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## Neutrophil Extracellular Traps and Its Correlation with Several Pathological Conditions: Prosperities and Deleterious Implications

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Neutrophil extracellular traps (NETs) are immune components found in a variety of pathological states. It has been shown to have either beneficial or harmful implications, depending on how it is controlled and has been particularly observed in three major scenarios: infection, autoimmune disease, and cancer. In this article, we compiled some of the roles of NETs in pathological conditions, as well as the benefits of targeting them for improved patient outcomes. The role of NETs were primarily positive in infectious disease, whether caused by bacteria, virus, or fungal infection. In non-infectious inflammatory scenarios, on the other hand, it's the complete opposite, with the effects being mainly deleterious and even worse than the original disease states. Targeting NETs directly or indirectly may help to prevent complications and improve patient outcomes. A plethora of compounds, including immunomodulators, anti-thrombosis, nicotinamide adenine dinucleotide phosphate (NADPH)/reactive oxygen species (ROS) inhibitors, nuclease, and other compounds, may be used to accomplish the therapeutic goals.

**Keywords:** autoimmune disease, cancer, DNase, infection, neutrophil extracellular traps

### Introduction

Neutrophils, also known as polymorphonuclear leukocytes (PMN), are the most plentiful leukocytes in the blood involved in the pathogen defensive system.<sup>1</sup> Several activities, including the fabrication of neutrophil extracellular traps

(NETs), extracellular webs made up of nuclear and granular parts of the cell that arise from decondensed chromatin, have been discovered per its action.<sup>2</sup> The formation of NETs (NETosis) for limiting and removing external infections is a different approach in comparison with necrosis and apoptosis.<sup>3-5</sup> In many circumstances, bacteria, fungi, and

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viruses are susceptible to NETs, indicating that NETs play a beneficial role.<sup>6</sup> On the contrary, a lack of proper NETs controls may result in immunothrombosis, which can have serious consequences for the patient.<sup>7</sup>

As previously stated, the role of NETs is a double-edged sword: at certain levels and conditions, this molecule acts as a non-specific immune response component; however, under uncontrolled conditions, it can cause tissue damage, initiate inflammatory cells, and contribute to pathological processes such as systemic inflammatory response syndrome (SIRS), acute lung injury, thrombosis, autoimmune diseases, multiple sclerosis, and cancer.<sup>2,8-10</sup> Tissue damage is mostly linked to increased levels of NETs and a reduction in DNase synthesis.<sup>11,12</sup> Through stimulating CD4<sup>+</sup> T cells, NETs play a role in the adaptive immune system.<sup>13</sup> NETs can also activate naive CD4<sup>+</sup> T cells and memory CD4<sup>+</sup> T cells, which can then induce CD8<sup>+</sup> T cell activation via interleukin (IL)-2, resulting in higher interferon (IFN)-1 production.<sup>13,14</sup> Intracellular proteins are exposed to the extracellular space during NETosis and have the potential to serve as autoantigens against the host, resulting in a vicious circle.<sup>15</sup> Thus, a greater understanding of the role of NETs and their targeting potential are required in a variety of situations, whether infectious or non-infectious disease. Regarding this condition, we briefly summarize some of NETs' roles in pathological conditions, as well as the benefits of its targeting for improving patient outcomes.

## NETosis

NETosis is a kind of cell death that is distinct from necrosis and apoptosis, and its mechanism is unknown, reflecting the neutrophil's key involvement in various inflammatory conditions.<sup>16,17</sup> Microscopic organisms, inflammation, and endogenous stimuli can all trigger this occurrence. Neutrophil signaling can lead to adhesion towards the Toll-like receptor (TLR), Fc receptors, and complement receptors, which can lead to the formation of NETs, which involve cytokines including IL-8, tumor necrosis factor (TNF), and IFN- $\gamma$ .<sup>2</sup>

Suicidal NETosis (programmed cell death) and vital NETosis (either reactive oxygen species (ROS)-dependent or ROS-independent) are the two primary forms of NETosis. Suicidal NETosis is the most prevalent type of NETosis, which begins when neutrophils are triggered by an inductor material, the most efficient of which is phorbol 12-myristate 13-acetate (PMA), in addition to lipopolysaccharides

