REVIEW ARTICLE

Formation of neutrophil extracellular traps (NETs) in periapical lesions: an integrative review

Formação de armadilhas extracelulares de neutrófilos (NETs) em lesões periapicais: uma revisão integrativa

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Abstract

Objective: to map and synthesize evidence on the formation of neutrophil extracellular traps (NETs) in periapical lesions. **Method:** a bibliographic study was conducted following the integrative review method. Searches were carried out in the MEDLINE/PubMed, Web of Science, Scopus, Embase, and Google Scholar databases using a search strategy based on descriptors and key terms. Both experimental studies (animal models) and studies using samples from human periapical lesions were eligible, with no restrictions regarding publication date or language. **Results**: after the screening, 1,135 references were retrieved, and 3 were included. The analysis of these studies demonstrated that NET formation is a mechanism present in experimental periapical lesions (confirmed by different methods), being associated with granular protease activation, persistent inflammation, and bone resorption. It was also observed that NET formation was correlated with the expression of REDOX enzymes in periapical tissues, indicating a probable involvement of oxidative stress in this process. Furthermore, the degradation of these structures by DNase I resulted in reduced osteolysis and local inflammation, demonstrating their likely proinflammatory role during periapical aggression by microorganisms. **Conclusion**: based on the literature, it can be inferred that NETs are formed in periapical lesions and may represent a potential therapeutic target for controlling inflammation and bone destruction in the periapical region.

Keywords: apical periodontitis; neutrophil extracellular traps; inflammation; bone resorption; innate immunity.

Resumo

Objetivo: mapear e sintetizar as evidências sobre a formação de armadilhas extracelulares de neutrófilos (NETs) em lesões periapicais. **Método**: foi realizado um estudo bibliográfico seguindo o método de revisão integrativa. As buscas foram conduzidas nas bases de dados MEDLINE/PubMed, Web of Science, Scopus, Embase e Google Scholar, utilizando uma estratégia de pesquisa baseada em descritores e termos-chave. Foram elegíveis tanto estudos experimentais (modelos animais) quanto estudos com amostras de lesões periapicais humanas, sem restrições quanto à data de publicação ou ao idioma. **Resultados**: após o rastreamento, 1.135 referências foram recuperadas e 3 foram incluídas. A análise desses estudos demonstrou que a formação de NETs é um mecanismo presente em lesões periapicais experimentais (confirmado por diferentes métodos), estando associada à ativação de proteases granulares, inflamação persistente e reabsorção óssea. Também foi observado que a formação de NETs esteve correlacionada à expressão de enzimas REDOX nos tecidos periapicais, indicando um provável envolvimento do estresse oxidativo nesse processo. Além disso, a degradação dessas estruturas pela DNase I resultou em redução da osteólise e da inflamação local, evidenciando seu provável papel pró-inflamatório durante a agressão periapical por microrganismos. **Conclusão**: com base na literatura, pode-se inferir que as NETs são formadas em lesões periapicais e podem representar um potencial alvo terapêutico para o controle da inflamação e da destruição óssea na região periapical.

Palavras-chave: periodontite apical; armadilhas extracelulares de neutrófilos; inflamação; reabsorção óssea; imunidade inata.

INTRODUCTION

It is well established that, during pathogen-induced insults, neutrophils represent the first line of cellular defense of the innate immune system, employing various strategies to combat infections (e.g., phagocytosis, degranulation, and production of reactive oxygen species)[1,2]. In addition to these strategies, neutrophils are also capable of defending the host through the formation of neutrophil extracellular traps (NETs), a process known as NETosis. In fact, NETs are structures composed of decondensed chromatin, often of nuclear origin, and proteins with antimicrobial potential, especially neutrophil elastase (NE) and myeloperoxidase (MPO), as well as histones and other proteins commonly found in the neutrophil cytoplasm[1,3,4].

