

Participation of endotoxin in root canal infections: A systematic review and meta-analysis

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ABSTRACT

This systematic review and meta-analysis aimed to evaluate the relationship between endotoxin levels and presence of clinical signs/symptoms and radiographic features in patients with endodontic infection. Electronic searches were performed on Medline/PubMed, Embase, Cochrane Library, Scielo, Science Direct, Web of Knowledge and Scopus databases for identification of relevant studies published up to December 2016. Grey literature was searched in Google Scholar. The selected literature was reviewed independently by two authors. Clinical studies evaluating the levels of endotoxin and the presence of clinical and radiographic features were included in this review. In order to determine the relationship between endotoxin levels and presence of clinical signs/symptoms and radiographic features meta-analyses were performed. Among the 385 articles identified in the initial search, 30 were included for full-text appraisal and only eight studies met the inclusion criteria for this systematic review. Meta-analysis revealed that individuals having teeth with tenderness to percussion (TTP) ($P = 0.04$; $I^2 57\%$) and previous episode of pain (PEP) ($P = 0.001$; $I^2 81\%$) had higher levels of endotoxin than their counterparts. Size of radiographic lesion >2 mm ($P = 0.02$; $I^2 68\%$) and presence of root canal exudation (EX) ($P = 0.0007$; $I^2 0\%$) were associated with higher levels of endotoxin. This systematic review and meta-analyses provided a strong evidence that endotoxin are related with the presence of clinical signs/symptoms and radiographic features in patients with endodontic infection.

Key words: Endodontic treatment, endotoxins, meta-analysis, systematic review

INTRODUCTION

A great diversity of Gram-negative bacteria has been identified in root canal infection,^[1-5] especially species belonging to *Prevotella*, *Porphyromonas* and *Fusobacterium spp.*^[6,7] The cell wall of Gram-negative bacteria contains lipopolysaccharide (LPS), a complex molecule, which is generally referred to as endotoxin.^[8]

Clinical studies have detected endotoxins in infected root canals,^[1-3,9-18] and most of them have

suggested a correlation between endotoxin levels and development of clinical symptoms whereas others have not demonstrated this association.^[4,9,12,13,15,19-22]

Endotoxins are potent inflammatory agents, which activate classical and alternative pathways of complement system.^[23,24] Complement activation releases biologically active peptides, which mediate a number of aspects of the inflammatory process.^[23] LPS may evoke pain through activation of the Hageman

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factor or through neurotoxic properties when acting on presynaptic nerve terminals,^[24] direct sensitization of nociceptors,^[25] sensitization and up-regulation of the transient receptor potential cation channel, sub-family V, member 1 (TRPV1).^[26,27]

Endotoxins have been found to stimulate stimulation of bone resorption in tissue culture.^[28-31] Some clinical studies have demonstrated a positive correlation between higher levels of endotoxin in infected root canals and larger area of periapical bone destruction, whereas others have failed.^[4,18,20,32] A more complex network between different inflammatory mediators seems to be implicated in the development of clinical symptoms and radiographic features.^[32] The LPS released from infected root canal triggers the synthesis of IL-1 and TNF-alpha,^[30,33] which in turn up-regulates the production of MMP (matrix Metalloproteinase) by macrophages to promote periapical bone resorption.^[30] Furthermore, LPS stimulates bone resorption by enhancing RANKL (receptor activator of NF-κB ligand) through activation of toll-like receptor-2 in osteoblasts^[31] as well as inhibitory effect on osteoblast differentiation.^[29]

It is important to highlight that an overall analysis of data from studies correlating presence of endotoxins with clinical and radiographic features has not been performed yet. One of the main advantages of a systematic review with a statistical approach, such as meta-analysis, is to address sources of bias in order to produce the most valid and precise estimate of effect as possible.^[34] Therefore, the main purpose of this study was to conduct an extensive systematic review of the literature on endotoxin in endodontic infections in order to elucidate the relationship between endotoxin levels and presence of clinical signs/symptoms and radiographic features in patients with endodontic infection.

MATERIALS AND METHODS

Review question

Is there a relationship between endotoxin levels and presence of clinical/radiographic symptoms in patients with periapical periodontitis?

