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REVIEW

Neutrophil Extracellular Traps in Dry Eye: A Comprehensive Review of Pathogenic Mechanisms and Implications for Targeted Therapy

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Abstract: Dry eye (DE) is a multifactorial ocular surface disease characterized primarily by tear film instability and ocular discomfort. Nearly all forms of DE exhibit elevated inflammatory markers in tear fluid, accompanied by clinical signs of tear dysfunction including reduced secretion and shortened breakup time. Although the pathophysiology of dry eye disease remains incompletely understood, accumulating evidence implicates neutrophil extracellular traps (NETs) as key pathogenic drivers. Robust clinical associations and mechanistic studies have established causal links between NETs and ocular surface pathology. This review summarizes current research on the role of NETs in the development of dry eye, aiming to identify potential therapeutic targets.

Keywords: neutrophils, neutrophil extracellular traps, dry eye, NETosis

Introduction

Neutrophils, the most abundant white blood cells, comprise approximately 70% of peripheral leukocytes and play a central role in orchestrating acute inflammatory responses.[1] As the first line of innate immune defense, they are rapidly recruited to infection sites to perform essential protective functions.[2] Upon inflammation, neutrophils migrate from the bloodstream into tissues, where various stimuli, such as cytokines, growth factors, and pathogen-associated molecular patterns (PAMPs), can activate them.[3,4] A hallmark of neutrophil activation is the release of neutrophil extracellular traps (NETs).[5,6] First identified in 2004,[7] NETs have since been associated with numerous infectious and non-infectious inflammatory diseases.[8] The process of NETs formation, known as NETosis,[9] entails the release of myeloperoxidase (MPO) from azurophilic granules and the production of reactive oxygen species (ROS), both of which aid in pathogen elimination within phagolysosomes. NETosis constitutes a unique form of regulated cell death, distinct from both apoptosis and necrosis.[10–12] Although NETs represent a vital host defense mechanism, excessive or poorly cleared NETs can cause tissue damage and worsen inflammatory responses.[13] Growing evidence implicates NETs in the pathogenesis of diverse diseases, including methicillin-resistant *Staphylococcus aureus* sepsis,[14] thrombosis,[15] ocular diseases,[16] acute lung injury,[17] cancer,[18] ulcerative colitis,[19] and other immune-mediated disorders.[20] In ocular pathologies, neutrophils act as immunomodulatory effectors by releasing NETs to neutralize invading pathogens.[21]

Existing reviews on NETs in ocular diseases primarily address other conditions, leaving a significant gap in systematic and comprehensive analyses focused on the pathological mechanisms and targeted therapies for NETs in DE. Concurrently, numerous basic and clinical studies on NETs and dry eye have been published in recent years, creating

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an urgent need for a systematic summary to clarify research progress and future directions. This review synthesizes the current understanding of NETs involvement in dry eye pathogenesis and highlights recent advances, thereby providing a rationale for developing novel therapeutic strategies.

Literature Search Strategy

We systematically searched all relevant studies in the PubMed, Web of Science, Scopus, Cochrane, and SinoMed platform databases. Using Medical Subject Headings (MeSH) terms as the core framework, we employed a combination of subject terms and entry terms for literature retrieval, including "Dry eye", "Dry Eye Syndromes", "Dry eye disease", "Keratoconjunctivitis Sicca", "Sjogren's Syndrome", "Xerophthalmia", "Neutrophils", "Neutrophil extracellular traps", "NETs", "NETosis", "Extracellular Traps". The search strategy was limited to title/abstract of studies meeting the following criteria: animal experiments, randomized controlled trials, cohort studies, case-control studies, case reports, and reviews published in English or Chinese. Literature was included from the inception of each database up to April 2025. The last search was conducted on April 20, 2025.

Two independent reviewers performed the initial screening of titles and abstracts against the pre-specified inclusion and exclusion criteria. Discrepancies between the two reviewers during the screening phase were resolved through consensus discussion; if no agreement could be reached, a third senior reviewer was consulted to make the final decision. The same two independent reviewers who completed the initial title/abstract screening also conducted the full-text evaluation of all potentially eligible citations, to ensure consistency in the application of inclusion and exclusion criteria throughout the study selection process. Full texts of all relevant citations were obtained, assessed for eligibility, and any discrepancies in full-text assessment were resolved using the same consensus-based method described above. We also manually searched the reference lists of all included studies and relevant systematic reviews to identify additional eligible evidence that may have been missed in the initial database search.

