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ORIGINAL RESEARCH

Involvement of Root Canal Treatment in Pro-Inflammatory Processes – A Real-World Study

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Purpose: Endodontic inflammation typically results from root canal infections and sensitizations to filling materials after root canal treatment (RCT), potentially leading to systemic inflammation and disease. We therefore aimed to characterize the inflammatory alterations after RCT as well as the inflammatory molecule levels following tooth extraction or renewed RCT.

Patients and Methods: All (a total of 2585) walk-in patients with or without RCT history were included in this retrospective study. During the 3-year observation period, blood levels of RANTES/CCL5 (regulated on activation, normal T-cell expressed and secreted/chemotactic cytokine ligand 5), C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and interleukin-10 (IL-10) were measured before and after tooth extraction or renewed RCT. Control patients did not undergo any dental treatment.

Results: A total 49.38% of patients had a history of at least one RCT. In RCT patients, RANTES/CCL5 values were significantly reduced by both tooth extraction (p=0.03) and renewed dental RCT (p=0.038), while a non-significant increase was observed in untreated patients. TNF-α levels were reduced by tooth extraction (p=0.014) but not by renewed RCT and no intervention. CRP levels were not significantly changed by either treatment. Tooth extraction significantly lowered IFN-γ levels (p=0.003), while in control patients, IFN-γ levels did not change. IL-10 levels were non-significantly reduced by tooth extraction (p=0.061). In a subcohort of RCT patients, the lymphocyte transformation test revealed an allergic reaction to at least one of the root filling materials in 39.46% of patients, with raw gutta percha (56%) and eugenol (19%) being frequent triggers.

Conclusion: Here, we demonstrate the involvement of root-treated teeth in inflammatory processes, as tooth extraction and renewed RCT could significantly reduce individual cytokine levels. Our data support the use of biomarkers for in vivo monitoring of treatment success.

Keywords: endodontically treated teeth, root canal, inflammation, thioether/mercaptans, RANTES/CCL5, root canal filling materials

Introduction

Endodontic inflammation, in particular chronic apical periodontitis, typically results from root canal infections and sensitizations to root-filling materials. There is increasing evidence that the inflammatory response to infections is not exclusively restricted locally to the affected area but may contribute to a systemic response, potentially leading to increased systemic inflammation and disease.[1] On the other hand, there is emerging evidence that successful root canal treatment (RCT) has beneficial effects on systemic health by lowering the inflammatory burden.[2]

Recently, we demonstrated that both tooth extraction and RCT in patients with bone marrow defects of the jawbone significantly reduce serum levels of the proinflammatory cytokine RANTES/CCL5 (regulated on activation, normal T-cell expressed and secreted/chemotactic cytokine ligand 5).[3] RANTES/CCL5 levels were up to 30-fold increase in these patients, while other cytokines such as the C-reactive protein (CRP) and Tumor Necrosis Factor-α (TNF-α)

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remained stable.[4,5] These chronically elevated RANTES/CCL5 levels have been shown to initially develop from localized inflammation due to tissue damage by, for example, root resection and dental implantation.[6]

Another source of inflammatory triggers includes bacteria persisting in root-treated teeth and producing toxic compounds such as methyl mercaptans and thioether compounds.[7] These toxins can be indirectly assessed in blood samples by the analysis of both interferon-γ (IFN-γ) secreted by type 1 T-helper (Th1) cells and interleukin-10 (lL-10) produced by regulatory T-cells.[8–10]

In addition, several components present in root canal filling materials may potentially trigger sensitization and late-type allergy involving allergen-specific T-lymphocytes that might induce local symptoms and systemic inflammatory reactions. Allergic sensitization to root filling material has been shown to be involved in several clinical conditions.[11–15]

Although there is considerable evidence of the importance of root-treated teeth for the occurrence of the pro-inflammatory mechanisms mentioned before, little is known about the significance of corresponding diagnostic analyses in routine dental practice.

To further characterize the involvement of root-treated teeth in pro-inflammatory processes, we assessed RANTES/CCL5, CRP, TNF-α, IFN-γ, and IL-10 levels in 2585 patients undergoing routine dental care. Laboratory diagnostics were performed in patients with and without root-treated teeth as well as before and after interventional dental procedures on root-treated teeth such as tooth extraction and renewed RCT. In a subcohort of patients with root-treated teeth, potential allergic sensitization to root filling materials was evaluated.

Materials and Methods

Study Design

The study was conducted retrospectively, analyzing data collected between 16.11.2020 and 31.12.2023 in a functional medicine practice in Luxembourg. All patients who visited the practice during this period were eligible to participate if they completed a dental history interview and underwent an examination of their oral cavity (no exclusion criteria). During the initial anamnesis, the number of RCT was assessed on the basis of the patient's panoramic X-ray, and participating patients were divided into study groups with and without RCT. Laboratory diagnostics were performed as appropriate (see below).

