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Proteins Associated with Persistent Apical Periodontitis: A Scoping Review

Weslay Rodrigues da Silva [a,b*], Ana Paula Veras Sobral [a,b], Kaline Romeiro [c], Cleiton Rone dos Santos Lima [d], Marina da Cunha Isaltino [a,e], Christianne Tavares Velozo Telles [a,e], Diana Santana de Albuquerque [a,e]

a Dental College of Pernambuco, Postgraduate Program in Dentistry, University of Pernambuco (UPE), Recife, PE, Brazil; b Oswaldo Cruz University Hospital (UPE), Integrated Anatomic Pathology Center, Recife, PE, Brazil; c Postgraduate Program in Dentistry, University of Grande Rio (UNIGRANRIO), Rio de Janeiro, RJ, Brazil; d Postgraduate Program in Dentistry, University Federal of Goias (UFG), Goiânia, GO, Brazil; e Department of Restorative Dentistry and Endodontics, Dental College of Pernambuco, University of Pernambuco (UPE), Recife, PE, Brazil

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**Corresponding author*: Weslay Rodrigues da Silva, Postgraduate Program in Dentistry, School of Dentistry, University of Pernambuco, R. Arnóbio Marques, 310, 50100-130, Recife, Pernambuco, Brazil.

E-mail: weslay.rodriguess@upe.br

This scoping review aimed to assess immunohistochemical markers associated with the physiopathogenesis of Persistent Apical Periodontitis. The protocol was adapted from the Joanna Briggs Institute Reviewer's Manual (2017) and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines for Scoping Reviews. A total of 239 articles were considered potentially eligible, and their full texts read by two reviewers. Six articles were included. The included articles were published between 1999 and 2017. A total of 12 biomarkers were identified, forkhead box P3, cluster of differentiation (CD)3, CD8, CD450, CD68, transforming growth factor alpha, transforming growth factor-beta1, matrix metalloproteinase-9, receptor activator of nuclear factor kappa beta ligand, osteoprotegerin, CD90 and sex-determining region Y-box 2; categorized according to their applicability. Among the biomarkers identified, receptor activator of nuclear factor kappa beta ligand and osteoprotegerinare were related to bone remodeling in apical periodontitis and may also be associated with persistent apical periodontitis.

Keywords: Apical Periodontitis; Biomarkers, Immunohistochemistry; Persistent Apical Periodontitis; Scoping Review

Introduction

Apical periodontitis (AP) is a chronic infectious-inflammatory periradicular tissue disease caused by etiological agents of endodontic origin. Cases that do not regress following endodontic treatment are considered refractory and categorized as persistent or secondary [1-3].

Persistent apical periodontitis (PAP) cases are caused by surviving microorganisms in treated root canals following intracanal disinfection procedures [4]. Secondary infections, in turn, are generally associated with microorganisms introduced into root canals by breaking the aseptic chain [4, 5]. Despite being clinically similar, PAP etiopathogenesis cases are still hardly studied [1].

Microorganisms in the root canal system can come into contact with the periradicular tissues through the apical foramen, lateral canals and recesses, causing immune responses that induce AP [6-8]. In this regard, anti-inflammatory and pro-inflammatory mediators, cytokines, and growth factors are released during the inflammatory process of periradicular tissues [9].

The continuance of this response in periapical tissues can lead to the accumulation of lymphocytes, plasma cells, macrophages, neutrophils, and eosinophils [10], as well as virulence factors from gram-negative bacteria, such as lipopolysaccharides, which induce the production of cytokines and matrix metalloproteinases (MMP), favoring bone resorption [11]. Many mediators may be involved in the bone resorption process [transforming growth factor alpha (TGF-α), transforming growth factor-beta1 (TGF-

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β1), MMP-9, receptor activator of nuclear factor kappa beta ligand (RANK-L), and osteoprotegerin (OPG)], also inducing and/or activating mesenchymal stem cells [cluster of differentiation (CD) 90 and SRY-box transcription factor 10] in the periradicular region, contributing to the persistence of AP through different cell signaling pathways [7, 10, 12-15].

Inflammatory stimuli are associated with molecular mechanisms, interfering with post-treatment AP repair and leading to AP persistence [4, 16]. In this context, the identification of biomarkers involved in PAP pathophysiology that participate in inflammatory response processes and consequent tissue repair is paramount [16, 17].