(LPS), or calcium ionophores (CaI).<sup>18</sup> Neutrophil activation would enhance protein kinase C activity, resulting in nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) engagement in the plasma and granular membranes. After that, chromatin decondensation is undertaken.<sup>4,15,19-21</sup> Several enzymes, such as neutrophil elastase (NE), myeloperoxidase (MPO), and peptidyl arginine deiminase 4 (PAD4), play an important part in this process.<sup>22</sup> Simultaneously, vesicles formed on the nuclear membrane, producing membrane damage, while the cytoplasmic granular membrane disintegrates, allowing granular contents and chromatin to merge.<sup>21,23</sup> After that, these elements will be liberated into the extracellular environment, and the NETosis will be completed when the plasma membrane disintegrates.<sup>4,20,24</sup> The process is simplified in Figure 1.

The formation of NETs begins with ligand binding to neutrophils via TLRs or IgG-Fc receptors, complement or cytokines.<sup>3</sup> Activation of neutrophils through the protein kinase C (PKC)/Raf-MEK-ERK kinase and Rac2 pathway will stimulate the NOX complex.<sup>20,21,25</sup> In addition, PKC isoenzymes can also inhibit histone deamination and activate the enzyme PAD4 in complement with an increase in Ca<sup>2+</sup> in the cytosol. PAD4 causes histone hypercitrullination which results in chromatin decondensation (by weakening DNA-histone electrostatic bonds).<sup>21,26,27</sup> In addition to PAD4, chromatin decondensation is aided by NE and MPO. MPO focuses on expanding chromatin condensation while NE degrades H1 and processes histone nuclei. The molecular key to the establishment of NETs is PAD4-dependent Ca<sup>2+</sup> citrullination of histone H3.<sup>20</sup>

ROS-dependent vital NETosis is associated with neutrophil priming using the granulocyte-macrophage colony-stimulating factor (GM-CSF). GM-CSF is activated by LPS or C5a and releasing mitochondrial DNA simultaneously with granular proteins that depend on ROS production.<sup>2,15,21,28</sup> The involvement of ROS that can be considered as a signaling molecule rather than the cytotoxic antimicrobial activity has implications in the formation of NETs.<sup>4</sup> Meanwhile, the ROS-independent condition of vital NETosis is linked to the rapid release of nuclear DNA while maintaining the nuclear neutrophil function, allowing them to complete the phagocytosis and classical bacterial killing process.<sup>29</sup> All vital NETosis processes allow neutrophils to remain active and capable of carrying out phagocytosis and consist of growth of the nuclear envelope, decondensation of chromatin, and destruction of the nuclear membrane.<sup>24</sup>

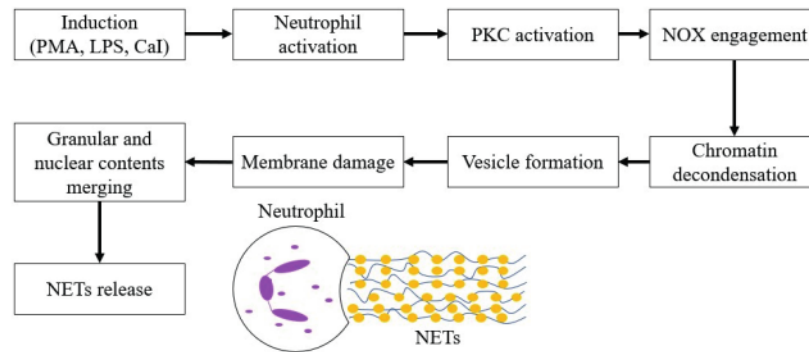


Figure 1. NETs formation (NETosis).

### NETs role in non-specific (innate) immune response system and its control of release

As pointed out previously, NETs are sourced from neutrophils, which are cellular components of non-specific immune systems (along with macrophage, mast cell, and dendritic cell). Its function in innate immune systems is related to the trapping of pathogenic microbes, which prevents microorganism spread, limits organ damage, and increases the concentration of released microbicidal agents in a specified target.<sup>30</sup> NETs can also cooperate with B and T lymphocytes, communicating the innate and adaptive immune systems and encouraging their activation (via NET/cell interaction and TCR signaling).<sup>13,31</sup> Excessive NETs secretion (which is linear with neutrophil expansion) can, on the contrary, lead to adverse disease progression, such as worse clinical manifestations, a higher likelihood of clinical instability, a longer length of hospital stay, and increased 30-day all-cause mortality events.<sup>32,33</sup> As a result, the good or bad aspect of NETs are reliant on sustaining a rigid balance of its release and removal.<sup>2</sup>