Within the study group with RCT, the intervention group was defined as patients who underwent tooth extractions of root-treated teeth or renewed RCT during the study period and for whom pre-and post-treatment laboratory data were available (inclusion criteria). Post-treatment values were obtained at least 1 month after the intervention. The control group was defined as patients who did not undergo any dental intervention during between the two collections of laboratory data.

Ethics Approval

No patient was treated for the purpose of this study, and all patients participating gave their informed consent. Study approval was provided by the responsible ethics committee (Luxembourg National Research Ethics Committee, CNER). The study complies with the standards of the Declaration of Helsinki.

Procedures

Dental Procedures

Interventional dental procedures were carried out by the dentists responsible as part of routine treatment and in accordance with the current standard procedures and complied with the applicable quality standards.[16,17]

Laboratory Techniques

Peripheral blood was drawn pre- and post-treatment (interventional group) or any time during the study period (control group), with at least one month between the two assessments. Serum levels of RANTES/CCL5, CRP, TNF-α were obtained by standard laboratory techniques. Additionally, a typing of effector cells regarding mercaptans and thioethers was realized. Positive cytokine results (IFN-γ, TH1-response and IL-10, T_{reg}-response) are reliably indicating a sensitization to mercaptan and thioether compounds.[7,8]

Lymphocyte Transformation Test

The lymphocyte transformation test has been described elsewhere.[18] In brief, 10 mL of heparinized venous blood from each patient of a study subcohort was processed by Ficoll density gradient centrifugation to obtain peripheral blood mononuclear cells (PBMC). PBMC were then incubated with root canal filling materials (raw gutta-percha, colophonium, Peru balsam, eugenol, polydimethylsiloxane (PBMS), bisphenol A, epichlorohydrin, bismuth oxide, silver, paraformaldehyde, triethanolamine, rosin, turpentine oil, silicone oil, peanut oil) for 6 days at 37 °C and 5% CO_2 atmosphere. Stimulations were performed in triplicates. PBMC were then labeled by the addition of 3H-thymidine for 12 hours. After cell harvesting, incorporated 3H-thymidine was measured (counts per minute, cpm), and the results of each stimulation were calculated as the cpm-ratio of stimulated PBMC (mean of triplicates) and unstimulated control PBMC.

Statistics

Most of the statistics were descriptive (mean ± standard deviation). The two-sample *t*-test was used to determine significant differences between independent variables. In addition, nonparametric Wilcoxon signed-rank tests were applied to assess differences between pre- and post-treatment values. The software "R" (version 4.3.3) was used for statistical calculations.

Results

Patient Flow and Patient Characterization

The anonymized database comprised eligible 2585 subjects (1818 female, 1067 male) with an average age of 45.74 ± 16.15 years. Numbers of RCT were evaluated in 2432 patients. A total of 49.38% of patients (n = 1201) had at least one RCT (mean RCT per patient = 1.35; min = 1, max = 18). Most patients had received between one and four RCT (Figure 1). In the intervention group, an average of 3.26 root-canal-treated teeth were extracted during the study period (min = 1, max = 8).