During the activation of the innate immune system by pathogens, the NET formation is a response associated with the presence of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), as well as inflammatory cytokines, antigen-antibody complexes,

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and byproducts[5-7]. Regarding their function, it is also known that NETs contribute significantly to the host's antimicrobial response by trapping and neutralizing invading pathogens through their toxic components, thereby containing their spread and enabling their elimination by the immune response. These contributions have been demonstrated across a wide range of microorganisms, including bacteria, fungi, viruses, and parasites[5,8,9].

However, NETosis has also been shown to exhibits functional duality in the context of inflammation and infection. Excessive or dysregulated NET formation by neutrophils can amplify the degree of inflammation and lead to significant tissue damage, impairing host homeostasis. Moreover, diverse pathogens have evolved evasion strategies against NETs, notably the release of nucleases that degrade NETs and the expression of surface proteins that inhibit the activity of enzymes such as neutrophil elastase and myeloperoxidase[5,7,8].

The relevance of investigating NET formation in pathological processes is supported by evidence indicating the involvement of these structures in autoimmune inflammatory conditions, such as lupus, arthritis, and psoriasis, where NETs contribute to the exposure of autoantigens, activation of the complement system, and tissue damage (e.g., vascular epithelium)[4,6]. In addition, NETs also play a role in cardiovascular diseases and metabolic disorders such as hepatic steatosis and atherosclerosis by contributing to thrombus formation (thrombosis) and the chronicity of the inflammatory proces[s3,8]. Lastly, NET formation has also been investigated in highly complex diseases with significant epidemiological relevance, such as cancer, where it is associated with tumor progression by facilitating metastasis, microenvironment remodeling, and immunosuppression[5,10], and COVID-19, where it is linked to the amplification of the inflammatory response, coagulopathies, and multiple organ failure[11,12].

Therefore, considering the relevance of NET formation in immune-mediated pathological processes, these structures are regarded as promising therapeutic targets in various diseases and health conditions. Multiple pharmacological strategies have aimed to inhibit their assembly or promote their degradation, with promising results observed in preclinical approaches and experimental trials involving infectious, autoimmune, metabolic, and neoplastic diseases[8-10]. Within this context, a question arises: what is the role of NET formation in periapical lesions?

This question becomes relevant when considering that periapical lesions result from the aggression of periapical tissues by microorganisms originating from the oral cavity, constituting a clinical condition of apical periodontitis. These pathogens, especially gram-negative anaerobic bacteria, invade the dental pulp following its exposure to the microbiota during other pathological processes (e.g., carious lesions), inducing a progressive inflammatory response that leads to pulp necrosis if left untreated[13,14]. Pulp necrosis, in turn, allows microbial products and toxins to reach the periapical tissues through the apical foramen, triggering a local immune response. As a consequence, the activation of immune cells, particularly neutrophils, macrophages, and lymphocytes, promotes the release of proinflammatory cytokines and other mediators that induce periapical bone resorption, leading to the formation of osteolytic lesions in the periapex[15,16].

Understanding the cellular and molecular dynamics involved in the development of periapical lesions is relevant because apical periodontitis is a highly prevalent disease worldwide, affecting many individuals throughout life. Moreover, this inflammatory condition has been associated with systemic effects due to the continuous release of inflammatory mediators into the bloodstream, making the development of periapical lesions a potential contributor to low-grade systemic inflammation and highlighting its clinical relevance beyond the oral cavity. In addition, molecular links have been demonstrated between apical periodontitis and other pathological processes involving immunity, sharing immunopathological mechanisms with autoimmune, cardiovascular, hepatic, and metabolic diseases[17-19].

Therefore, given the correlations between pathological processes, it is reasonable to assume that NETosis also plays a role in the development of periapical lesions, contributing to the understanding of the cellular and molecular mechanisms involved in apical periodontitis and aiding in the identification of a meaningful therapeutic target for intervention. Indeed, the involvement of NETs in the pathogenesis of oral diseases has become an area of growing interest in the scientific literature, with evidence reported in conditions such as gingivitis, pulpitis, periodontitis, peri-implantitis, and candidiasis[20-22]. However, periapical lesions have not been properly explored. For this reason, the objective of this literature review was to map and synthesize evidence on the NET formation in periapical lesions.