Biomarkers are substances related to a normal biological process, pathological processes or biological responses to a therapeutic intervention [18, 19]. Immunohistochemistry (IHC) is used for identifying and localizing biomarkers with clinical relevance, as well as understanding the etiopathogenesis of some *lesions* [20 ,18]. There is a lack of studies evaluating the immuno-expression of biomarkers involved in PAP, and to this day, there has been no systematic reviews on the subject. Therefore, a scoping review was carried out to identify, compile, and analyze studies using immunohistochemical evaluations in order to identify biomarkers involved in the pathogenesis of PAP, and identify any gaps in scientific knowledge on this topic. The following research question was formulated: Which immunohistochemical markers are associated with the pathogenesis of persistent apical periodontitis in humans?

Methods

Research question

The addressed research question was "Which immunohistochemical markers are associated with the pathogenesis of persistent apical periodontitis in humans?"

Eligibility criteria

Persistent apical periodontitis lesions are classified as persistent even after endodontic retreatment, thus excluding primary and secondary AP [1, 4]. Only cross-sectional studies that analyzed markers associated with PAP pathogenesis through IHC expression analyses were included, and experimental studies performed on animals, review articles, *in vitro* studies, case reports, case series, editor letters and studies evaluating protein expressions by other techniques were excluded.

Information sources

Searches were performed up to January 2024 at the PubMed, Web of Science, Scielo, Scopus, Cochrane Library, Embase, and Science Direct databases. Grey literature was also searched in the Google Scholar database. No language or publication date restrictions were applied in searching the aforementioned electronic databases.

Search strategy

All literature searches were performed in the advanced search field. The following Boolean descriptors and operators were used to create the search:

("periapical tissue" OR "periapical lesion" OR apicoectomy OR "endodontic surgery" OR "periradicular surgery" OR "tooth extraction" OR retreatment OR "periapical curettage" OR "chronic periapical periodontitis" OR "periapical granuloma" OR "radicular cyst" OR "persistent periradicular lesions" OR "persistent infection" OR "acute apical periodontitis") AND ("molecular markers" OR "molecular biomarkers" OR "immunohistochemical markers" OR immunohistochemistry OR "IHC markers" OR IHC).

The design of the search strategy was supported by key inclusion criteria, categorized according to the population-concept-context framework recommended by the Joanna Briggs Institute (JBI) for scoping reviews [21], as a less restrictive alternative to the Population, Intervention, Comparator, and Outcome framework recommended for systematic reviews. The population, concept and context terms consisted of Population (PAP); Concept (the expression of proteins involved in the pathogenesis); Context (Immunohistochemistry).

Selection of evidence sources

According to the applied eligibility criteria, two researchers (WRS and KR), blinded to each other, and independently, assessed the titles and abstracts of the retrieved papers, and subsequently, the full text of each. In case of a disagreement, the opinion of a third reviewer (APVS) was requested. The Rayyan software was used, and a manual search was also performed. Duplicates were removed using the Rayyan software.

Data charting process and data items

Data extraction was performed jointly by two reviewers in order to determine the list of variables to be extracted. Data from eligible studies were organized in Microsoft Excel spreadsheets in the following categories: study characteristics, characteristics of the studied population, the antibody used, and synthesis of results.

Critical appraisal of individual evidence sources

The JBI Critical Appraisal Checklist for Analytical Cross-sectional Studies was used for quality assessment[22]. No cut-off points to determine good study quality were available in the abovementioned JBI critical appraisal checklist. The checklist is applied for reviewing the appropriateness of each assessment item against the quality assessment of other research, such as the JBI Critical Appraisal Checklist for Randomized Control/Pseudo-randomized Trial,