Inclusion and exclusion criteria

Studies were included based on the following inclusion criteria:

1. Clinical studies in humans
2. Endodontic infection must be present
3. Quantification of endotoxin and description of the test

4. Evaluation of clinical/radiographic symptoms
5. Publication in English.

Studies were eliminated if the inclusion criteria were not met or if they presented any of the following exclusion criteria:

1. *In vitro* or animal study, case report, review article, or opinion article
2. Lack of mean level of endotoxin and standard deviation, or data that allow their calculation
3. Lack of evaluation of the endotoxin levels
4. Lack of description of the type of endodontic infection
5. The publication was based on a population from another study
6. Study evaluating endotoxin in primary teeth
7. The same levels of endotoxins described by an author in more than one study
8. Lack of clear definition of the method used for endotoxin detection.

Search strategy

The literature was searched to identify published articles analysing the presence of endotoxin in endodontic infection. Electronic searches were performed on Medline/PubMed, Embase, Cochrane Library, Scielo, Science Direct, Web of Knowledge and Scopus databases and also in grey literature on Google Scholar from where we have analysed the first 100 articles for identification of relevant studies published up to December 2016. The search was conducted by using Medical Subject Heading (MeSH) terms and other free terms as follows: "Periapical periodontitis, OR periapical diseases, OR dental pulp diseases, OR apical periodontitis, OR endodontic infection AND endotoxin, OR LPS."

All references were tabulated by using the software EndNote X6. Duplicate references were excluded. Titles, abstracts and key-words were screened by two reviewers (DDR and LLF) based on inclusion and exclusion criteria. The cases of disagreement between authors were solved after discussion. After initial screening of titles and abstract, the full articles were evaluated by the same two reviewers. In addition to the electronic search, a hand search of the reference lists of the selected articles was performed. Pre-defined data collection worksheets were used for assessment of each selected publication.

Data extraction

Relevant data were extracted from the selected articles based on study description (i.e., setting, sample),

endotoxin detection method, type of endodontic infection (i.e., acute or chronic) and presence of clinical or radiographic features. Furthermore, in order to conduct the meta-analysis, the mean level and standard deviation of endotoxins were gathered.

The authors were contacted when further clarifications regarding the study methodology or results were required. Data were extracted by two reviewers (DDR and LLF) independently by using pre-piloted data extraction forms. In case of disagreement, discussions were held to resolve and reach consensus. All the stages of this systematic review were supervised by a third reviewer (GGN) who has expertise in systematic review methodology.

Quality assessment

The articles were systematically evaluated and the quality of the methodology was assessed. For each study, the following parameters recorded: authors' names, date of publication, study design, sample size and included subjects. The evidence level was determined according to guidelines provided by The Centre for Evidence-Based Medicine at Oxford.

Statistical analysis

Five different meta-analyses were conducted in order to evaluate the levels of endotoxin in the presence of each clinical symptom as follows: pain on palpation (POP), tenderness to percussion (TTP), previous episode of pain (PEP), exudation (EX) and size of radiographic lesion greater than 2 mm (SRL). For each model, the standardized mean difference was obtained with fixed- and random-effect models. If heterogeneity was present ($P < 0.05$; $I^2 \geq 50\%$), the random-effect model was employed.^[35] Median and range values were converted into mean and standard deviation according to Hozo *et al.*^[36] The analyses were conducted by using the software RevMan 5.3 (The Nordic Cochrane Center, Copenhagen, Denmark, 2014).

Methods used to quantify endotoxins

With regard to the methods used to quantify endotoxins, two studies used Gel Clot LAL assay,^[19,20] two used Endpoint Chromogenic LAL assay,^[9,13] four used Kinetic Turbidimetric LAL assay.^[1,2,11,12] All these LAL tests use the principle of a serine protease catalytic coagulation cascade activated by Factor C, the first component in the cascade which is a protease zymogen activated by endotoxin binding.^[37]

This pathway activates downstream a proclotting enzyme into a clotting enzyme (i.e., coagulogen

into coagulin).^[37] The Gel Clot LAL assay and Kinetic Turbidimetric LAL assay use coagulogen by monitoring its conversion into coagulin, which begins to form a gel clot, thus increasing the turbidity. The Endpoint Chromogenic LAL assays uses synthetic peptide-pNA substrate, which is cleaved by the clotting enzyme, imparting a yellow color to the solution. The strength of the yellow color (determined at an optical density [OD] = 405 nm) resulting from chromogenic LAL substrate and turbidity (determined at an OD = 340 nm) due to the conversion of coagulogen is correlated with the endotoxin concentration from the standard curve.

