, Palmblad K, van Venrooij WJ, Klareskog L, et al. Citrullinated proteins have increased immunogenicity and arthritogenicity and their presence in arthritic joints correlates with disease severity. *Arthritis Research & Therapy* 2005; 7(3): 1-10.
- [54]. Khandpur R, Carmona-Rivera C, Vivekanandan-Giri A, Gizinski A, Yalavarthi S, Knight JS, et al. NETs are a source of citrullinated autoantigens and stimulate inflammatory responses in rheumatoid arthritis. *Science Translational Medicine* 2013; 5(178): 178.
- [55]. Caturegli P, De Remigis A, Rose N. Hashimoto thyroiditis: clinical and diagnostic criteria. *Autoimmunity reviews* 2014; 13(4-5): 391-97.
- [56]. Lorini R, Gastaldi R, Traggiai C, Perucchin PP. Hashimoto's thyroiditis. *Pediatric endocrinology reviews: PER* 2003; 12 (S 2): 205-11.
- [57]. Pedersen IB, Knudsen N, Jørgensen T, Perrild H, Ovesen L, Laurberg P. Thyroid peroxidase and thyroglobulin autoantibodies in a large survey of populations with mild and moderate iodine deficiency. *Clinical Endocrinology* 2003; 58(1):36-42.
- [58]. Xiao F, Jiang Y, Wang X, Jiang W, Wang L, Zhuang X, et al. NETosis may play a role in the pathogenesis of Hashimoto's thyroiditis. *International Journal of Clinical and Experimental Pathology* 2018; 11(2): 537.
- [59]. Tillack K, Naegele M, Haueis C, Schipling S, Wandinger KP, Martin R, et al. Gender differences in circulating levels of neutrophil extracellular traps in serum of multiple sclerosis patients. *Journal of Neuroimmunology* 2013; 261(1-2):108-19.
- [60]. Mutukula N, Man Z, Takahashi Y, Martinez FI, Morales M, Carreon-Guarnizo E, et al. Generation of RRMS and PPMS specific iPSCs as a platform for modeling Multiple Sclerosis. *Stem Cell Research* 2021; 53: 102319.
- [61]. Calabrese R, Zampieri M, Mechelli R, Annibali V, Guastafierro T, Ciccarone F, et al. Methylation-dependent PAD2 upregulation in multiple Sclerosis peripheral blood. *Multiple Sclerosis Journal* 2012; 18(3): 299-304.
- [62]. Wang Y, Xiao Y, Zhong L, Ye D, Zhang J, Tu Y, et al. Increased neutrophil elastase and proteinase 3 and augmented NETosis are closely associated with β -cell autoimmunity in patients with type 1 diabetes. *Diabetes* 2014; 63(12): 4239-248.
- [63]. Moscarello MA, Mastronardi FG, Wood DD. The role of citrullinated proteins suggests a novel mechanism in the pathogenesis of multiple Sclerosis. *Neurochemical Research* 2007; 32(2): 251-56.
- [64]. O'Malley D, Dinan TG, Cryan JF. Altered expression and secretion of colonic interleukin-6 in a stress-sensitive animal model of brain-gut axis dysfunction. *Journal of neuroimmunology* 2011; 235(1-2):48-55.
- [65]. Qin J, Fu S, Speake C, Greenbaum C, Odegard J. NETosis-associated serum biomarkers are reduced in type 1 diabetes in association with neutrophil count. *Clinical & Experimental Immunology* 2016; 184(3): 318-22.
- [66]. Parackova Z, Zentsova I, Vrabcova P, Klocperk A, Sumnik Z, Pruhova S, et al. Neutrophil extracellular trap induced dendritic cell activation leads to Th1 polarization in type 1 diabetes. *Frontiers in immunology* 2020; 11(4):661.
- [67]. Huang J, Xiao Y, Xu A, Zhou Z. Neutrophils in type 1 diabetes. *J Diabetes Investig.* 2016; 7(5): 652-63.
- [68]. Wang S, Demers M, Wagner D. Diabetes primes neutrophils to undergo NETosis which severely impairs wound healing. *Nat Med* 2015; 21(7): 815-19.
