# Correlation between Volume of Apical Periodontitis Determined by Cone-beam Computed Tomography Analysis and Endotoxin Levels Found in Primary Root Canal Infection

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## Abstract

**Introduction:** This clinical study was conducted to correlate the levels of endotoxins and bacterial counts found in primary endodontic infection with the volume of periapical bone destruction determined by cone-beam computed tomography (CBCT) analysis. Moreover, the levels of bacteria and endotoxins were correlated with the development of clinical features. **Methods:** Twenty-four root canals with primary endodontic disease and apical periodontitis were selected. Clinical features such as pain on palpation, pain on percussion, and previous episode of pain were recorded. The volume (cubic millimeters) of periapical bone destruction was determined by CBCT analysis. Endotoxins and bacterial samplings were collected by using sterile/apyrogenic paper points. Endotoxins were quantified by using limulus amebocyte lysate assay (KQCL test), and bacterial count (colony-forming units [CFU]/mL) was determined by using anaerobic culture techniques. Data were analyzed by Pearson correlation and multiple logistic regression ($P < .05$). **Results:** Endotoxins and bacteria were detected in 100% of the root canal samples (24 of 24), with median values of 10.92 endotoxin units (EU)/mL (1.75–128 EU/mL) and $7.5 \times 10^5$ CFU/mL ($3.20 \times 10^5$–$8.16 \times 10^6$ CFU/mL), respectively. The median volume of bone destruction determined by CBCT analysis was 100 mm$^3$ (10–450 mm$^3$). The multiple regression analysis revealed a positive correlation between higher levels of endotoxins present in root canal infection and larger volume of bone destruction ($P < .05$). Moreover, higher levels of endotoxins were also correlated with the presence of previous pain ($P < .05$). **Conclusions:** Our findings revealed that the levels of endotoxins found in root canal infection are related to the volume of periapical bone destruction determined by CBCT analysis. Moreover, the levels of endotoxin are related to the presence of previous pain. *(J Endod 2015;41:1015–1019)*

## Key Words

Bacteria, CBCT, endotoxins, infection

Apical periodontitis is an inflammatory disorder established in periapical tissues as a result of root canal infection that culminates in periapical bone destruction (1). The gradual decrease in bone mineral density mediated by apical periodontitis appears on the radiograph as a radiolucent area around a root apex (2, 3).

Lipopolysaccharide (LPS), also known as endotoxin, an outer membrane component of gram-negative bacteria predominantly involved in root canal infection (4, 5), is an important inflammatory mediator in apical periodontitis (6–8). The LPS molecule has been shown to interact with local tissue cells via toll-like receptors (TLRs), both TLR-2 and TLR-4 (8), which in turn recognize the LPS molecule and activate multiple downstream signaling pathways (5, 9, 10). The binding of LPS to TLR-4 leads to the activation of p38 mitogen-activated protein kinase (an upstream effector common to many inflammatory cytokines), whereas the nuclear factor kappa B transcription factor (central to several immune and inflammatory responses) leads to the release of bone resorptive mediators that participate in bone destruction (5, 9–11).

Over the years, many clinical studies have attempted to investigate the presence of endotoxins in root canal infection with apical periodontitis (6–8, 12, 13) and to correlate its contents with the development of clinical features (7, 8, 14) and size of bone destruction (6–8, 11). Higher levels of bacteria and endotoxins (6, 11, 12) have been found in teeth with larger size of radiolucent area. However, all these previous studies determined the size of periapical radiolucency by using 2-dimensional (2D) periapical radiographs, which is quite insufficient, especially because of the lack of capacity of this 2D method in assessing the "depth" (buccolingual size) of a lesion (15–17).

With the introduction of the 3-dimensional (3D) reconstruction of an anatomic area at a relatively low radiation dose, it became possible to use cone-beam computed tomography (CBCT) (15, 17). As an advantage, CBCT imaging can distinguish which roots are involved in the lesion as well as their exact location and volume of bone destruction (15, 18). To our knowledge, however, there has been no clinical report

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assessing the volume of apical radiolucency determined by CBCT and correlating it with the levels of endotoxins and bacteria present in root canal infection.

Considering the limitations of 2D analysis used to determine periapical destruction in previous investigations and the advantage of volumetric analysis of bone destruction determined by CBCT image, this clinical study was conducted to correlate the levels of endotoxins and bacterial counts found in primary endodontic infection with the volume of periapical bone destruction determined by CBCT analysis. Moreover, the levels of bacteria and endotoxins were correlated with the development of clinical features.

## Materials and Methods

### Patient Selection

Twenty-four patients attending the São José dos Campos Dental School (UNESP), São José dos Campos (SP), Brazil, for primary endodontic treatment were included in the present study. The age of the patients ranged from 22 to 45 years. A detailed dental history was obtained from each patient. Those who had received antibiotic treatment during the past 3 months or who had any general disease were excluded. The Human Research Ethics Committee of the São José dos Campos Dental School (UNESP) approved the protocol describing the sample collection for this investigation, and all the volunteer patients signed an informed consent form.

For a greater standardization of the sample, all the single-rooted teeth were maxillary teeth with primary endodontic infection showing presence of 1 root canal and absence of periodontal pockets deeper than 4 mm. Teeth that could not be isolated with rubber dam were also excluded.