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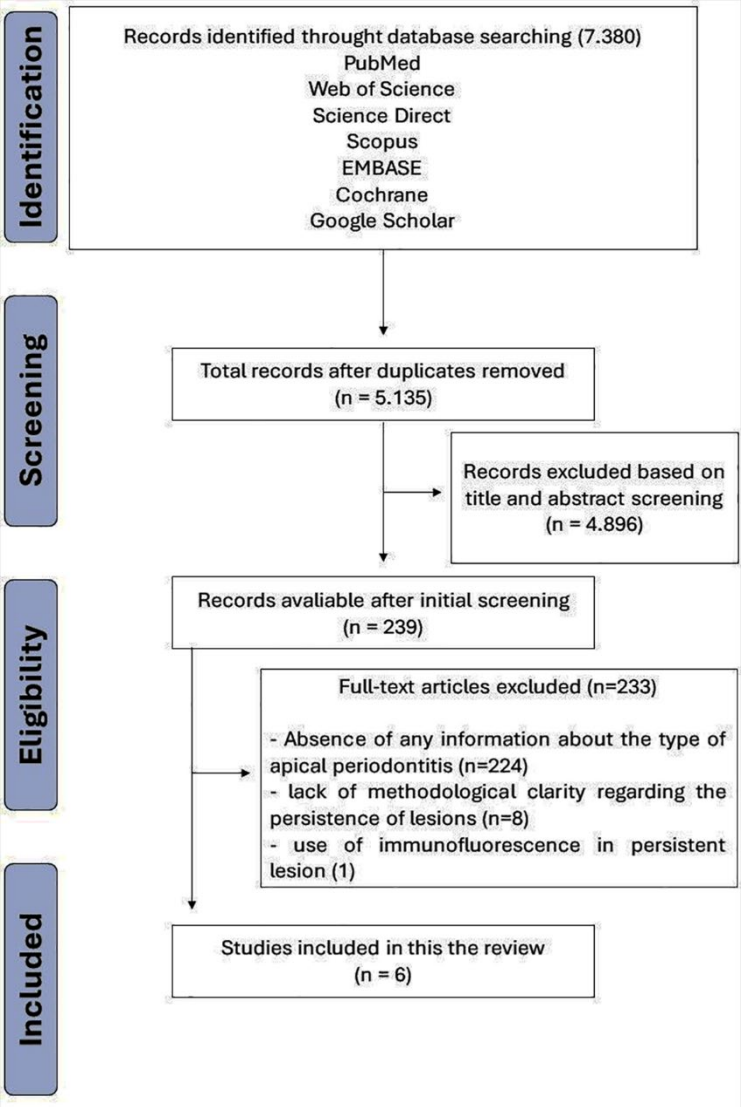

Figure 1. Flowchart article selection process

the JBI Critical Appraisal Checklist for Comparable Cohort/Case Control, or the JBI Critical Appraisal Checklist Assessment Checklist for Descriptive Series/Case Control [23, 24]. The same criterion was applied in the present study. The questionnaire contains eight questions answered with yes, no, or not clear. "Yes" scores of > 5, 3-4, and 0-2 were considered as high, moderate, and low methodological quality, respectively [25]. Two independent reviewers (WRS and KR) assessed whether each study met each of the eight checklist items, indicating "yes", "no", "unclear" or "not applicable". Disagreements were resolved by a third evaluator (APVS).

Synthesis of results

The results are presented and discussed according to the expression of immunohistochemistry biomarkers in PAP cases.

Results

Evidence source selection and characterization

The broad search carried out at different databases retrieved a total of 7,380 articles. Duplicates were removed using the Rayyan software. After reading the titles and abstracts, a total of 239 articles were considered potentially eligible, and their full texts were read by two reviewers (WRS and KR). Six articles that met all the inclusion criteria were selected for the final assessment [7, 12, 14-16, 26]. Among all selected studies, a total of 113 PAP cases were analyzed by immunohistochemistry. No studies were included following the manual search. The flowchart of the article selection process is depicted in Figure 1. All selected articles were in English.

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Evidence source characteristics

Article characteristics

Most articles were published in the last 10 years (*n*=3) [15, 16, 26], with the highest number of publications noted in 2017, with 2 articles [15, 26]. The country that published the most studies was Brazil (*n*=5)[7, 14-16, 26] (Table 1).

Study characteristics

Persistent apical periodontitis cases were diagnosed by clinical and imaging features. All included studies were cross-sectional. Two out of 6 articles employed IHC exclusively [14, 15]. Other approaches used alongside IHC assessments were *in situ* hybridization (*n*=1) [12], polymerase chain reaction (PCR); (quantitative reverse transcription polymerase chain reaction (qRT-PCR), RT-PCR; *n*=2) [7, 16] and histochemistry through gram's stain and radiographic examination (*n*=1) [26].