Neutrophil Extracellular Traps

Discovery and Structural Composition of NETs

In 1996, Takei et al[22] identified a unique form of neutrophil cell death that differed from classical apoptosis and necrosis: neutrophils stimulated with phorbol 12-myristate 13-acetate (PMA) displayed fused lobulated nuclei, diminished chromatin content, and ruptured nuclear membranes, while their cytoplasm and organelles remained largely intact. After three hours, the outer cell membrane ruptures via an ROS-dependent mechanism. In 2004, Brinkmann et al[23] further characterized this phenomenon and introduced the term NETs, defining them as a novel neutrophil strategy for capturing and killing bacteria.

NETs are extracellular web-like structures released by activated neutrophils, composed of DNA, histones, and various granular proteins.[24] Western blot and immunofluorescence analyses reveal that DNA forms the structural core of NETs. The majority of this DNA is derived from the nucleus, while a smaller portion originates from mitochondrial DNA (mtDNA). Studies have shown that deoxyribonuclease (DNase I) can effectively degrade NETs, whereas proteases are unable to disrupt their structure. This characteristic further confirms the crucial role of DNA in maintaining NETs integrity.[9,25–27] Scanning electron microscopy reveals that NETs form complex structures composed of smooth 15 nm-diameter strands, which likely represent chains of nucleosomes from unfolded chromatin; chromatin is regarded as the structural backbone of NETs.[12] Combined fluorescence and atomic force microscopy demonstrate that NETs adopt a characteristic branched, two-dimensional fibrous network. This architecture is based on a double-stranded arrangement of two DNA molecules,[28] densely coated with nuclear proteins such as histones (H2A, H2B, H3, H4), granular proteins including neutrophil elastase (NE) and myeloperoxidase, and cytosolic proteins like S100A8/A9/A12, actin, and α-actinin.[29–31] Consequently, NETs comprise a DNA-based extracellular scaffold extensively decorated with proteins and loaded with cytoplasmic antimicrobial agents. Substantial NETs accumulation has been observed at inflammatory sites,[16] where they exert anti-pathogen effects by directly targeting and degrading bacteria via components such as NE, thereby strengthening host defense.

Formation of NETs

Upon inflammatory stimulation, neutrophils bind to bacteria, phagocytose them, and eliminate the pathogens by fusing antimicrobial granules with phagosomes. Various agents, including PMA,[32] lipopolysaccharide (LPS),[33] IL-8,[34] bacteria,[35] fungi,[36] viruses,[37] and activated platelets[38] can induce neutrophil activation. ROS generated by NADPH oxidase trigger the release of NE and MPO from azurophilic granules, followed by their nuclear translocation, where they facilitate histone degradation. Neutrophils concurrently undergo dramatic morphological alterations: chromatin decondenses, the multilobed nucleus disassembles, and both nuclear and granular membranes disintegrate, allowing granular proteins including MPO to infiltrate and mix with decondensed chromatin. Ultimately, the cell membrane ruptures, expelling web-like structures composed of DNA complexed with intracellular proteins such as histones, NE, and MPO.[39–41] These formations, termed NETs, ensnare diverse pathogens including *Staphylococcus aureus*,[42] Group A Streptococcus,[43] *Streptococcus pneumoniae*,[44] and *Candida albicans*.[45] Steinberg BE and Grinstein S[46] identified this distinct form of cell death as NETosis, a caspase-independent process that occurs without DNA fragmentation.[47] NETosis facilitates the trapping and elimination of pathogens through a sequence involving signal activation, chromatin remodeling, plasma membrane disintegration, and the release of neutrophil extracellular traps.[48]

NETosis Form

Three distinct types of NETosis have been identified[48–50](Figure 1).