It is important to point out that the sensitivity of these tests depends on the time point (single or multiple time point) of the progress of the LAL reaction leading to the coagulogen conversion recorded. In the Endpoint Chromogenic LAL assay, OD is recorded at single time (at ≈ 16 minutes) (0.1-1 EU/mL). In Kinetic Turbidimetric LAL assay, OD is read at multiple time points because the reaction proceeds with no termination step (≈ 60 minutes), which allows the concentration of endotoxin to be quantified over a wider range sensitivity (0.01-100 EU/mL in the turbidimetric methods).

Assessment of clinical and radiographic features

Percussion is a diagnostic procedure used to assess the condition of a body part by means of tapping, with a painful response indicating periradicular inflammation.^[38]

Palpation is a diagnostic procedure used to assess the condition of periapical inflammation by performing a firm pressure with the pad of the finger or with a cotton swab buccal or lingual gingiva apical to a suspected tooth. A painful response may indicate periapical inflammation.^[38]

According to the AAE glossary,^[38] percussion is a diagnostic procedure used to assess the condition of a body part by means of tapping, with a painful response indicating periradicular inflammation. The tooth, which is sensitive to percussion, has a periapical diagnosis of symptomatic apical periodontitis.^[39,40]

In the diagnostic of the periapical periodontitis, marked visual signals may leave the clinician uncertain as to whether the structures have in fact normal morphology or pathological alterations.^[40] Strategies for the radiographic diagnosis of periapical pathosis take this doubt into account through calibration of the observers and plans for handling of borderline and

disagreement cases.^[40] Therefore, for this study we have agreed that a 2 mm radiolucency in periapical radiograph would be a parameter to determine the presence of a major radiographic lesion.

RESULTS

Electronic search revealed 385 articles. After removing 141 duplicate articles, 244 articles were included for title and abstract screening. Twenty-nine articles were included for full-text appraisal, including one article identified in the reference list. Twenty-one articles were excluded after full-text assessment. Subsequently, eight studies met the inclusion criteria in this systematic review. Figure 1 displays the flowchart of the study selection. The samples in the included studies totaled 231 individuals. Six studies were conducted in Brazil,^[1,2,9,11-13] one in Japan^[20] and one in the United States^[19] [Table 1]. With regard to the methods used to quantify endotoxins, two studies used Gel Clot LAL assay,^[19,20] two used Endpoint Chromogenic LAL assay,^[9,13] four used Kinetic Turbidimetric LAL assay^[1,2,11,12] [Table 1]. Meta-analysis revealed that individuals with tenderness to percussion (TTP) ($P = 0.04$; $I^2 57\%$) and previous episode of pain (PEP) ($P = 0.001$; $I^2 81\%$) presented higher levels of endotoxin than their counterparts [Figure 2]. No association between endotoxin concentration and pain on palpation (POP) was noted ($P = 0.87$; $I^2 45\%$). A higher level of endotoxins

was associated with the presence of exudation in infected root canals (EX) ($P = 0.0007$; $I^2 0\%$) and larger size of radiographic lesion (SRL >2 mm) ($P = 0.02$; $I^2 68\%$). Meta-analysis and graph for symptoms and radiographic features are shown in [Figure 2].

DISCUSSION

To the best of our knowledge, this is the first systematic review and meta-analysis evaluating the relationship between endotoxin levels and presence of clinical

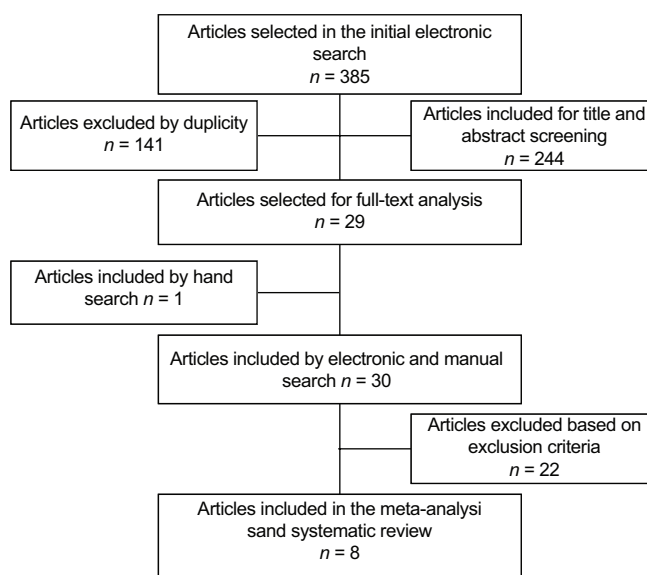


Figure 1: Flowchart of the articles screened in the review process

Table 1: Studies included in the systematic review. Study design, subjects and sample size, diagnosis of symptoms, method of endotoxin detection and evidence level