Following the preceding statement, NETs control and regulation must be implemented to avoid negative consequences. Although the precise molecular mechanisms regulating NETs formation are still largely unknown, several mediators have been identified to play a role. The primary regulatory compounds are NE and MPO which is activated by ROS, causing NETosis to occur. NE and MPO then migrated to the nucleus. NE cleaves histones and encourages chromatin decondensation, whereas MPO binds to chromatin later, promoting further decondensation. NE and MPO can also conspire to promote chromatin decondensation, resulting in cell destruction and NETs

release.<sup>34</sup> Other regulatory compounds are PAD4 (mediator of histone citrullination), nicotinamide adenine dinucleotide phosphate/NADPH (inhibitor of PMA-induced NETs formation), mammalian target of rapamycin (mTOR), protein kinase A (PKA), and PKC.<sup>35</sup> The signaling pathways that regulate NET formation varied depending on the stimulant and warrants further investigation.<sup>36</sup> The summary regarding NETs role in innate immune system and its control is depicted in Figure 2.

### NETs role in several pathological conditions

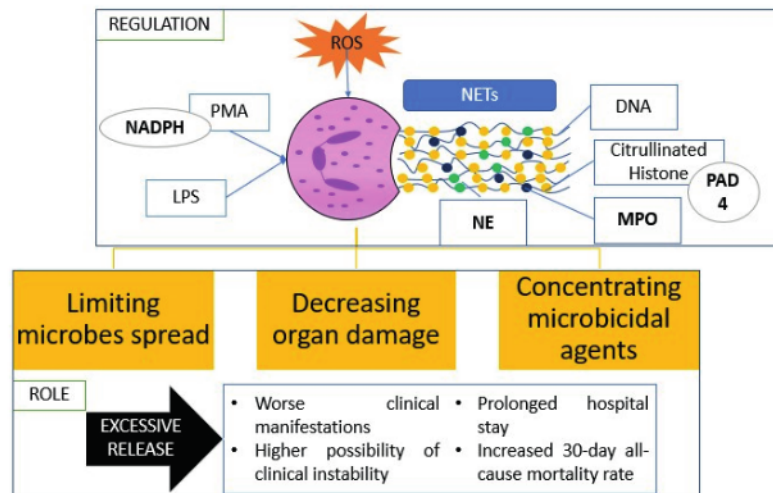
#### Infection

NETs have been demonstrated to have a role in a variety of infections, including those caused by viruses, bacteria, protozoa, helminths, and fungal invasion, among others.<sup>37-40</sup> The role was mainly positive, such as killing microbes directly or interacting with numerous antimicrobial peptides, such as histones and granule-resident antimicrobial proteins (NE, MPO, Gasdermin D (GSDMD), cathepsin G,  $\alpha$ -defensins, and lactoferrin), which have been studied extensively in bacterial infection.<sup>41,42</sup> Microbial entrapment, toxin degradation, and virulence factors breakdown, also played a role in the struggle against the infection.<sup>43</sup> Some ions, like iron and zinc, may interact with NETs to prevent microbial proliferation.<sup>44</sup>

#### Virus

Viral infections, including the Chikungunya, Human Immunodeficiency Virus (HIV), Dengue, Respiratory Syncytial Virus (RSV), and Influenza have been associated with the NETs. Beneficial effects of NETs have been observed to promote viral neutralization which is dependent to the TLR7 (an endolysosome able to recognize single-





**Figure 2. The role of NETs in the innate immune system and its control particles.** NETs have three main mechanisms that are responsible for cellular protection; however, excessive release can result in some negative effects, as shown in the illustration. Several regulatory compounds have been identified and are linked to a variety of inducer proteins, including NADPH (for PMA), PAD4 (for citrullinated histone), NE, and MPO (for ROS). The regulatory compounds are denoted by words in bold type.