METHODS

Approach

To achieve the proposed objective, an integrative review was conducted to compile scientific evidence from studies employing different research methods, integrating the findings to provide a synthesis of the current state of knowledge. The bibliographic analysis procedure was based on established methodological frameworks for this type of literature review[23,24]. The steps carried out were: (1) formulation of the primary and secondary research questions; (2) establishment of eligibility criteria; (3) selection of databases and definition of search strategies; (4) screening; (5) data extraction; (6) evidence synthesis.

Research Questions

The conduction of this bibliographic analysis was guided by a primary question: Are neutrophil extracellular traps (NETs) formed in periapical lesions? As a development of this initial

inquiry, a secondary guiding question was formulated: What is the role of neutrophil extracellular traps (NETs) formation in periapical lesions: proinflammatory or anti-inflammatory?

Eligibility

To address the research questions, two profiles of investigations were considered: (1) in vivo experimental approaches using animal models to induce periapical lesions; (2) observational approaches using human samples of periapical lesions obtained through surgical procedures during endodontic interventions (e.g., apicoectomies and apical curettage). Regarding the eligibility criteria for the first profile, studies were considered if they used valid animal models for the experimental induction of periapical lesions and approached, either directly or indirectly, the identification of NETs in pulp and/or periapical tissues after exposure to the oral microorganisms. For the second profile, studies involving human subjects with apical periodontitis confirmed by clinical and radiographic criteria were considered, provided the individuals had no systemic conditions that could alter the natural course of the disease and, similar to the first profile, the identification of NETs in the collected samples was approached. Additionally, for both profiles, no restrictions were applied regarding language, location, or publication period.

Search Strategy

The screening of eligible investigations was carried out in the following databases: MEDLINE/PubMed, Web of Science, Scopus, Embase, and Google Scholar (first 100 hits). Then, electronic search strategies for all databases were developed using descriptors from Medical Subject Headings (MeSH) and commonly used keywords in related literature, systematically combined using Boolean operators (“OR” and “AND”). Preliminary searches were conducted to explore the scope and determine the best approach to cover both investigation profiles. Chart 1 presents a detailed overview of the search strategies applied in all databases.

Chart 1. Search strategies applied in all databases.

Database	Search strategy	References
MEDLINE/PubMed	("neutrophil extracellular trap" OR "neutrophil extracellular traps" OR “NET” OR “NETs” OR “NETosis” OR “neutrophil” OR “neutrophils”) AND ("periapical lesion" OR "periapical lesions" OR "apical periodontitis" OR "periapical disease")	154
Web of Science	("neutrophil extracellular trap*" OR “NET*” OR “NETosis” OR “neutrophil*”) AND ("periapical lesion*" OR "apical periodontitis" OR "periapical disease*")	100
Scopus	("neutrophil extracellular trap*" OR “NET*” OR “NETosis” OR “neutrophil*”) AND ("periapical lesion*" OR "apical periodontitis" OR "periapical disease*")	376
Embase	("neutrophil extracellular trap" OR "neutrophil extracellular traps" OR “NET” OR “NETs” OR “NETosis” OR “neutrophil” OR “neutrophils”) AND ("periapical lesion" OR "periapical lesions" OR "apical periodontitis" OR "periapical disease")	405
Google Scholar	("neutrophil extracellular trap*" OR “NET*” OR “NETosis” OR “neutrophil*”) AND ("periapical lesion*" OR "apical periodontitis" OR "periapical disease*")	100

Screening

The references retrieved through the electronic search strategies applied in the databases were exported (including author, year, journal, title, and abstract) to EndNoteTM software (Clarivate, USA) for integration, management, and identification of potential overlaps between databases (duplicates). Duplicate titles were removed, and non-duplicates proceeded to content assessment. An initial exploratory reading of the titles was conducted to assess alignment with the research questions. Non-eligible titles were excluded, and those deemed potentially relevant had their abstracts qualitatively evaluated. Abstracts that did not meet the eligibility criteria were excluded, while eligible ones advanced to full-text reading, where the decision to include or exclude each reference within the scope of this literature review was made, providing a reasonable justification for exclusions at this stage.