A critical appraisal within evidence sources

The present study employed the criteria established by the JBI Critical Appraisal Checklist for Analytical Cross-Sectional Studies to assess the quality of the studies included in this scoping review. All articles presented a low risk of bias [7, 12, 14-16, 26] (Table 2).

Clinicopathological characteristics

Only one study reported the number of evaluated patients [26]. Patient ages ranged from 15 to 55 years old, with most patients being female (*n*=40). Five articles reported no comorbidities in the evaluated patients [7, 12, 14, 16, 26]. The PAP treatment method was periapical surgery in five articles. Four articles histologically classified PAP cases as periapical granulomas (PG) (*n*=45) and radicular cysts (RC) (*n*=29) [12-16]. One article [5] classified PAP lesions as epithelized (*n*=19) and non-epithelialized (*n*=18), and another article did not specify the histopathological lesion type [26]. These findings are compiled in Table 1.

Results of the individual evidence sources

Six studies [7, 12, 14-16, 26] evaluated 12 immunohistochemistry PAP markers associated to some degree with the pathophysiology of this condition. The studied markers were classified according to their specificity as pro and anti-inflammatories cell markers, namely forkhead box P3 (FOXP3), CD3, CD8, CD450, CD68; markers associated with bone remodeling, namely TGF-α, TGF-β1, MMP-9, RANK-L and OPG; and markers with mesenchymal stem cell profile, namely CD-90, sex-determining region Y-box 2 (SOX2). The most employed markers were RANK-L (*n*=3), OPG (*n*=3), CD68 (*n*=3), and CD3 (*n*=2) (Table 3).

Pro- and anti-inflammatories cell markers

Among the seven retrieved studies, four evaluated inflammatory cell markers [10, 11 ,13, 22]; for example, observed higher FOXP3+expression in PAP samples compared to primary PA samples ($P<0.001$), and a reduced number of CD68+cells in PAP samples compared to primary PA lesions ($P<0.001$). Similar numbers of other lymphocyte populations (CD3+, CD45RO+, and CD8+cells) were observed in both PAP and primary PA lesions ($P>0.05$). No difference in inflammatory cells was, however, demonstrated when comparing PAP lesions morphologically classified as RC or PG ($P>0.05$). Similar data were reported by Menezes *et al.* [11] and Carneiro *et al.* [22] (Table 3).

Markers associated with bone remodeling

Only the study of Tyler *et al.* [9] reported the immunoexpression of TGF-α and TGF-β1 in RC and PG cases. All leukocytes expressed TGF-β1 in PG cases, while TGF-α was not expressed in eosinophils. The RC lining epithelium was immunostained for both proteins. These findings may suggest an association between the inflammatory response and remodeling of periapical tissues [9]. Only Carneiro *et al.* [7] evaluated the immunohistochemical expression of MMP-9. Cytoplasmic immunopositivity for MMP-9 was observed in all PAP cases, both epithelialized and non-epithelialized. Macrophage-like cells were predominantly immunoreactive for MMP-9, although some fibroblast-like cells, polymorphonuclear neutrophils and endothelial cells also exhibited immunoexpression. Carneiro *et al.* reported a predominance of MMP-9+/total cells in non-epithelialized lesions compared to epithelialized lesions ($P<0.0001$) [7]. Thus, both metalloproteinases were noted to be expressed in different types of inflammatory cells in PAP lesions and may be involved in extracellular matrix degradation." to: "These inflammatory mediators were expressed in PAP and may be involved in extracellular matrix degradation.

The immunoexpression of RANK-L and OPG was demonstrated by Carneiro *et al.* [7], Menezes *et al.* [14], and Estrela *et al.* [13], with immunopositivity noted predominantly in macrophage-like cells in both RC and PG. Epithelial RC cells demonstrated reactivity for RANK-L and OPG, with the number of RANK-L cells and the RANK-L/total cell ratio greater than OPG/total cells in both PG ($P<0.0012$) and RC ($P<0.0001$) [14]. However, fibroblast-like cells, polymorphonuclear neutrophils, and endothelial cells also exhibited Congo Red and gram's staining. These findings suggest that PAP demonstrates potential for bone resorption [14]. Estrela *et al.* [16] did not find a statistically significant difference between the immunoexpression of RANK-L and OPG between PAP and primary AP, or between cases of cyst and granuloma.