stranded RNA virus) activation and ROS production. The virus has been inactivated by the aggregation with the free DNA and citrullinated histone H3 (H3cit) which is responsible for capturing the virus particles in the studies simulating Chikungunya virus.<sup>45</sup> A study also concluded that NETs were able to inhibit HIV-1 proliferation in macrophage, which is responsible for almost 80% reduction without affecting the survivability of the macrophage. The inhibition of viral replication mostly caused by the interference in HIV-1-DNA integration, an early critical step in viral reproducibility, in addition to causing decreased viral fitness and facilitating macrophage production of  $\beta$ -chemokine.<sup>46</sup> In the study which is assessing the protective effect of NETs to the RSV, it is concluded that low concentration of NETs were beneficial to counteract the virus. It is hypothesized to be associated with direct viral capturing or viral neutralization through the interaction with the myeloperoxidase, histones, defensins. But, in high concentration, NETs were responsible for massive cellular damage (which is greater than the virus effect).<sup>47</sup>

However, although NETs have provided some beneficial effects in countering the viral infection, it is also potential to induce some detrimental effects. In the RSV infection, although NETs have been considered to prevent

viral infection and its binding to the epithelial cell, it is also able to induce airway obstruction which is leading to severe features of the disease.<sup>48</sup> The high level of NETs level in the plasma was also correlated with the worse features of the disease caused by the infection of Influenza virus. Acute Lung Injury (ALI), which was caused by the interaction between Influenza virus and NETs was associated with increased alveolar epithelial protein permeability.<sup>49</sup> Dengue virus, on the other hand, was able to induce NETs formation, which is responsible for promoting pathogenic effects, increased vascular permeability, an important mechanisms for the progression of severe dengue infection.<sup>50</sup>

NETs-associated biomarkers, histone DNA (his-DNA) complexes, and NETs-specific MPO-DNA complexes were discovered to be markedly elevated in plasma samples and/or tracheal aspirates from a critically ill COVID-19 patient population (including those who undergo mechanical ventilation).<sup>51,52</sup> As an outcome, an increase in NETs concentration is related to a worsening of the disease, which can be observed through imaging studies or autopsies.<sup>51,52</sup> The association of NETs with at least seven acute-phase protein genes (IL6, TNF, CRP, CXCL8, IL-1 $\beta$ , IL-17A, and IL-1) was also aligned with disease deterioration.<sup>53</sup> In severe COVID-19 contexts,

several pathological mechanisms that underlie the poorer outcome include a hyperinflammatory state (cytokine storm), immunothrombosis, and microvascular endothelial injury, which may also be arbitrated by NETs.<sup>54</sup>

### **Bacteria**

NETs role mainly observed in sepsis cases. It has been determined to promote the *Escherichia coli* capturing, which is originating from the platelet TLR4 activation.<sup>55</sup> The intravascular NETs were responsible for acute protection of *E. coli* invasion to the liver cells and hematogenous spread of the bacteria to the distant organs. The histones are also responsible for the immuno-neutralization activity, leading into reduced organ damage and enhanced survival in the sepsis and endotoxemia cases. However, some pathological findings have been observed, which is correlated with the hepatic damage, induced by NETs.<sup>56</sup> In the observation using the *Staphylococcus aureus*, it was found that NETs, in companion with MPO have significant effect in bacterial killing, when combined with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). It is mainly related to the oxidative damage produced by H<sub>2</sub>O<sub>2</sub> activity, which is a two-sided phenomenon, either beneficial or detrimental.<sup>57</sup>

As stated before, NETs have potential benefits and also some harmful effects in the battle against bacterial infection. For the example, a study was assessing *Pseudomonas aeruginosa*-associated keratitis. It was found that NETs were responsible for preventing *P. aeruginosa* dissemination to the brain by the dead-zone formation, which is related to the type-3 secretion system (T3SS). However, prevention of brain dissemination poses worse pathological effect to the eye and antimicrobial resistance by the biofilm formation.<sup>58</sup> In the Ventilator-associated pneumonia cases, NETs, measured as MPO-DNA complexes, are highly detected in alveolar space of the critically ill patients which is associated with greater bacterial burden and neutrophilic inflammation process. This process could lead into Acute Respiratory Distress Syndrome (ARDS) which is negatively affect patient's prognosis.<sup>59</sup> Another case in pneumococcal meningitis have shown that NETs did not causing any reduction in bacterial viability. The bacteria was able to disperse into other organs and also the brain. Dissemination of the bacteria to the brain was related to an increased bacterial load and Blood Brain Barrier (BBB) leakage.<sup>60</sup>