Title and abstract screening was performed by a single researcher on April 17, 2025. Full-text reading was conducted independently by four other researchers to validate the initially proposed scope. Discrepancies were resolved by consensus or, when necessary, with the involvement of the researcher who conducted the initial screening (titles and abstracts). No automatic filters or refinement operators were applied in the databases during the screening process. Additionally, a manual search was carried out in the reference lists of the included studies to identify potentially relevant studies that may not have been retrieved through the previously outlined electronic search strategy.

Extraction

After the scope of studies to be included in this literature review was defined, the information relevant to the research questions was retrieved by a single researcher. Once the data had been

compiled, four other researchers independently validated the accuracy of the information and indicated necessary corrections. Discrepancies were resolved in the same manner as in the screening stage. To guide the retrieval process, spreadsheets were structured with the following variables of interest: (1) study identification (author, year of publication, and journal); (2) methodological characteristics (design, sample, type of approach, and type of microscopic and molecular analysis); (3) eligibility for participants (human subject approaches) or selection of animal models (experimental approaches); (4) techniques used for identifying NETs or NET-related markers; (5) main outcomes related to NET formation in periapical lesions, with focus on the research questions previously proposed.

Synthesis

The synthesis of the data retrieved from the references included in this review was organized in two stages: (1) presentation of the theoretical and methodological information from the evaluated studies, and (2) critical reflection, discussion, and development of response texts addressing the proposed guiding questions. These stages were conducted with the aim of integrating the findings from the included studies, despite methodological heterogeneity among them, which allowed for the identification of gaps, convergences, and divergences to adequately synthesize the current state of knowledge regarding the relationship between NET formation in periapical lesions. All researchers contributed to the development of the synthesis.

RESULTS AND DISCUSSION

The initial search retrieved 1,135 references. After removing 562 overlapped references, 573 remained. Of these, 344 were deemed ineligible based on title analysis, and another 221 were excluded after abstract analysis. Eight references remained eligible for full-text reading, of which five were excluded for not meeting the eligibility criteria. Therefore, three studies were included in the qualitative synthesis within the scope of this literature review.

Chart 2 summarizes the general characteristics of the studies included in the synthesis. Although each study employs distinct approaches, they collectively converge on a central theme: exploring the involvement of NETs in the development of periapical lesions. The study conducted by Guilherme-Neto et al. (2023) focused on the functional assessment of NETs, exploring their direct impact on bone resorption and inflammatory processes, including through the inhibition of NET assembly using DNase I (deoxyribonuclease type I enzyme)25. In contrast, the study conducted by Massoni et al. (2025) aimed to characterize the expression of specific NET markers across different phases of experimentally induced periapical inflammation, without the application of external interventions26. Lastly, the study conducted by Silva et al. (2025) integrated the analysis of NET markers expression in periapical tissues with the evaluation of the REDOX system, thereby proposing a more comprehensive investigation of the molecular mechanisms underlying the pathogenesis of periapical lesions[27].

Chart 2. General characteristics of the studies included in the synthesis.