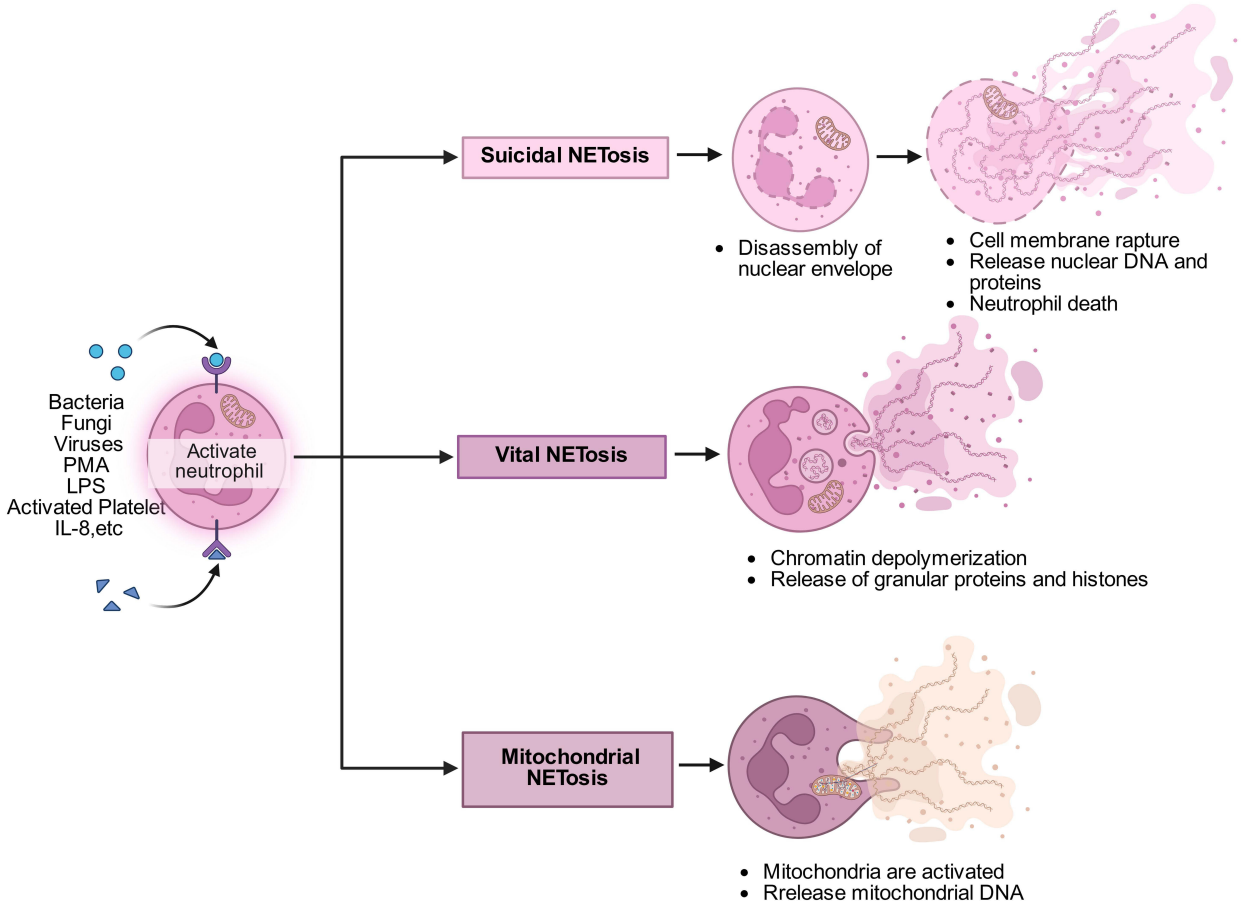

Figure 1 Three distinct types of NETosis. There are three distinct types of NETosis: suicidal NET cell death, viable NET cell death, and mitochondrial NET cell death. (**A**) Suicidal NETosis: Activated neutrophils undergo cell membrane rupture, chromatin and proteins are released into the extracellular space to form NETs, the neutrophils eventually die. This process takes several hours; (**B**) Vital NETosis: Activated neutrophils undergo chromatin disintegration, which is encapsulated and transported to the extracellular space through nuclear membrane vesicles, forming NETs. After release, the neutrophils maintain their vitality and normal functions. This process takes usually within 15–60 minutes; (**C**) Mitochondrial NETosis: Activated neutrophils release mitochondrial DNA and proteins into the extracellular space. After release, the neutrophils maintain their vitality and normal functions throughout the process. This process takes usually within 15 minutes. Created with BioRender.com.
Abbreviations: NETs, Neutrophil Extracellular Traps; PMA, phorbol 12-myristate 13-acetate; LPS, lipopolysaccharide.