### **Fungal**

*Candida albicans* was the most commonly observed fungal organism in determining NETs function. *C. albicans*

infestation is reliant on the ability of the organism to switch between cellular and hyphal (even pseudohyphal) forms, which is fundamental for the transformation from candidemia to tissue invasion.<sup>61-63</sup> Phagocytosis can remove cellular (blastoconidia) forms, but hyphal forms are too big to be ingested, interfering this characteristic antimicrobial host protective mechanism. Thus, an alternative defensive method, NETs, could play a role in the battle against *C. albicans* (in both cellular and hyphal forms).<sup>64</sup> The involvement of NETs in regulating complement receptor type 3 (CR3) in modulating the host defenses to fungal pathogen-associated molecular patterns (PAMP), which is dependent on the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) pathway, was significant.<sup>65</sup> Unopsonized *C. albicans* elicits NOX-independent NETosis via dectin-2 and its downstream signaling pathway (Syk-Ca<sup>2+</sup>-PKC $\delta$ -PAD4/NE), which aids in limiting fungal dissemination and transmission to the subsequent organs, according to a study.<sup>66</sup> NETs are also triggered by various *C. albicans* components, including the principal cell wall-building polysaccharides (mannans and  $\beta$ -glucans) and secreted aspartic proteases (Saps). Sap4 and Sap6, which are proven to be released by fungal hyphae via ROS-dependent ( $\beta$ -Glucans) and ROS-independent (Mannans) signaling pathways, notably through linkages with the CD11b receptor, are the most potent NET-releasing responses.<sup>67</sup>

Although NETs are substantially effective in combating fungal infection, *C. albicans* has developed some defensive schemes to resist their effects. The extracellular matrix-mediated inhibitory pathway produced by *C. albicans* biofilms may impede neutrophil response, resulting in impaired NETs release and diminished fungal termination.<sup>68</sup> Furthermore, the production of DNase I or catalase, as well as cytochalasin D, may dramatically decrease NET function, allowing *C. albicans* to evade NETs capturing and killing.<sup>69</sup>

### **Autoimmune Disease**

Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis (RA), psoriasis, and antiphospholipid syndrome are all autoimmune conditions in which NETs play an important role.<sup>70,71</sup> Proteins located in NETs, such as MPO and proteinase 3 (PR3) was hypothesized as the source of essential autoantigens.<sup>72</sup> Anti-myeloperoxidase and anti-protein 3 in anti-neutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (AAV), anti-double-stranded DNA (dsDNA) in SLE, anti-citrullinated protein antibodies

(ACPAs) in RA, and human cathelicidin LL-37 in psoriasis have all been found to play an autoantigenic position.<sup>73,74</sup> Production of ROS and its interplay with pro-inflammatory cytokines such as TNF- $\alpha$ , IL-1, IL-8, and IL-17 are thought to produce NET formation.<sup>75</sup> NETs have been shown to have a major impact on the severity of autoimmune disorders and to hasten their progression<sup>73,76</sup> due to chemicals existing in the NETs or NETs degradation product by DNase I which operating as auto-antigens, or due to overwhelming NET degradation failure<sup>77</sup>.

As stated above, NETs have been considered as the source of autoantigens in SLE.<sup>78,79</sup> NETs production have been associated with low-density granulocyte (LDG) activity which significantly correlated with the cell-free DNA (cfDNA) concentration, a biomarker of active SLE.<sup>80</sup> In the lupus nephritis patient, in addition to increased cfDNA concentration, higher level of quantitative 24-hour urinary protein and suppression of creatinine clearance rate also found.<sup>78</sup> Neutrophils from SLE patients with active disease may induce the release of NETs, resulting in overexpression of the stress-related protein regulated in development and DNA damage response 1 (REDD1). SLE-associated NETs were decorated with the tissue factor (TF) and IL-17A, which pioneered thrombin production and the fibrotic process in discoid skin lesions, glomerular and tubulointerstitial compartments.<sup>81</sup> In addition, lower ability of SLE patient's serum to degrade NETs may be associated with severe disease feature, attributed to an activation of complement systems, mainly involved C3 and C4.<sup>82</sup>