Authorship	Ýear	Journal	Objective
Guilherme-Neto, et al.[25]	2023	Journal of Endodontics	To evaluate the role of NETs in bone resorption observed in pulp infection–induced apical periodontitis in mice
Massoni, et al.[26]	2025	Archives of Oral Biology	To evaluate the expression of NET markers in experimental apical periodontitis induced in mice
Silva, et al.[27]	2025	Clinical Oral Investigations	To evaluate and correlate the expression of REDOX enzymes and NET markers during the development of periapical lesions

Chart 3 presents the methodological characteristics of the studies included in the synthesis. Regarding the study design, all three studies employed *in vivo* experimental approaches, inducing periapical lesions through pulp exposure of lower molars to the oral microbiota in wild-type mice[25-27]. While Massoni et al. (2025)[26] and Silva et al. (2025)[27] exclusively used murine models, the study conducted by Guilherme-Neto et al. (2023)[25] also incorporated periapical lesion samples obtained from human biopsies, adding a translational aspect to the investigation, despite the limited human sample size.

Regarding the samples, there was consistency in the selection of the wild-type murine strain (C57BL/6, male, aged from 6 to 8 weeks). However, differences were observed in sample size and the distribution of experimental groups[25-27]. Guilherme-Neto et al. (2023)[25] used 60 animals, divided into groups treated with DNase I and control groups, with lesion induction maintained for 7 days before euthanasia. Massoni et al. (2025)[26] employed 55 mice, allocated into six groups: one negative control (no pulp exposure) and five experimental time points, with euthanasia performed at 2, 5, 7, 21, and 42 days after pulp exposure. Similarly, Silva et al. (2025)[27] used 42 mice, divided into a negative control (no pulp exposure) and five experimental periods with euthanasia at 2, 5, 7, 21, and 42 days following pulp exposure.

Chart 3. Methodological characteristics of the studies included in the synthesis.

Authorship	Design	Sample	Analyses
Guilherme-Neto et al.[25]	Mixed (Experimental and human subject samples)	Mice (N = 60) C57BL/6 (male), between 6-8 weeks + Apical granulomas from adults (N = 3)	Microscopy (hematoxylin and eosin staining with histomorphometry), tartrate-resistant acid phosphatase, immunohistochemistry, antibody colocalization, micro-computed tomography, gene expression, serum levels, and use of a NET inhibitor
Massoni et al.[26]	Experimental	Mice (N = 55) C57BL/6 (male), between 6-8 weeks	Microscopy (hematoxylin and eosin staining with histomorphometry), immunohistochemistry, antibody colocalization (overlay), and gene expression
Silva et al.[27]	Experimental	Mice (N = 42) C57BL/6 (male), between 6-8 weeks	Microscopy (hematoxylin and eosin staining with histomorphometry), immunohistochemistry, antibody colocalization (overlay), and gene expression.

Regarding the analyses, all three included studies performed histological and histomorphometric assessments (microscopic approaches) using hematoxylin and eosin (H&E) staining, complemented by immunohistochemical labeling or immunofluorescence technique to detect NET markers. At the molecular level, all studies applied qRT-PCR (quantitative reverse transcription polymerase chain reaction) to evaluate gene expression of NET components. In the case of Silva et al. (2025)27, qRT-PCR was also used to assess the expression of the REDOX system enzymes. NET identification was conducted using classical markers such as MPO (myeloperoxidase), citrullinated histone H3 (H3Cit), ELANE (neutrophil elastase – NE), PAD4 (peptidyl arginine deiminase 4), and CatG (cathepsin G), assessed through antibody colocalization[25-27].

Moreover, it was observed that Guilherme-Neto et al. (2023)[25] established a functional relationship between the inhibition of NETs and the reduction of osteoclastogenesis to the extent of tissue damage (osteolytic-related area). Massoni et al. (2025)[26] focused on the temporal dynamics of NETs expression, detailing the biological behavior of these markers over time, while Silva et al. (2025)[27] explored underlying molecular mechanisms by correlating NETs markers with enzymes involved in REDOX homeostasis: NOS2 (inducible nitric oxide synthase type 2), classified as an oxidant enzyme; and the antioxidant enzymes SOD1 (superoxide dismutase type 1), CAT (catalase), and GSR (glutathione reductase).