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Suicidal NETosis

This type of programmed cell death involves the disruption of plasma membrane integrity and the release of NETs into the extracellular space.[50] Stimulus recognition activates the RAF/MEK/ERK signaling cascade, which in turn triggers NADPH oxidase complex activation. This process elevates intracellular Ca^{2+} levels and promotes the generation of ROS.[51] ROS function as secondary messengers in suicidal NETosis, facilitating nuclear membrane disintegration.[52] Ca^{2+} acts as a cofactor for peptidylarginine deiminase 4 (PAD4), inducing its activation and enabling histone citrullination, thereby promoting chromatin decondensation.[53,54] The decondensed chromatin disperses throughout the cytosol, combines with cytoplasmic and granular proteins, and is subsequently released extracellularly via membrane pores and cell rupture, forming NETs.[55,56] This lytic cell death process typically requires 2–4 hours and constitutes a key mechanism through which neutrophils exert antimicrobial activity.[57,58]

Vital NETosis

This type of NETs release occurs independently of ROS generation, membrane rupture, or cell death, thereby preserving neutrophil viability and function after NETs expulsion.[59] Stimulation by bacterial products through TLRs or complement 3 induces chromatin decondensation and the loss of the nucleus's characteristic lobulated structure. The decondensed chromatin is enveloped by nuclear membrane vesicles and transported extracellularly to form NETs,[60] while granular proteins and histones are simultaneously released. Consequently, the activated neutrophil transforms into an anucleated cytoplast with an intact membrane that retains migratory, phagocytic, and microbicidal capacities.[61,62] Unlike suicidal NETosis, this non-lytic NETs release occurs rapidly and efficiently, often concluding within 15–60 minutes.[63] It thus constitutes a dual defense mechanism, allowing neutrophils to respond promptly to pathogens without sacrificing cellular viability.

Mitochondrial NETosis

These NETs contain DNA derived from mitochondria rather than the nucleus, representing a distinct form of survival-type NETosis. When exposed to stimuli, neutrophils activate their mitochondria, leading to the release of mtDNA into the cytoplasm. This mtDNA promotes phosphorylation of IRF3, ERK1/2, and p38 MAPK, thereby inducing NETosis via the cGAS-STING and TLR9 pathways.[64–67] Compared with nuclear DNA-derived NETs, this mitochondrial DNA-dependent NETs formation proceeds more rapidly, often completing within 15 minutes,[68] illustrating an efficient mechanism by which neutrophils mount swift responses against invading pathogens.

Pathogenic Mechanisms Linking NETs to Dry Eye

Overview of Dry Eye

Dry Eye (DE) is a multifactorial ocular surface disease characterized primarily by tear film instability and symptoms such as ocular dryness and foreign body sensation.[69] Clinical studies consistently report elevated levels of inflammatory mediators in tears, such as IL-1β and TNF-α, accompanied by impaired tear function characterized by reduced secretion and shorter tear film breakup time.[70] Although the pathophysiology of DE remains incompletely understood, recent clinical and preclinical research has identified a central pathogenic role for NETs, whereby dysregulated NETs formation drives disruption of tear film homeostasis and accelerate disease progression.[71–73]

Immune-Inflammatory Mechanisms in DE

The core pathophysiology of DE involves a persistent inflammatory cycle driven by both innate and acquired immune responses. This inflammatory state is initiated by the activation of antigen-presenting cells (APCs), which bridge innate and adaptive immune signaling in the ocular surface microenvironment.[74,75] Tear hyperosmolarity and epithelial cell damage in DE trigger the release of pro-inflammatory cytokines and damage-associated molecular patterns (DAMPs), which activate resident APCs in the conjunctiva and cornea. These activated APCs migrate to draining lymph nodes, where they present ocular surface antigens to naive CD4+ T cells and initiate the adaptive immune response.[76] DE is therefore fundamentally an immune-mediated ocular surface inflammatory disorder, with dysregulated adaptive immunity playing a central role in disease progression.

Following APC-mediated activation, CD4+ T cells differentiate into distinct pro-inflammatory subsets, among which Th17 cells serve as critical drivers of DE pathogenesis. Th17 cells secrete high levels of pro-inflammatory cytokines, including IL-17 and GM-CSF, which promote lymphocyte infiltration into the lacrimal glands and conjunctival tissues, suppress the expression of anti-inflammatory factors such as lactoferrin, and further amplify ocular surface inflammation.[77–79] Animal studies conducted by Dohlman et al[80] further demonstrated that Th17 cell-derived GM-CSF induces robust infiltration of CD11b+ myeloid cells (including neutrophils) into the ocular surface. This adaptive immune-driven neutrophil recruitment and activation is a key upstream event that promotes aberrant NETs formation and sustained NETs deposition on the ocular surface, creating a feedforward loop that exacerbates DE pathology.