Authors	Year	Country	Study design	Subjects	Diagnosis of symptoms	Method of Detection	Evidence level according to Oxford Centre for Evidence Based on Medicine
Schein & Schilder	1975	USA	Cross-sectional	20 Patients with Primary Infection	SRL, PEP	Gel Clot LAL Assay	D
Horriba <i>et al.</i>	1991	Japan	Cross-sectional	30 Patients with Primary Infection	EX, SRL, PEP	Gel Clot LAL Assay	D
Jacinto <i>et al.</i>	2005	Brazil	Cross-sectional	50 Patients with Primary Infection	TTP, POP, PEP	Endpoint Chromogenic LAL Assay	D
Martinho & Gomes	2008	Brazil	Cross-sectional	24 Patients with Primary Infection	TTP, POP, PEP	Endpoint Chromogenic LAL Assay	D
Martinho <i>et al.</i>	2010a	Brazil	Cross-sectional	21 Patients with Primary Infection	TTP, POP, EX, SLR	Kinetic Turbidimetric LAL Assay	C
Martinho <i>et al.</i>	2011a	Brazil	Cross-sectional	21 Patients with Primary Infection	TTP, POP, EX	Kinetic Turbidimetric LAL Assay	C
Endo <i>et al.</i>	2012	Brazil	Cross-sectional	15 Patients with Primary Infection	TTP, POP, SRL	Kinetic Turbidimetric LAL Assay	C
Gomes <i>et al.</i>	2012	Brazil	Cross-sectional	30 Patients with Primary and Secondary Infection	TTP, POP, SRL	Kinetic Turbidimetric LAL Assay	C

TTP: Tenderness to percussion, POP: Pain on palpation, SRL: Size of radiographic lesion, EX: Exudation, PEP: Previous episode of pain