Chart 4 presents the main outcomes of the studies included in the synthesis. Based on an integrated analysis, it was possible to address the research questions, since all of the studies performed inferences regarding the expression of NETs markers and the formation of periapical lesions:

(Research Question 1) Are neutrophil extracellular traps (NETs) formed in periapical lesions?

Yes, all three studies included in this literature review provided direct evidence of NET formation in periapical lesions. Guilherme-Neto et al. (2023)[25] confirmed this finding through immunofluorescence, demonstrating colocalization of MPO and H3Cit in both murine models and human biopsies of apical granulomas. The visualization of these structures directly demonstrated NET assembly in the inflammatory context induced by pulp exposure to the oral microbiota. Massoni et al. (2025)[26] also demonstrated NET formation, with emphasis on extracellular colocalization of MPO/H3Cit at experimental time points of 5, 21, and 42 days after pulp exposure. The increased gene expression of these markers over time further supported continuous NETosis activation. Notably, this study also identified a second expression peak for PAD4 and CatG at 21 and 42 days, suggesting possible reactivation of NET formation during the chronic phase of periapical inflammation. Silva et al. (2025)[27] corroborated these findings through immunohistochemical colocalization of MPO/H3Cit and ELANE/H3Cit from day 5 (acute phase) through to days 21 and 42 (chronic phase). The increased expression of MPO, ELANE, and H3Cit, as assessed by qRT-PCR, further confirmed the release of typical NET components.

(Research Question 2) What is the role of neutrophil extracellular traps (NETs) formation in periapical lesions: proinflammatory or anti-inflammatory?

The formation of NETs appears to play a predominantly

proinflammatory role in the development of periapical lesions. The study by Guilherme-Neto et al. (2023)[25] provided the most direct evidence addressing this question. The authors demonstrated that inhibition of NET assembly using DNase I led to a significant reduction in periapical lesion size, osteoclast numbers, and the expression of genes related to osteoclastogenesis (Acp5, Rankl, Ctsk). These findings indicate that the presence of NETs exacerbates local inflammation and promotes periapical bone resorption, thus characterizing a proinflammatory role for NETs in periapical lesion formation.

It is also important to highlight that Massoni et al. (2025)[26] and Silva et al. (2025)[27], although they did not address this question directly, provide important supporting information. Massoni et al. (2025) may support this proinflammatory interpretation, as NETs markers (PAD4, MPO, H3Cit, and CatG) showed peak gene expression during the later stages of inflammation, particularly at 21 and 42 days, when the periapical inflammatory infiltrate and extent of tissue damage (osteolytic-related area) were most advanced. The temporal alignment between NET marker peaks and maximal lesion severity provides indirect support for their proinflammatory involvement.

The findings of Silva et al. (2025)[27] also may support the proinflammatory role of NETs in the context of periapical lesion development. Through qRT-PCR and immunohistochemistry, the study demonstrated elevated expression of NET markers (MPO, ELANE, and H3CIT) following pulpal exposure, with expression peaks at 5 days and sustained presence during the chronic inflammatory phases at 21 and 42 days. Notably, the gene expression of H3CIT was significantly correlated with the periapical lesion area, suggesting that NET formation contributes directly to the extent of bone destruction. Additionally, the study revealed positive correlations between NET markers and oxidant enzymes (particularly NOS2), as well as significant associations between NET components and antioxidant enzymes, indicating a REDOX imbalance. These findings suggest that NET formation, also driven by oxidative stress, amplifies local inflammation and periapical tissue damage. Therefore, the molecular interplay between NETs and REDOX pathways provides further mechanistic evidence of the proinflammatory contribution of NETs in periapical lesion formation.

Chart 4. Main outcomes of the studies included in the synthesis.