### CBCT Analysis: Volume of Periapical Bone Destruction (Cubic Millimeters)

The CBCT scans were made at the Department of Radiology of the São José dos Campos Dental School (UNESP). The occlusal plane of the patient was oriented in parallel to the axial scanning plane, according to the manufacturer's recommended protocol. All the preoperative scans were made by using the I-cat Next Generation (Imaging Science International, Hatfield, PA) with 8 cm field of view, 120 kVp, 36.15 mA, and 12 bit depth. The scanning parameters were kept similar for all patients, and the resulting data were exported with a 0.25-mm voxel size to obtain an identical spatial resolution for all of the images. All the scans were converted into digital imaging and communications in medicine format (DICOM) and then exported. The DICOM data of every scan were saved and then imported and evaluated with the NEMOTEC software (Nemotec, Madrid, Spain). Two independent and calibrated examiners (1 endodontist and 1 radiologist) assessed all the scans separately. The examiners scrolled through the entire reconstructed volume of every scan to assess the presence of periapical radiolucency associated with root apices, which were at least twice the width of the periodontal ligament (17, 19, 20). The volume of each periapical radiolucency was measured by both examiners following the same segmentation procedure in the NEMOTEC software before being saved in Excel file format (Excel Software, Henderson, NV). The segmentation procedure and the volumetric measurements were made by locating the slice in each of the 3 planes (ie, axial plane, anteroposterior and bucco-palatinal measures; sagittal plane, anteroposterior and superoinferior measures; and coronal plane, superoinferior and bucco-palatinal measures) in which these slices must be passed to the high-dimensional region of the periapical lesions (Fig. 1). After creating the measures at the 3 planes, it was determined a polygon covering the whole limit of the lesion (with security margin) to format the 3D reconstruction of the periapical radiolucency at a threshold of 350 Hounsfield units. The threshold for volume generation was chosen on the basis of a pilot study in which different Hounsfield unit options were tested, with the most optimal value being consensually set by radiologist and endodontist to better represent the lesion. A 2D segmentation was performed in all 3 planes to select the radiolucent area, and the limits of the lesion were checked to find whether they were within the polygon generated, with corrections being made whenever necessary. Next, a 3D reconstruction of the radiolucency was created by expanding the selected areas into the 3 slices 3-dimensionally. The borders of the selected volume were inspected in all slices and corrected when necessary. Finally, the "volume detect" option of the software was used to automatically calculate the selected volumes in cubic centimeters. Then the volume was converted into cubic millimeters (Fig. 1).

### Sampling Procedures

Files, instruments, and all materials used in this study were treated with Co60 gamma radiation (20 kGy for 6 hours) for sterilization and

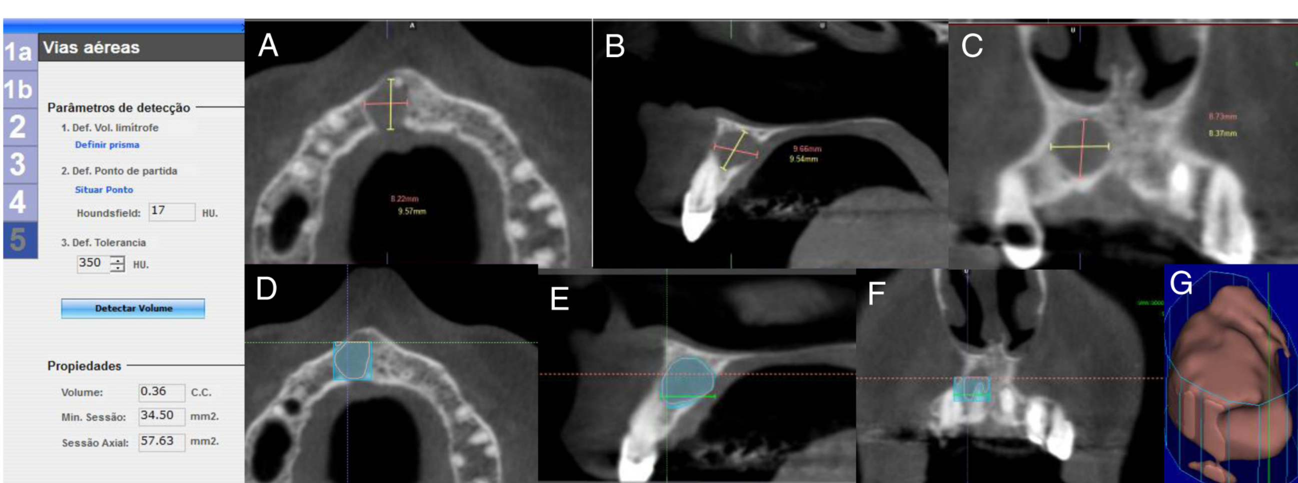


**Figure 1.** DICOM data of preoperative verification transferred to NEMOTEC software and 2D segmentation of apical periodontitis axial planes (*A*), sagittal planes (*B*), coronal planes (*C*), and 3D reconstruction in the axial planes (*D*), sagittal (*E*), and coronal (*F*), 3D reconstruction of apical periodontitis (*G*).