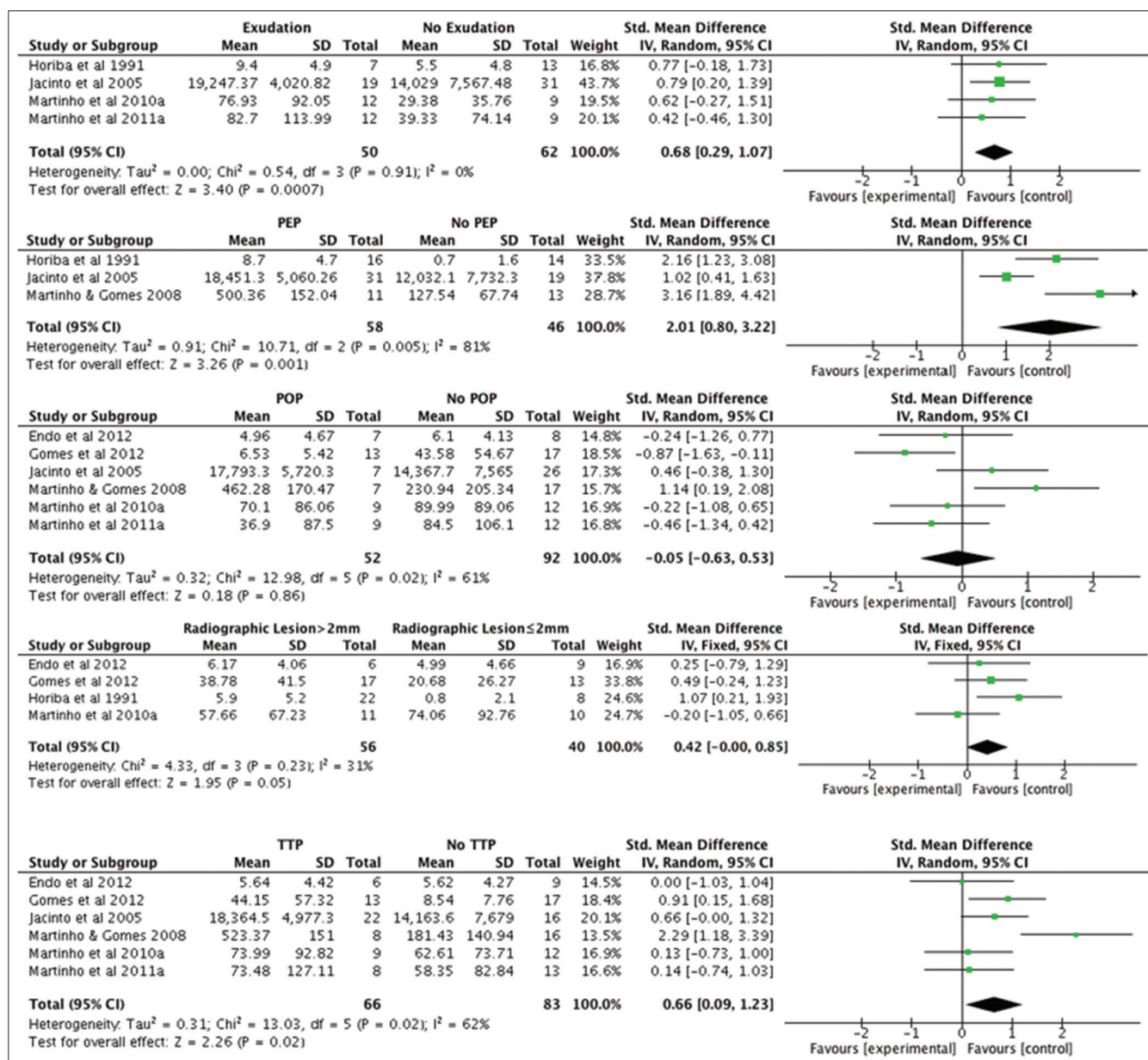


Figure 2: Forest plot with the evaluation of exudation, previous episode of pain, pain on palpation, size of radiographic lesion, and tenderness to percussion. Endotoxin concentration (mean and standard deviation values in EU/mL)

signs/symptoms and radiographic features in patients with endodontic infection. The data obtained in this study revealed that individuals having teeth with tenderness to percussion as well as previous episode of pain showed higher levels of endotoxin than their counterparts. Additionally, larger size of radiographic lesion and presence of root canal exudation were associated with higher levels of endotoxin.

The data obtained in this study revealed that individuals with tenderness to percussion showed higher contents of endotoxins than their counterparts. Also, a correlation was found between higher levels of endotoxins and patients with previous episode

of pain. These correlations are consistent with the hypothesis that LPS in clinical infections is related to the production of pain and mechanical allodynia. This hypothesis is strengthened by preclinical studies demonstrating that injection of LPS into rats produces nocifensive behavior and mechanical allodynia.^[41]

There are different mechanisms that can evoke pain due to bacterial LPS.^[25-27] It is well accepted that the innate immune response against bacterial contents leads to the release of different inflammatory mediators, including interleukin 1- beta (IL-1β), prostaglandin E2 (PGE2) and tumor necrosis factor alpha (TNF-α), which can sensitize nociceptors. Higher levels of PGE2 have been

related to teeth with clinical symptomatology.^[3,42,43] Higher levels of PGE2 were found in macrophage supernatants stimulated with bacterial contents from teeth showing tenderness to percussion.^[3] Thus, higher contents of IL-1 β have also been detected in teeth with clinical symptomatology.^[44,45] Martinho *et al.*^[1] not only indicated higher levels of endotoxins in teeth with tenderness to percussion, but also found higher levels of IL-1 β in macrophage supernatants stimulated with bacterial contents from teeth with this clinical symptomatology. Thereby, IL-1 is a potent stimulus for PGE-2 release.^[46]

Besides, increased sensitization and up-regulation of TRPV1 constitutes a potential mechanism by which TNF-alpha mediates inflammatory hyperalgesia and pain. It has been demonstrated that trigeminal neurons express both LPS receptors (TLR4 and CD14) leading to the hypothesis that bacterial byproducts might directly activate or sensitize trigeminal nociceptors. The sensitization and up-regulation of transient receptor potential cation channel, sub-family V, member 1 (TRPV1), has been addressed.^[26,27] Ferraz *et al.*,^[25] by using cultures of rat trigeminal neurons, demonstrated that pre-treatment with LPS produced a significant increase in the capsaicin-evoked release of calcitonin gene-related peptide (CGRP) compared to vehicle pre-treatment, thus showing sensitization of the capsaicin receptor TRPV1 by LPS. Additionally, the authors showed co-localization of the LPS receptor (toll-like receptor 4, TLR4) with CGRP-containing nerve fibers.

Diogenes *et al.*^[26] found that (i) LPS binds to receptors in trigeminal neurons by means of competitive binding, (ii) LPS evokes a concentration-dependent increase in the intracellular calcium accumulation (Ca(2+))(i) and inward currents, and (iii) LPS significantly sensitizes TRPV1 to capsaicin measured by (Ca(2+))(i) release of calcitonin gene-related peptide and inward currents. All these together imply that LPS is capable of directly activating trigeminal neurons and sensitizing TRPV1 via a TLR4-mediated mechanism.