Authorship	Outcomes
Guilherme-Neto et al.[25]	(1) NETs were identified in periapical lesions of mice and in human biopsies; (2) Exposure to DNase I significantly reduced the size of the periapical lesion and the number of osteoclasts; (3) Gene expression of Acp5, Rankl, and Ctsk decreased following NET inhibition; (4) Lower serum levels of NETs (MPO-DNA) were observed after DNase I exposure.
Massoni et al.[26]	(1) Gene expression of NET markers (PAD4, MPO, H3Cit, and CatG) was significantly increased after 5 days of pulp exposure, with variations throughout the experimental timeline; (2) PAD4 and CatG showed a second expression peak at 21 and 42 days, suggesting their involvement in the chronic phase of inflammation; (3) Extracellular colocalization of MPO and H3Cit was observed by immunohistochemistry, confirming NET formation, especially at 5, 21, and 42 days; (4) Immunohistochemical staining of PAD4 and CatG was more intense at 21 and 42 days, predominantly localized in the apical region and periapical lesions.
Silva et al.[27]	(1) Gene expression of NET markers (MPO, ELANE, and H3Cit) significantly increased after 5 days of pulp exposure, with H3Cit remaining elevated through the later time points (21 and 42 days); (2) Immunohistochemical colocalization of MPO/H3Cit and ELANE/H3Cit confirmed NET formation in periapical tissues, especially at 5, 21, and 42 days; (3) H3Cit was the marker most strongly associated with periapical lesion area and REDOX system markers, suggesting a relevant and persistent role of NETs in inflammatory progression; (4) A significant positive correlation was found between NET markers and the antioxidant enzymes CAT and GSR, indicating a possible interaction between oxidative stress and NET formation.

This literature review mapped and integrated evidence regarding the formation of NETs in periapical lesions. Experimental approaches confirmed that NETosis occurs following the exposure of dental pulp to the oral microbiota, as part of the host's innate immune response. Another key finding was the likely proinflammatory role of NET formation in periapical lesion formation in animal models. From a molecular perspective, as also discussed by Guilherme-Neto et al. (2023), Massoni et al. (2025), and Silva et al. (2025)[25-27], it is important to consider the mechanisms associated with NETosis that may intensify inflammation and bone resorption, particularly the excessive release of granular proteases, such as NE and MPO, which contribute to extracellular matrix degradation and osteoclastogenesis[3].

Another central mechanism involves the activity of the PAD[4] enzyme, which is responsible for histone citrullination, an essential step in NET formation. The activation of

this enzyme promotes the exposure of modified nuclear antigens (autoantigens), which can trigger sustained tissue inflammation. Moreover, the extracellular presence of NET-associated components, especially histones, can function as DAMPs, amplifying immune responses and activating additional bone resorptive pathways. One such pathway is the NLRP3 inflammasome (NOD-like receptor family pyrin domain-containing 3), which upregulates the expression of IL-1β (interleukin-1 beta), a cytokine widely recognized as a potent inducer of bone resorption[8,10]. Supporting this, it has previously been demonstrated that inhibition or knockout of NLRP3 significantly reduces IL-1β expression and the extent of periapical osteolysis[14]. Together, these findings underscore the pathogenic potential of NETs as amplifiers of inflammation and bone resorption in periapical lesions.

Beyond the formation of periapical lesions, Guilherme-Neto et al. (2023)[25] also reported that DNase I treatment reduced the concentration of circulating NETs in peripheral blood (serum levels), suggesting that NET assembly during periapical lesion development may contribute to the systemic dissemination of inflammation, in addition to its local effects at the periapical region. This finding raises important questions about the potential systemic implications of localized endodontic-related infections. In particular, it highlights a gap in our understanding of the molecular pathways that may link apical periodontitis to broader immune-mediated diseases, such as cardiovascular, rheumatologic, or metabolic disorders, as previously discussed[17-19]. Further studies are warranted to explore whether the persistent activation of NETs in periapical tissues may serve as a source of systemic inflammatory mediators, contributing to disease beyond the oral cavity.