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Table 1. Summary of included studies

First author, year Country	Sample Age average (extremes) Sex (n)	Comorbities	Treatment	Histopathology (n)	Marker	Results	Conclusion
Tyler, 1999 USA	17 44 yrs; F(10) and M (7)	No evidence of systemic disease	Unclear	Periapical granuloma (9); Periradicular cyst (9)	TGF-alpha, TGF-beta1	No detected TGF-α in the granulomas. Detection of TGF- α protein in eosinophil surrounding the periapical cysts. Detection of TGF-β1 protein in eosinophils surrounding the periapical granuloma and cysts. Detection of TGF- β1 in lymphocytes, fibroblasts, and monocytes.	The presence of TGF-alpha and TGF-beta1, suggests a mechanism by which the host inflammatory response may participate in the repair and remodeling of periapical tissues.
Menezes, 2006 Brazil	20 26 yrs (15-55); N/E	Non-contributory medical history and not under medication	Periapical surgery	Periapical granuloma (10); Periapical cyst (10)	RANKL, OPG, CD68	RANKL and OPG stained macrophage-like cells in both lesions. CD68 staining support the nature of the macrophage-like cells stained for RANKL and OPG. Positive staining of fibroblast-like, PMN neutrophil, and endothelial-like cells in periapical granulomas and cysts. RANKL and OPG stained epithelial cells in periapical cysts.	The findings indicate the presence of RANKL and OPG in cysts and granulomas, strongly suggesting the involvement of these gene products in the development of periapical lesions.
Carneiro, 2009 Brazil	20 36.13 yrs (15-55); N/E	N/E	Periapical surgery	Without epithelium (10); Presenting epithelium (10)	MMP-9, CD68	Detection of immunopositive cytoplasmatic for MMP-9 in all the specimens. Macrophage-like cells were predominantly MMP-9 immunostained in both lesions. Stained of some fibroblast-like, PMN–like and endothelial-like cells in both types of AP lesions. Thin strands of epithelial cells stained with MMP-9. Macrophage-like cells could be an important source of MMP-9 in periapical lesions environment.	Several inflammatory cells, mainly CD68+cells, in the MMP-9 expression in apical periodontitis lesions. MMP-9 could be actively enrolled in the ECM degradation in apical periodontitis lesions
Estrela, 2016 Brazil	20 32 yrs; F(8) and M(12)	All patients were considered clinically healthy with no history of systemic diseases.	Periapical surgery	Periapical granuloma (10); Periapical cyst (10)	CD3, CD8, CD45RO, CD68, FoxP3, RANKL, OPG	Higher expression of FoxP3+ cells in PAP than in PPL. Reduction of +cells in PAP than PPL. Similar number of other lymphocyte populations in PAP and PPL. No differences in the RANKL, OPG, and immune-inflammatory cells.	PAP, as biologically active lesions had potential of bone resorption and are characterized by an immune-inflammatory cell profile; it suggests a suppressive/regulatory environment favorable to more chronic clinical behavior.
Carneiro, 2017 Brazil	20 (19-41); F (13) and M (7)	Non-contributory medical history	Periapical surgery	N/E	RANKL, OPG, CD3	No significant difference between the expression for RANKL, OPG and RANKL/OPG ratio in different groups.	Gram-negative bacteria may play an important role in OPG activity in the symptomatic group.
Estrela, 2017 Brazil	16; N/E N/E	All patients in this study were clinically healthy and had no history of systemic disease	N/E	Periapical cyst (10); Pepiapical granuloma (6)	CD90, Sox2	Expression of CD90 in 68.5% of PAP cases. CD90 staining was predominantly found in the vascular endothelial cells of PPL. Significantly higher expression of CD90 in PAP than PPLs. Expression of Sox2 in all cases of PAP and PPL. No correlation between Sox2 expression and histopathological diagnoses, inflammatory cell infiltrate intensity, or acute/chronic inflammatory cell infiltrate.	Mesenchymal stem cells may contribute to the immunosuppressive environment in PAP. Additionally, distinct stem cell sources may be associated with the chronic nature of PAP and the development of PPLs.