Our results revealed that higher levels of endotoxins were associated with the presence of exudation in infected root canals. Exudate is defined as fluid, cells and plasma proteins which escaped from the vascular system and accumulates in a tissue or tissues, usually being the result of inflammation.^[38] The presence of exudation in root canal reflects an acute inflammation in periapical lesion.^[42]

Gram-negative bacterial LPSs are one of the mainly potent stimuli for macrophage cells in the release of PGE2.^[47,48] A positive correlation between number of Gram-negative bacterial species and levels of PGE2 macrophage secretion was revealed.^[3] PGE2 is both directly and indirectly implicated in most of the inflammatory and destructive changes occurring in apical lesions (e.g., vasodilatation), thus increasing vascular permeability and collagen degradation.^[49] Takayama *et al.*^[42] stated that the PGE2 concentration in periapical exudate could reflect the state of the disease activity in periapical periodontitis. Additionally, IL-1 β and TNF- α are also implicated in exudation.^[50-53] Therefore, a more complex network among these and other different cytokines seems to play a role in exudation.^[32]

Higher levels of endotoxin have been found in root canals with larger size of radiolucent (>2 mm). Previous studies demonstrated that the endotoxin content of teeth with radiolucent areas is five times as great as that of teeth without them.^[54,55] Such a correlation is not inconsistent with the hypothesis that LPS in clinical infections is related to bone destruction.

This hypothesis is strengthened by pre-clinical studies showing that injection of LPS into animal tissues induces periapical bone destruction.^[30,56,57] The LPS released from the infected root canal triggers the synthesis of IL-1 and TNF- α from macrophages, which in turn up-regulates the production of MMP-1^[30] and serves primarily for degrading non-mineralized extracellular matrix.^[58,59] Stimulation of osteoclastogenesis by MMP-1 and generating collagen degradation fragments on bone surfaces has also been proposed.^[60]

Studies have also demonstrated that MMP-1-expressing macrophages increase consistently as the lesion expands, which implies the involvement of MMP-1 in the production of macrophages in periapical destruction.^[61,62] These authors have also shown that LPS induces the expression of inducible nitric oxide synthesis (iNOS) and transforming growth factor- β 1 (TGF- β 1) genes.^[63,64] Furthermore, LPS stimulates bone resorption by enhancing the receptor activator of NF- κ B ligand (RANKL) through activation of toll-like receptor 2 in osteoblasts^[31] as well as by inhibiting osteoblast differentiation.^[29] *Porphyromonas endodontalis* LPS inducing RANKL by osteoblast^[65] and *Porphyromonas gingivalis* exacerbating ligature-induced RANKL-dependent alveolar bone resorption via differential regulation of toll-like receptor 2 (TLR2)

and TLR4^[66] are reported. It was demonstrated that the complex interplay between different cytokines plays a role in periapical bone destruction, including significant (-) correlations between IL-6 and IL-1 β as well as PGE2 and (+) correlation between TNF- α and PGE2.^[32]

It is important to highlight that not only the levels of endotoxin are implicated with the presence/development of symptoms and severity of bone destruction, but also the bacterial community involved in the infection, its interplay (synergism/antagonism),^[2,6,67] and consequently, the type of bacterial LPS and its lipid A structure.^[68,69] Among the Gram-negative bacteria, black-pigmented anaerobes, including *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Prevotella nigrescens* have been implicated as pathogens associated with the presence/development of symptoms and severity of bone destruction.^[2,6,7,67] Importantly, the LPS structure shows considerable heterogeneity among bacterial species,^[69-71] thus evoking different patterns of inflammatory response.^[33,50,69] It is worth to point out that the lipid A structure of the LPS molecule (i.e., hydrophobic component located in the outer leaflet of the outer membrane, responsible for the toxic effects of infections with Gram-negative bacteria)^[72] can suffer modulation from the environment, particularly, regarding hemin concentration^[70] and temperature.^[73] Hence, LPS molecule can also vary among different strains of single species, and consequently exhibit different inflammatory potentials.^[70,71,73]

Overall, this meta-analysis provides strong evidence that endotoxin are related with the presence of clinical signs/symptoms and radiographic features in patients with endodontic infection.

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Conflicts of interest

There are no conflicts of interest.

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