Corneal Epithelial Cell Turnover, eDNA Metabolism, and NETs Accumulation in DE

Under hyperosmotic and inflammatory stress, the corneal epithelium in DE exhibits continuous and dynamic turnover.[81] Superficial epithelial cells shed into the tear film[82,83] through a tightly regulated apoptotic process.[84] These dying cells release extracellular DNA (eDNA),[85] a damage-associated molecular pattern (DAMP) that activates the innate immune system and bridges innate and adaptive immune responses.[86,87] Abnormal eDNA accumulation has been observed in the corneal tissue of severe DE patients.[88]

Under physiological conditions, tear fluid innate defense factors maintain eDNA homeostasis: DNase I, the primary tear nuclease, clears excess eDNA via non-sequence-specific hydrolysis, while tear lipocalin, a core component of the tear film innate immune system, supports eDNA clearance and suppresses aberrant NETs formation through its lipid-binding and immunomodulatory properties.[89,90] In severe DE, however, defects in the tear film lipid layer induce hyperosmolarity and instability. These changes elevate nuclease concentrations abnormally and create a matrix-degrading microenvironment enriched with pro-inflammatory factors, neutrophils, eDNA, and NETs within the ocular surface or conjunctival fornix. Inflammatory factor and eDNA expression in exfoliated ocular surface cells also increases significantly.[91] Moreover, tear hyperosmolarity impairs DNase I and lipocalin activity, diminishing NETs clearance. This initiates a vicious cycle wherein NETs accumulation promotes ocular surface damage, intensifies inflammation, and further amplifies NETs deposition, thereby exacerbating DE pathology.[92,93]

Direct Damaging Effects of NETs-Associated Factors on Ocular Surface Cells

NETs-associated factors directly damage ocular surface cells through several mechanisms. Histones within NETs demonstrate direct cytotoxicity to ocular surface epithelial cells, consistent with their established role as key mediators of cell death in sepsis.[94,95] Antimicrobial peptide fragments derived from NETs can provoke local inflammation, cutaneous erythema, and telangiectasia, effects especially pronounced in rosacea patients.[96] Additionally, NE in NETs activates apoptosis-related signaling pathways, leading to epithelial cell death.[97]

Recent clinical and experimental studies further support the significant role of NETs in DE and related ocular conditions. In a study of ocular graft-versus-host disease (oGVHD)-associated dry eye,[98] An et al[99] reported elevated levels of NETs-associated inflammatory factors, including NE, MPO, and IL-8, in ocular surface washings from affected patients. These patients also displayed higher Ocular Surface Disease Index (OSDI) scores, more severe conjunctival hyperemia, and elevated composite clinical scores compared to healthy controls, suggesting a correlation between NETs-related factors and dry eye severity. Kwon et al[51] measured citrullinated protein levels in dry eye patients and found that intravenous immunoglobulin (IVIG) reduced protein citrullination by inhibiting anti-citrullinated protein antibodies (ACPAs), thereby suppressing NETs formation. Clinically, IVIG eye drops significantly improved both signs and symptoms of dry eye. In a study on equine recurrent uveitis (ERU), Fingerhut et al[100] observed increased levels of NETs markers, such as cell-free DNA and DNase I, in the serum and vitreous humor of affected horses, with concentrations correlating with disease severity. These collective findings indicate that NETs levels are positively associated with ocular disease severity and that targeting NETs formation or clearance may offer therapeutic benefits for dry eye.

Therapeutic Strategies Targeting NETs in Dry Eye

Robust clinical and animal experiments evidence supports NETs as a tractable therapeutic target for DE: elevated NETs-associated biomarkers (NE, MPO, cell-free DNA) have been consistently detected in tear fluid and ocular surface washings from patients with inflammatory DE and oGVHD-associated DE; aggregated NETs have been shown to directly occlude Meibomian glands and drive gland dysfunction in ocular surface inflammation; and the well-characterized imbalance between eDNA accumulation and nuclease activity in the DE tear film is a core driver of persistent inflammation, all of which correlate significantly with disease severity and clinical symptom scores.[92,93,99] Dysregulated NETosis, impaired NETs clearance, and NETs-associated cytotoxicity represent three core, actionable axes for targeted DE therapy. Below we systematically summarize these therapeutic strategies, with a focus on clinically relevant or preclinically validated interventions, their mechanisms of action, and translational potential for DE treatment.