- [69]. Diana J, Simoni Y, Furio L, Beaudoin L, Agerberth B, Barrat F, et al. Crosstalk between neutrophils, B-1a cells and plasmacytoid dendritic cells initiates autoimmune diabetes. *Nature Medicine* 2013; 19(1): 65-73.
- [70]. de Bont CM, Stokman ME, Faas P, Thurlings RM, Boelens WC, Wright HL, et al. Autoantibodies to neutrophil extracellular traps represent a potential serological biomarker in rheumatoid arthritis. *Journal of Autoimmunity* 2020; 113: 102484.
- [71]. Fox S, Leitch AE, Duffin R, Haslett C, Rossi AG. Neutrophil apoptosis: relevance to the innate immune response and inflammatory disease. *Journal of Innate Immunity* 2010; 2(3): 216-27.
- [72]. Zheng Y, Ley SH, Hu FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nature Reviews Endocrinology* 2018; 14(2): 88-98.
- [73]. Njeim R, Azar WS, Fares AH, Azar ST, Kassouf HK, Eid AA. NETosis contributes to the pathogenesis of diabetes and its complications. *Journal of Molecular Endocrinology* 2020; 65(4): 65-76.
- [74]. Nijhuis J, Rensen SS, Slaats Y, Van Dielen FM, Buurman WA, Greve JWM. Neutrophil activation in morbid obesity, chronic activation of acute inflammation. *Obesity* 2009; 17(11): 2014-2018.
- [75]. Wang Q, Xie Z, Zhang W, Zhou J, Wu Y, Zhang M, et al. Myeloperoxidase deletion prevents high-fat diet-induced obesity and insulin resistance. *Diabetes* 2014; 63(12): 4172-185.
- [76]. Talukdar S, Oh DY, Bandyopadhyay G, Li D, Xu J, McNelis J, et al. Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. *Nature Medicine* 2012; 18(9): 1407-412.
- [77]. Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. *Nature Medicine* 2018; 24(7): 908-22.
- [78]. Luci C, Bourinet M, Leclère PS, Anty R, Gual P. Chronic inflammation in nonalcoholic steatohepatitis: molecular mechanisms and therapeutic strategies. *Frontiers in Endocrinology Front Endocrinol.* 2020; 11(12): 597648.

- [79]. Antonucci L, Porcu C, Timperi E, Santini SJ, Iannucci G, Balsano C. Circulating neutrophils of nonalcoholic steatohepatitis patients show an activated phenotype and suppress T lymphocytes activity. *J Immunol Res.* 2020; 6: 4570219.
- [80]. Hwang S, He Y, Xiang X, Seo W, Kim SJ, Ma J, et al. Interleukin-22 ameliorates neutrophil-driven nonalcoholic steatohepatitis through multiple targets. *Hepatology* 2020; 72(2): 412-29.
- [81]. Du J, Zhang J, Chen X, Zhang S, Zhang C, Liu H, et al. Neutrophil extracellular traps induced by proinflammatory cytokines enhance procoagulant activity in NASH patients. *Clinics and Research in Hepatology and Gastroenterology* 2022; 46(1): 101697.
- [82]. Randhawa PK, Singh K, Singh N, Jaggi AS. A review on chemical-induced inflammatory bowel disease models in rodents. *The Korean journal of physiology & pharmacology* 2014; 18(4):279-88.
- [83]. Wallace KL, Zheng LB, Kanazawa Y, Shih DQ. Immunopathology of inflammatory bowel disease. *World Journal of Gastroenterology* 2014; 20(1): 6-15.
- [84]. dos Santos Ramos A, Viana GCS, de Macedo Brigido M, Almeida JF. Neutrophil extracellular traps in inflammatory bowel diseases: Implications in pathogenesis and therapeutic targets. *Pharmacological Research* 2021; 171: 105779.
- [85]. Stephens M, Liao S, Von der Weid PY. Mesenteric lymphatic alterations observed during DSS induced intestinal inflammation are driven in a TLR4-PAMP/DAMP discriminative manner. *Front Immunol.* 2019; 10(3): 557.