yrs=years; *M=male; F=female; IHC=Immunohistochemistry; N/E=Not especified; PAP=Persistent apical periodontits; PPL=Primary periapical lesion; ECM=Extracellular matrix; TGF- α=TGF-alpha; TGF- β1= TGF-beta1*

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Table 2. Analysis of the risk of bias of the articles included in the review according to JBI Critical Appraisal Checklist for Analytical Cross-Sectional Studies

Reference	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Total
Tyler *et al.* (1999)	Y	Y	Y	N	N	N	Y	Y	5
Menezes *et al.* (2006)	Y	Y	Y	Y	N	N	Y	Y	6
Carneiro *et al.* (2009)	Y	Y	Y	Y	N	N	Y	Y	6
Estrela *et al.* (2016)	Y	Y	Y	Y	N	N	Y	Y	6
Estrela *et al.* (2017)	Y	N	Y	Y	N	N	Y	Y	5
Carneiro *et al.* (2017)	N	Y	Y	Y	N	N	Y	Y	5

N, No; Y, Yes; Q1, Were the criteria for inclusion in the sample clearly defined?; Q2, Were the study subjects and the setting described in detail?; Q3, Was the exposure measured in a valid and reliable way?; Q4, Were objective, standard criteria used for measurement of the condition?; Q5, Were confounding factors identified; Q6, Were strategies to deal with confounding factors stated; Q7, Were the outcomes measured in a valid and reliable way; Q8, Was appropriate statistical analysis used?

Table 3. Immunohistochemical analysis used in the selected studies

First author, year	Marker	Immunohistochemical analysis
Tyler, 1999	TGF-alpha, TGF-beta1	Not especificied.
Menezes, 2006	RANKL, OPG, CD68	The number of positively stained cells for each antibody was counted per 10 consecutive microscopic fields (magnification: 100×) over totally counted cells. The epithelial cell area of periapical cysts was separated from the nonepithelial area regarding cell counting.
Carneiro, 2009	MMP-9	The number of cells positively stained for MMP-9 was counted in 10 consecutive microscopic fields (magnification: 100×) over total cells counted. The epithelial cell area was separated from the nonepithelial area, for the purpose of cell counting.
Estrela, 2016	CD3, CD8, CD45RO, FoxP3, CD68, RANKL, and OPG	The quantitative analysis was based on the number of positive cells for CD3, CD8, CD45RO, FoxP3, and CD68. Cells were counted (cells/ mm2) under a light microscope by using an integration graticule in 10 alternate high-power fields under 400× magnification. A total area of 0.3125 mm^2 was evaluated for each sample. Only microscopic fields with strong staining were selected for analysis. RANKL and OPG immunoexpression was evaluated according to the percentage of positive staining cells in relation to the whole area under examination. For RANKL and OPG analysis, staining was scored by the percentage of positive cells into 5 categories: no staining=0, 1%–24%=1, 25%–49%=2, 50%– 74%=3, and >75%=4.
Estrela, 2017	CD90, SOX2	Immunohistochemical scores were classified in 4 categories to estimate staining intensity (0, no staining; 1, weak; 2, moderate; and 3, strong) and 6 categories to estimate the proportion of positive cells (0, expression in <1%; 1, in 1%–5%; 2, in 6%–10%; 3, in 11%–25%; 4, in 26%–50%; and 5, in >50% of the cells). CD90 and Sox2 immunostaining was classified as positive in inflammatory cells and mesenchymal cells. Sox2 staining was classified as positive in odontogenic epithelium cells of epithelialized periapical granulomas and periapical cysts. CD90 and Sox2 final scores were calculated by multiplying the intensity values by the proportion values, which generated a score ranging from 0 to 12.
Carneiro, 2017	RANKL, OPG, CD3	For RANKL and OPG, the area fraction of positive cells was measured, and the percentage of RANKL and OPG immunopositive areas relative to the total area of the microscopic field was calculated. The RANKL/OPG ratio was also determined. For gram staining, the number of gram-negative cells per microscopic field was counted, as previously described (Ahmed *et al.*, 2013). The RANKL/OPG ratio was also determined.