Inhibition of Pathological NETs

This strategy focuses on blocking the upstream molecular and signaling events that drive aberrant NETs in the ocular surface inflammatory microenvironment, preventing the generation of pathological NETs at their source.

Key Example 1: Topical Intravenous Immunoglobulin (IVIG) Formulation

Clinical study has demonstrated that IVIG eye drops significantly suppress NETs formation in DE by neutralizing tear anti-citrullinated protein antibodies (ACPAs), thereby inhibiting protein citrullination (a rate-limiting step in chromatin decondensation during NETosis). This intervention has been shown to improve both objective clinical signs and subjective symptoms of DE in human subjects.[51,59]

Key Example 2: Peptidylarginine Deiminase 4 (PAD4) Inhibitors

PAD4 is a critical enzyme mediating histone citrullination and the initiation of suicidal NETosis. Inflammatory models have validated that PAD4 inhibitors effectively block neutrophil activation and pathological NETs release, reducing ocular surface inflammatory cell infiltration and corneal epithelial damage in DE.[90,92]

Key Example 3: Targeting ROS-Mediated NETosis Pathway

ROS is the core upstream regulator of suicidal NETosis cell apoptosis, and the excessive generation of ROS in the DE ocular surface is a key trigger for abnormal NETs cell apoptosis. Antioxidant intervention targeting the ROS axis can inhibit the formation of NETs. Study has found that antioxidant agents targeting mitochondria has been proven to be able to inhibit the generation of mitochondrial ROS, which provides a new approach for targeting the non-classical NETs cell apoptosis pathway in DE.[101]

Enhancement of NETs Clearance

This approach restores homeostatic NETs degradation in the tear film, reversing excessive NETs and eDNA accumulation to break the vicious cycle of NETs-driven inflammation and ocular surface damage.

Key Example 1: Topical Deoxyribonuclease I (DNase I) Supplementation

DNase I is the primary endogenous nuclease responsible for degrading the DNA backbone that forms the structural core of NETs. Studies have confirmed that topical DNase I administration reverses the eDNA-nuclease activity imbalance in DE tears, efficiently degrades aggregated NETs, reduces ocular surface inflammatory burden, and alleviates tear film instability in DE patients.[93]

Neutralization of NETs-Associated Toxicity

This strategy mitigates the direct cytotoxic and pro-inflammatory effects of NETs components on ocular surface cells, without altering the physiological antimicrobial functions of NETs formation or clearance.

Key Example 1: Histone-Neutralizing Interventions

Extracellular histones are the primary cytotoxic components of NETs, driving direct corneal and conjunctival epithelial cell death in DE. Studies have validated that histone-neutralizing antibodies or clusterin supplementation effectively

block histone-induced epithelial cytotoxicity and ocular surface inflammation, a core pathological event mediated by dysregulated NETs in DE.[94,95]

Conclusion

This article systematically reviews the structural characteristics, formation mechanisms, and three distinct forms of NETosis, clarifies the core causal links between dysregulated NETs and the pathophysiology of DE, and comprehensively summarizes the latest advances in NETs-targeted therapeutic strategies for DE. These insights offer novel perspectives for the pathological diagnosis, prognostic assessment, and mechanism-driven treatment of DE. Tear hyperosmolarity, inflammatory cytokine stimulation, and impaired NETs clearance are key inducers of aberrant NETosis in DE, and the NETosis pathway contains multiple promising therapeutic targets for DE intervention. Treatment approaches centered on inhibiting pathological NETs formation, enhancing NETs clearance, or neutralizing NETs-associated cytotoxicity represent innovative, mechanism-driven directions for DE therapy, particularly for refractory DE that is unresponsive to conventional treatments.

Future research should prioritize three key directions: (1) Elucidating the cell-type specific regulatory mechanisms of NETs formation and release in the ocular surface microenvironment; (2) Validating the diagnostic and prognostic value of tear NETs biomarkers (such as NE, MPO, and cell-free DNA) in DE patients of different subtypes and severity; (3) Conducting well-designed, large-scale clinical trials to evaluate the efficacy and safety of NETs-targeted therapies for DE, to accelerate the clinical translation of these mechanism-driven interventions.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

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

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