- [86]. Li T, Wang C, Liu Y, Li B, Zhang W, Wang L, et al. Neutrophil extracellular traps induce intestinal damage and thrombotic tendency in inflammatory bowel disease. *Journal of Crohn's and Colitis* 2020; 14(2): 240-53.
- [87]. Lin EYH, Lai HJ, Cheng YK, Leong KQ, Cheng LC, Chou YC, et al. Neutrophil extracellular traps impair intestinal barrier function during experimental colitis. *Biomedicine* 2020; 8(8): 275.
- [88]. Levy ML. Is spirometry essential in diagnosing asthma? *No. Br J Gen Pract.* 2016; 66(650): 485-92.
- [89]. Choy DF, Hart KM, Borthwick LA, Shikotra A, Nagarkar DR, Siddiqui S, et al. TH2 and TH17 inflammatory pathways are reciprocally regulated in asthma. *Science Translational Medicine* 2015; 7(301): 129-33.
- [90]. Douwes J, Gibson P, Pekkanen J, Pearce N. Non-eosinophilic asthma: importance and possible mechanisms. *Thorax* 2002; 57(7): 643-48.
- [91]. Chen F, Yu M, Zhong Y, Wang L, Huang H. Characteristics and role of neutrophil extracellular traps in asthma. *Inflammation* 2021: 1-8.
- [92]. Zhang S, Shen H, Shu X, Peng Q, Wang G. Abnormally increased low-density granulocytes in peripheral blood mononuclear cells are associated with interstitial lung disease in dermatomyositis. *Modern Rheumatology* 2017; 27(1): 122-29.
- [93]. Peng Y, Zhang S, Zhao Y, Liu Y, Yan B. Neutrophil extracellular traps may contribute to interstitial lung disease associated with anti-MDA5 autoantibody positive dermatomyositis. *Clinical Rheumatology* 2018; 37(1): 107-15.
- [94]. Duvvuri B, Pachman LM, Morgan G, Khojah AM, Klein-Gitelman M, Curran ML, et al. Neutrophil extracellular traps in tissue and periphery in juvenile dermatomyositis. *Arthritis & Rheumatology* 2020; 72(2): 348-58.
- [95]. Zhang S, Shu X, Tian X, Chen F, Lu X, Wang G. Enhanced formation and impaired degradation of neutrophil extracellular traps in dermatomyositis and polymyositis: a potential contributor to interstitial lung disease complications. *Clinical & Experimental Immunology* 2014; 177(1):134-41.
- [96]. Wójcik P, Garley M, Wroński A, Jabłońska E, Skrzydlewska E. Cannabidiol modifies the formation of NETs in neutrophils of psoriatic patients. *International Journal of Molecular Sciences* 2020; 21(18): 6795.
- [97]. Hu SCS, Yu HS, Yen FL, Lin CL, Chen GS, Lan CCE. Neutrophil extracellular trap formation is increased in Psoriasis and induces human β -defensin-2 production in epidermal keratinocytes. *Scientific Reports* 2016; 6(1): 1-14.
- [98]. Lin AM, Rubin CJ, Khandpur R, Wang JY, Riblett M, Yalavarthi S, et al. Mast cells and neutrophils release IL-17 through extracellular trap formation in Psoriasis. *The Journal of Immunology* 2011; 187(1): 490-500.
- [99]. Snoderly HT, Boone BA, Bennewitz MF. Neutrophil extracellular traps in breast cancer and beyond: current perspectives on NET stimuli, thrombosis and metastasis, and clinical utility for diagnosis and treatment. *Breast Cancer Research* 2019; 21(1): 1-13.
- [100]. Miller-Ocuin JL, Liang X, Boone BA, Doerfler WR, Singhi AD, Tang D, et al. DNA released from neutrophil extracellular traps (NETs) activates pancreatic stellate cells and enhances pancreatic tumor growth. *Oncoimmunology* 2019; 8(9): 1605822.
- [101]. Masucci MT, Minopoli M, Del Vecchio S, Carriero MV. The emerging role of neutrophil extracellular traps (NETs) in tumor progression and metastasis. *Frontiers in Immunology* 2020; 11(9): 1749.