In the patients with rheumatoid arthritis, NETs posed both pro and anti-inflammatory effects. A study have shown that it is modulated by the C1q and cathelicidin (LL-37).<sup>83</sup> The anti-inflammatory effects were attributed by the inhibition of IL-6 secretion and increased secretion of the immunomodulatory cytokine IL-10. Meanwhile, pro-inflammatory states were shown by the increase in IL-8 levels and up-regulation of CD11b.<sup>83</sup> Histopathological examination have shown that NETs may disrupt articular cartilage by its elastase activity which induces the release of membrane-bound peptidylarginine deiminase-2 by fibroblast-like synoviocytes (FLSs), in addition to activation of macrophage.<sup>84</sup> Targeting of NETs may be beneficial for RA treatment, which has been examined in a study using CI-amidine, a highly specific inhibitor of PAD4. Inhibition of NETs release significantly attenuated collagen-induced arthritis (CIA) and suppressed the stimulation of antigen-bearing dendritic cells (DCs).<sup>85</sup>

An increase of NETs level also observed in psoriasis patients. Its increase were related to the progressing disease severity. A model study stated that NETs, which is formed through the mast cells and neutrophils were responsible for an increased expression of IL-17.<sup>86</sup> It may provide a biologic basis for systemic features of psoriasis, and increase in atherosclerosis and mortality.<sup>87,88</sup> But, another study also suggested direct role of NETs in the activation of inflammatory response in keratinocytes, by involving the TLR4/IL-36R crosstalk.<sup>89</sup> NETs also able to create a vicious cycle of skin inflammation by making the complex with LL37 triggered TLR8/TLR13-mediated cytokine.<sup>74</sup> However, it can induce the production of human  $\beta$ -defensin-2 (HBD-2), an antimicrobial peptide which is overexpressed in epidermal keratinocyte of psoriasis patients. By this mechanisms, it can help in protecting the psoriasis plaque from being infected by secondary bacterial infections.<sup>88</sup> Inhibition of NETs, either by CI-amidine or DNase I was beneficial to decrease scaling, acanthosis, and inflammatory infiltrate in histopathological study of psoriatic skin.<sup>89</sup>

### Cancer

Neutrophils and NETs have been understood to have a role in tumor growth. NETs were solely accountable for tumor progression and proliferation, as well as distant metastasis.<sup>90,91</sup> Inflammation-induced awakening of quiescent cancer cells may be mediated via NETs and NET-mediated extracellular matrix (ECM) alteration. NETs can awaken cancer cells, allowing for successive proteolytic remodeling of laminin and integrin-mediated signaling in the tumor cells.<sup>92</sup> In addition to promoting tumor angiogenesis, hypercoagulation (through a rise in endogenous thrombin potential), and cancer-associated thrombosis, NETs were found to hinder cytotoxic lymphocytes from assaulting cancer cells.<sup>93-95</sup> Upon the stimulation of the ILK- $\beta$ -parvin-RAC1-CDC42 pathway, which can improve cell motility, the transmembrane protein CCDC25 was assumed to guide the metastatic process.<sup>96,97</sup> However, because of the neutrophil's antitumor and protumor (N1 and N2) capabilities, NETs may play a role in tumor formation or inhibition, depending on the context, position, and volume generated.<sup>98</sup>

NETs have been observed to poses significant association with the breast cancer stages. It has been found that the higher levels of NE-DNA complexes were examined in regional and distant stages as compared with localized disease.<sup>99</sup> NETs also poses some detrimental phenotypes which is strongly associated with pro-metastatic features of the cancer cells. NETs were able to induce the alteration of



morphology dan migratory pattern of the cancer cell line (MCF7), intensifying epithelial–mesenchymal transition (through cadherin switching), modifying the expression pattern of solid tumor stem cell markers, CD24 and CD44, in addition to intercalating pro-inflammatory markers, including IL-1 $\beta$ /IL1B, IL6, and IL-8/CXCL8, markers. All of these features collaborate to produce a more aggressive breast cancer subtypes.<sup>100</sup> A study have shown that PAD4 and nuclear factor kappa-B (NF- $\kappa$ B), which activated by the NETs were responsible for inducing inflammation and metastasis of the cancer cells. Application of NEMO-binding domain (NBD) for suppressing NETs were useful for preventing lung metastasis in mice model of breast cancer.<sup>101</sup>