Stem cell profile cell markers

Only one article employed stem cell markers (CD90 and SOX2) [15]. The cytoplasmic expression of CD90 in mesenchymal and endothelial cells was 68.5% in PAP cases, with higher expressions noted in samples displaying a predominance of chronic inflammatory cells (P<0.05). The expression of this marker was higher in PAP cases than in cases of primary AP (P<0.05). On the other hand, although SOX2 was expressed in all PAP cases, it was not significantly correlated to any variable.

Discussion

Few biomarkers have been analyzed by immunohistochemistry in PAP cases to assess their participation in the development of these lesions [7, 12-16, 26]. Some studies have employed the inflammatory cell markers CD68, CD3, CD8, CD45RO and FOXP3 to suggest association between the phenotypic profile of these cell populations with PAP cases [13, 14, 16, 26].

Some biomarkers are being studied by immunohistochemistry to assess their role in AP [27]. In clinicopathological practice, immunohistochemistry is relatively easy to apply and more accessible than other techniques [27]. The role of biomarkers through immunohistochemistry in the development of PAP in an animal model is studied [28]. However, these results cannot be extrapolated to humans, which is why only human studies were considered in the present study.

Systematic reviews are a type of synthesis of research conducted to identify and retrieve evidence that is relevant to a specific question or questions and to evaluate and synthesize the results of this research [29]. In some cases, a scoping review may be indicated,

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as it has more flexibility than the traditional systematic review and meta-analysis, and can include a diversity of literature and relevant studies using different methodologies. Despite the usefulness of systematic reviews, there are cases in which they are unable to meet the objectives or requirements necessary to be carried out [29]. Therefore, a scoping review is an appropriate alternative to a systematic review when the literature is vast and complex [30]. As a result, this synthesis was chosen to be of the scoping type, since the findings in the literature are scarce and not uniform; of the 6 studies, only 3 used the same biomarkers.

Several inflammatory mediators may be involved in PA, such as TGF-α and TGF-β1, as they can regulate inflammatory processes and carry out extracellular matrix replacement/remodeling and consequently, of periapical tissues [10, 12]. The expression of these proteins in leukocytes, except for TGF-α, which was not observed in eosinophils, may be associated with the bone healing process in PAP cases [12]. In addition, Menezes *et al.* [14] noted RANK-L and OPG immunoreactivity in cells similar to macrophages, confirmed by the immunoreactivity of CD68, suggesting the participation of these cells in bone remodeling processes.

Matrix metalloproteinases are also involved in bone matrix remodeling, responsible for extracellular matrix degradation and involved in inflammatory processes [13]. Furthermore, these metalloproteinases have also been associated with the bone resorption process, removing the collagen layer from the bone surface before demineralization [7, 13]. Carneiro *et al.* [7] observed cytoplasmic MMP-9 immunoexpression in all evaluated PAP cases, mainly in macrophage-like cells, suggesting the participation of this protein in apical extracellular matrix degradation.

Most of the retrieved articles morphologically classified PAP as RC or PG [12-16]. Persistent apical periodontitis comprises biologically active lesions that display the potential for bone resorption, and the inflammatory cells found in these lesions suggest the presence of a suppressive and regulatory environment favorable to chronic clinical conditions [15].

The bone resorption that occurs in PAs is triggered by the proliferation and differentiation of immature osteoclast precursor cells into mature osteoclasts that promote the degradation of organic and inorganic bone components [31]. Receptor activator of nuclear factor kappa beta ligand and OPG expressed in AP play an important role in the development of these lesions [31, 32]. The interaction between RANK and RANK-L is required for the activation and differentiation of osteoclasts, an event regulated by OPG which in turn, inhibits osteoclast differentiation by preventing RANK and RANK-L interactions [33]. Estrela *et al.* [16] demonstrated that the immunohistochemistry expression of RANK-L and OPG did not reveal statistically significant differences between PAP and primary AP. However, Menezes *et al.* [14] observed RANK-L immunolabelling in a greater number of cells in periapical cysts and granulomas than OPG in cases of PAP. These findings suggest that PAPs have bone resorption potential, although there was no statistically significant difference in the RANK-L/OPG ratio in both lesions.