- [102]. Cools-Lartigue J, Spicer J, McDonald B, Gowing S, Chow S, Giannias B, et al. Neutrophil extracellular traps sequester circulating tumor cells and promote metastasis. *The Journal of Clinical Investigation* 2013; 123(8): 3446-458.
- [103]. Demers M, Wagner DD. Neutrophil extracellular traps: A new link to cancer-associated thrombosis and potential implications for tumor progression. *Oncoimmunology* 2013; 2(2): 22946.
- [104]. Huang H, Zhang H, Onuma AE, Tsung A. Neutrophil elastase and neutrophil extracellular traps in the tumor microenvironment. *Adv Exp Med Biol.* 2020; 1263: 13-23.

- [105]. Tsai CY, Hsieh SC, Liu CW, Lu CS, Wu CH, Liao HT, et al. Cross-talk among polymorphonuclear neutrophils, immune, and non-immune cells via released cytokines, granule proteins, microvesicles, and neutrophil extracellular trap formation: a novel concept of biology and pathobiology for neutrophils. *International Journal of Molecular Sciences* 2021; 22(6): 3119.
- [106]. Alfaro C, Teijeira A, Oñate C, Pérez G, Sanmamed MF, Andueza MP, et al. Tumor-produced interleukin-8 attracts human myeloid-derived suppressor cells and elicits extrusion of neutrophil extracellular traps (NETs). *Clinical Cancer Research* 2016; 22(15): 3924-936.
- [107]. de Andrea CE, Ochoa MC, Villalba-Esparza M, Teijeira Á, Schalper KA, Abengozar-Muela M, et al. Heterogenous presence of neutrophil extracellular traps in human solid tumours is partially dependent on IL-8. *The Journal of Pathology* 2021; 255(2): 190-201.
- [108]. Buckland RL, Wilson AS, Brüstle A. Quantification of neutrophil extracellular traps isolated from mouse tissues. *Current Protocols in Mouse Biology* 2020; 10(3): 78.
- [109]. Rada B. Neutrophil extracellular traps. *NADPH Oxidases: Springer*; 2019, p. 517-28.
- [110]. Gavillet M, Martinod K, Renella R, Harris C, Shapiro NI, Wagner DD, et al. Flow cytometric assay for direct quantification of neutrophil extracellular traps in blood samples. *American Journal of Hematology* 2015; 90(12): 1155-158.
- [111]. Otawara M, Roushan M, Wang X, Ellett F, Yu YM, Irimia D. Microfluidic assay measures increased neutrophil extracellular traps circulating in blood after burn injuries. *Scientific Reports* 2018; 8(1): 1-9.
- [112]. Berends ET, Horswill AR, Haste NM, Monestier M, Nizet V, von Köckritz-Blickwede M. Nuclease expression by *Staphylococcus aureus* facilitates escape from neutrophil extracellular traps. *Journal of Innate Immunity* 2010; 2(6): 576-86.
- [113]. Skendros P, Mitsios A, Chrysanthopoulou A, Mastellos DC, Metallidis S, Rafailidis P, et al. Complement and tissue factor–enriched neutrophil extracellular traps are key drivers in COVID-19 immunothrombosis. *The Journal of Clinical Investigation* 2020; 130(11): 6151-157.
- [114]. Remijsen Q, Kuijpers T, Wirawan E, Lippens S, Vandenabeele P, Vanden Berghe T. Dying for a cause: NETosis, mechanisms behind an antimicrobial cell death modality. *Cell Death & Differentiation* 2011; 18(4): 581-88.
- [115]. Saitoh T, Komano J, Saitoh Y, Misawa T, Takahama M, Kozaki T, et al. Neutrophil extracellular traps mediate a host defense response to human immunodeficiency virus-1. *Cell Host & Microbe* 2012; 12(1): 109-16.
- [116]. Buhr Nd, Köckritz-Blickwede MV. Detection, visualization, and quantification of neutrophil extracellular traps (NETs) and NET markers. *Neutrophil: Springer*; 2020, p. 425-42.