Moreover, the expression of NET markers was also correlated with the expression of REDOX enzymes. As discussed by Silva et al. (2025)[27], this correlation can be explained by the active role of reactive oxygen species (ROS) in inducing NETosis, since intracellular ROS production triggers the activation of key enzymes involved in NET assembly, namely MPO, NE, and PAD4. Additionally, ROS-mediated activation of PAD4 contributes to histone citrullination, a fundamental step in chromatin decondensation required for NETosis. These findings demonstrate that oxidative stress is not merely a byproduct of inflammation but also a potent stimulus for NET formation[3,4,9].

It is important to consider the limitations of the reviewed literature when interpreting and applying the findings regarding the interface between NETs and periapical lesions. Although Guilherme-Neto et al. (2023)[25] included human samples of periapical lesions, the limited number of cases constrains the generalizability of their findings to human pathology. Additionally, recent studies have distinguished between different NET assembly pathways, as vital and non-vital mechanisms, which exhibit distinct molecular profiles and implications for tissue inflammation[8,9]. While the evidence reviewed focused primarily on the classical NETosis pathway (non-vital), it is plausible that the alternative NETosis pathway (vital) may also contribute to the formation of periapical lesions. This possibility highlights the need for future experimental approaches to explore these distinct pathways more comprehensively.

While such limitations are important, the current body of evidence nonetheless allows us to hypothesize broader mechanistic interactions that may underlie the persistence and severity of periapical inflammation. For these reasons, it is worth considering whether the widely described interactions among NETs, ROS, and inflammasomes represent a functional cluster of cellular and molecular mechanisms that sustain a persistent self-regulating inflammatory microenvironment within experimental periapical lesions. This perspective considers the continuous oxidative stress and the activation of signaling pathways that promote bone resorption, potentially advancing our understanding of both the local pathogenesis and the systemic implications associated with apical periodontitis[3,8,10].

Regarding the scope of the included studies, it is important to highlight a key limitation of the study by Guilherme-Neto et al. (2023)[25]: all analyses focused exclusively on the acute phase, encompassing only the first seven days after pulp exposure to the oral microbiota. As such, the study did not evaluate NET formation during the chronic stages of periapical lesion progression. Based on this limitation, it is reasonable to question whether the inhibition of NETosis observed in this study would produce the same anti-inflammatory effect once periapical osteolysis has already progressed into a chronic state. Moreover, the administration of DNase I was performed systemically, which raises further questions about whether a similar therapeutic effect could be achieved through localized delivery (e.g., intracanal medication), a critical consideration when evaluating the potential of NETs as a viable therapeutic target for periapical lesions.

Massoni et al. (2025)[26], while advancing the understanding of NET marker expression dynamics during the chronic phases of periapical inflammation, did not assess the effects of either stimulation or inhibition of NET assembly. Additionally, the study did not correlate NET marker expression with microscopic parameters such as periapical lesion size. A similar limitation is observed in the study by Silva et al. (2025)[27]. Although it demonstrated correlations between NET markers and REDOX enzymes, the impact of antioxidant exposure on periapical lesion formation, as well as specifically how such exposure might modulate NET formation during acute or chronic inflammation, was not evaluated.

For future investigations, there is a clear need for more comprehensive studies involving human samples of periapical lesions to validate the insights currently derived from animal models. In this context, it is essential to deepen our understanding of the molecular mechanisms involved in NET formation during periapical bone resorption, with the aim of identifying pharmacological strategies capable of controlling inflammation and promoting bone repair through NET assembly inhibition. Furthermore, future research should compare the

effects of local versus systemic interventions and evaluate the immunomodulatory potential of antioxidant agents on both periapical bone resorption and the NETosis process.

CONCLUSION

Based on the reviewed literature, it can be concluded that NETs are formed in experimental periapical lesions, with consistent microscopic and molecular evidence derived from murine models. The findings also support the conclusion that NETs likely play a predominantly proinflammatory role in this animal model, since their inhibition leads to a reduction in periapical bone resorption, characterized by decreased osteoclastic activity and diminished osteolysis. These results indicate that NETs actively contribute to the pathogenesis of periapical lesions.

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