Some immunohistochemical markers have been employed for the identification of mesenchymal stem cells, such as the surface marker CD90, the enzyme aldehyde dehydrogenase 1 and the transcription factors SOX2 and octamer-binding transcription factor 4 [34, 35]. Mesenchymal stem cells are multipotent progenitor cells capable of differentiating into mesenchymal and non-mesenchymal lineages [36]. These cells exhibit certain properties, such as proliferation, migration, differentiation, and immunomodulation. Mesenchymal stem cells have been detected in periodontal ligaments, inflamed dental pulps [17], and inflamed periapical tissue [17, 37]. In one study, Estrela *et al.* [15] evaluated the immunoexpression of both CD90 and SOX2 in PAP, reporting that CD90 expression was significantly higher in PAP cases than in primary lesions, while SOX2 did not present any statistically significant association, despite being more expressed in acute inflammatory cells. These findings suggest that stem cells may contribute to the local immunosuppression detected in PAP cases, contributing to its chronicity [15].

The present study analyzed the role of several biomarkers (FOXP3, CD3, CD8, CD450, CD68, TGFα, TGF-β1, MMP9, RANK-L, OPG, CD90 and SOX2) [7, 12, 14-16, 26] in PAP cases. The etiopathogenesis of the abovementioned lesions is, however, still poorly studied and to this day, no review on the subject is available. Thus, the identification of markers potentially associated with PAP development is paramount for a better understanding of the biological behavior of this condition and, therefore, the establishment of associations with its prognosis and/or treatment.

Despite the contribution of the present scoping review, certain limitations are noted. Only 4 studies [7, 12, 15, 16] state in their methodology that they are about PAP. Menezes *et al.* [14] state that cases of primary and secondary AP were excluded, and the teeth had satisfactory endodontic treatment, so we classify them as PAP. Carneiro *et al.* [26] only report that the teeth were endodontically retreated two years prior to the endodontic surgery; although they did not inform about excluding cases of primary and secondary AP, it was considered as PAP, since it was still necessary to complete endodontic periradicular surgery even after receiving endodontic treatment.

Although basic research articles were included in this scoping review, clinical information is important for a better

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clinicopathological understanding of the lesion studied [38, 39]. Despite this, only three studies report the symptomatology of the cases [14, 16, 26]. Only two studies report the tooth or region affected [12, 26]. Restorative treatment after endodontic therapy is also an important stage in the success of endodontic treatment since when it is compromised it can lead to failure through secondary infection [1]. None of the articles included a description of the quality of the coronal restorative treatment. Despite this, Menezes *et al.* [14] mention that cases of PAP were associated with endodontically well-treated teeth but did not describe which requirements were considered. Only Carneiro *et al.* [22] described the imaging aspects of PAP and none of the articles reported the time of evolution.

The pathogenesis of primary AP is different from PAP cases [1], we believe that it would be interesting to compare immunohistochemical expressions with primary AP lesions, as well as with normal tissue. Studies of Estrela *et al.* [15, 16] were the only studies comparing the immunohistochemical expression of persistent lesions with untreated primary lesions. Carneiro *et al.* [7] compared the immunoexpression of PAP cases with periodontal ligament samples. Tyler *et al.* [12] were not clear on their applied immunohistochemical analysis, making it difficult to carry out future studies. It is possible that many other proteins can be identified through IHC in cases of PAP. Only the following have been evaluated: FOXP3, CD3, CD8, CD450, CD68, TGF-α, TGF-β1, MMP-9, RANK-L, OPG, CD-90, and SOX2.

Conclusions

Twelve biomarkers were identified using IHC in human PAP. Few studies have evaluated protein expression through IHC in PAPs, which poses as a limitation; although they have contributed to better understanding the pathogenesis of the lesion, there are not enough studies to support the extrapolation of the results in terms of their clinical application. Despite this, two of the most studied biomarkers have been RANK-L and OPG, which are related to bone remodeling in AP, including PAP. Future well-reported longitudinal studies, with representative samples, using other biomarkers and other techniques may help to expand knowledge about the pathogenesis of PAP.

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Conflict of interest

None.

Author Contributions

Conceptualized the study and developed the protocol, research design, acquisition, analysis, data interpretation, scientific writing, and necessary corrections: SILVA WR, SOBRAL APV and ROMEIRO K. Obtaining, analyzing, and interpreting data: LIMA CRS, ISALTINO MC and TELLES CTV. Research design, analysis, data interpretation, writing and critical analysis: ALBUQUERQUE DS. All authors contributed to the study and approved the final manuscript.

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