In the colorectal cancer patients, NETs may play a direct tumor proliferative role and inhibit tumor cell apoptosis. The involvement of several factors, including the exosomes, IL-8, and mast cells may take a role in metastatic process, especially in the liver, lung, and peritoneum.<sup>102,103</sup> Procoagulant phenotype also promoted by excessive platelet-activated NETs release, involving the changes in human umbilical vein endothelial cells (HUVECs). It shows an increased risk of venous thromboembolism, as evidenced by significant correlation with thrombin-antithrombin (TAT) complexes and D-dimer.<sup>104</sup> A study also evaluated higher NETs production in patients with significant complications following colorectal cancer operation (Clavien Dindo classification  $\geq 3$ ). The differences also found in the patients with longer length of stay ( $>5$  days) and died, all of which with higher NETs level.<sup>105</sup> Following surgery, immune response will be activated and have an important role for reparative processes besides promoting the risk of tumor recurrence and distant metastasis.<sup>106</sup> However, in another study, tumor resection was observed to suppressed NETs formation, reduced apoptosis, and elevated phagocytosis.<sup>107</sup> The effect is not only limited for colon surgery, but also the surgery for metastatic cells in the liver. Interaction between NETs, high mobility group box 1 (HMGB1) and TLR9 activation following surgery was responsible for protumorigenic properties and tumor progression. Thus, the targeting of DNase and PAD4 may inhibit these events.<sup>108</sup>

### Implication for targeting NETs as therapeutic approach in several pathological conditions

As previously stated, NETs have favorable impacts in a variety of infectious conditions; however, conflicting

evidence exists on their impact in some non-infectious inflammatory disorders, such as autoimmune disease and cancer, leading to the conclusion that there are "bad" and "good" NETs.<sup>109</sup> The role of NETs in various pathological circumstances, as well as NETs control via drugs that either impair NETs formation or target NETs components such as DNA or neutrophil proteases, is still being investigated.<sup>110</sup> DNase administration was effective in preventing cancer metastasis<sup>111</sup>, decreasing thrombotic complications<sup>34</sup>, promoting wound healing and preventing scar formation<sup>112</sup>, in addition to inhibiting activation of alternative complement pathway and endothelial damage<sup>113,114</sup>.

Another strategy involving thrombomodulin was found to be protective against endothelial disorder in sepsis and have a favorable therapeutic capacity for sepsis-induced coagulopathy.<sup>115</sup> Thrombomodulin also hampered the induction of NETs and suppressed the epithelial to mesenchymal transition in cancer cells by degrading high mobility group box protein 1 (HMGB1), thus inhibiting cancer metastasis.<sup>116,117</sup> The use of recombinant human-soluble TM (rTM) can also completely inhibit NETosis, inhibiting nucleic acid and NETs-mediated coagulation both *in vivo* and *in vitro*.<sup>118,119</sup>

In summary, four mechanisms can be used to target NETs for therapeutic purposes: (1) NETs induction blockade (CXCR-ligands, IL-8, and G-CSF); (2) NETs formation pathway inhibition (NOX2, NE, MPO, or PAD4); (3) NETs structure destruction using DNase; and (4) NETs-tumor contact impediment (integrin, TLR9, or CCDC25).<sup>120</sup> Anti-inflammatory/immunomodulatory, anti-thrombosis, NADPH/ROS inhibitors, nuclease, and other substances (including probiotics and vitamin D) are among the anti-NETs treatment modalities. Hypercoagulation intervention, oxidative stress reduction, NETosis inhibition, endothelial damage minimization, and cell apoptosis decrease are among the primary priorities of the NETs inhibition.<sup>121</sup>

### Conclusion

Depending on the pathological states, NETs have both positive (primarily for infectious disease) and negative (for non-infectious disease) implications. As a result, to target the disease more specifically, it is necessary to carefully understand the types of stimulus and the signaling pathways in every pathological process, which requires more mechanistic and clinical study. Thus, NETs can be used as a potential marker for predicting patient prognosis and outcome. By directly targeting NETs destruction or

indirectly targeting NETs induction and formation, several adverse events that are even worse than the natural course of the disease can be prevented.

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