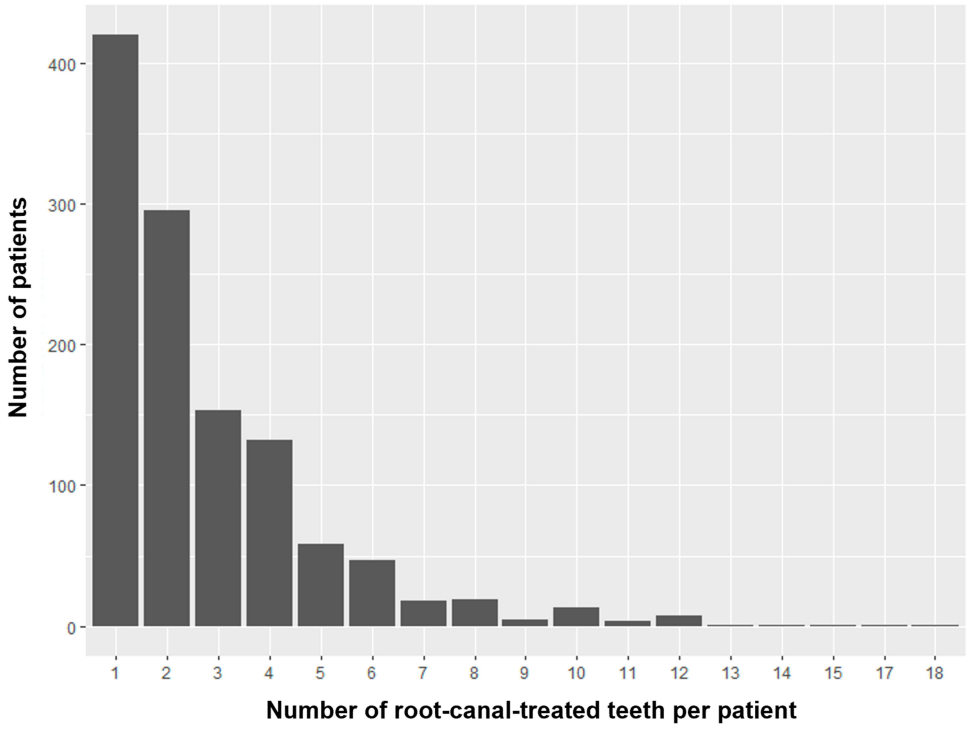

Figure 1 Root-canal treated teeth per patient.

Effects of Endodontic Treatments on RANTES/CCL5, TNF-α, and CRP Levels

In patients with RCT, RANTES/CCL5 serum values were significantly reduced by both tooth extraction (59.726 ± 40.472 ng/mL – 38.021 ± 23.221 ng/mL; p = 0.03; n = 39) and renewed dental RCT (57.856 ± 37.008 ng/mL – 34.683 ± 24.043 ng/mL; p = 0.038; n = 12). In the control group (n = 49), RANTES/CCL5 values increased from 41.222 ± 19.723 ng/mL to 51.696 ± 4.463 ng/mL; p > 0.05; n = 49; Figure 2).

For TNF-α, only tooth extraction caused a significant reduction (26.079 ± 50.594 pg/mL – 8.238 ± 7.036 pg/mL; p = 0.014; n = 13), whereas renewed dental RCT (n = 6, data not shown) and no intervention (n = 28) had no significant effect (p > 0.05; Figure 3). In contrast, CRP levels were not significantly changed by either tooth extraction or renewed dental RCT (data not shown).

Effects of Endodontic Treatments on IFN-γ and IL-10 Levels

Before endodontic interventions, IFN-γ and IL-10 levels of patients with RCT largely corresponded to those of patients without RCT and did not significantly differ (IFN-γ, p = 0.820; IL-10, p = 0.571). Out of 527 patients with RCT, 19.17% (n = 101) and 31.87% (n = 168) had increased stimulated IFN-γ and IL-10 levels, respectively. In 9.11% of patients (n = 48) both values were increased. Out of 228 patients without RCT, 19.29% (n = 44) and 29.82% (n = 68) had increased stimulated IFN-γ and IL-10 levels, respectively, and in 9.21% (n = 21), both IFN-γ and IL-10 levels were increased (Figure 4).

In patients with RCT (n = 25), tooth extraction significantly lowered IFN-γ levels from 1. 872 ± 3.309 to 0.072 ± 0.321 pg/mL (p = 0.003), while in untreated control patients, IFN-γ levels did not significantly change during the observation period (0.088 ± 0.164 pg/mL – 0.575 ± 1.74 pg/mL; p > 0.05). In the extraction group, IFN-γ levels even dropped to zero in 23 out 25 patients (Figure 5). Likewise, tooth extraction reduced IL-10 levels from 75.264 ± 103.546 to 27.236 ± 54.653 pg/mL but this difference was not significant (p = 0.061). In the control group, IL-10 levels non-significantly decreased from 97.438 ± 82.614 pg/mL to 18.075 ± 38.775 pg/mL (p > 0.05; Figure 6).

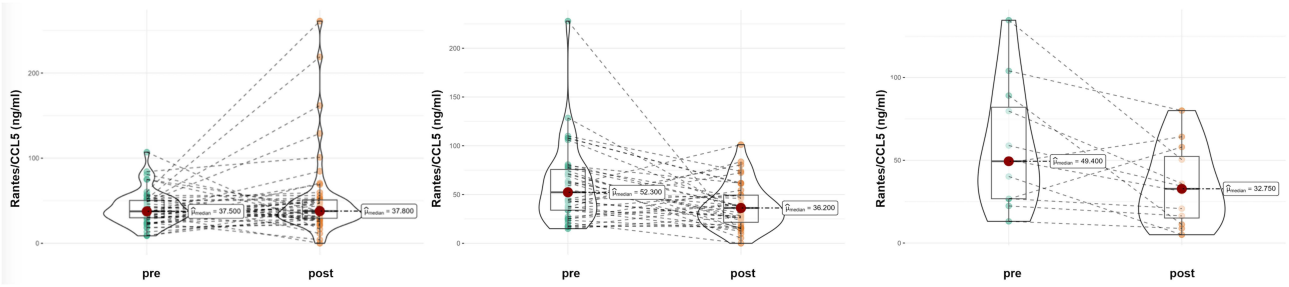

Figure 2 Evolution of RANTES/CCL5 without intervention (left), after tooth extraction (middle) and after root canal treatment renewal (right).

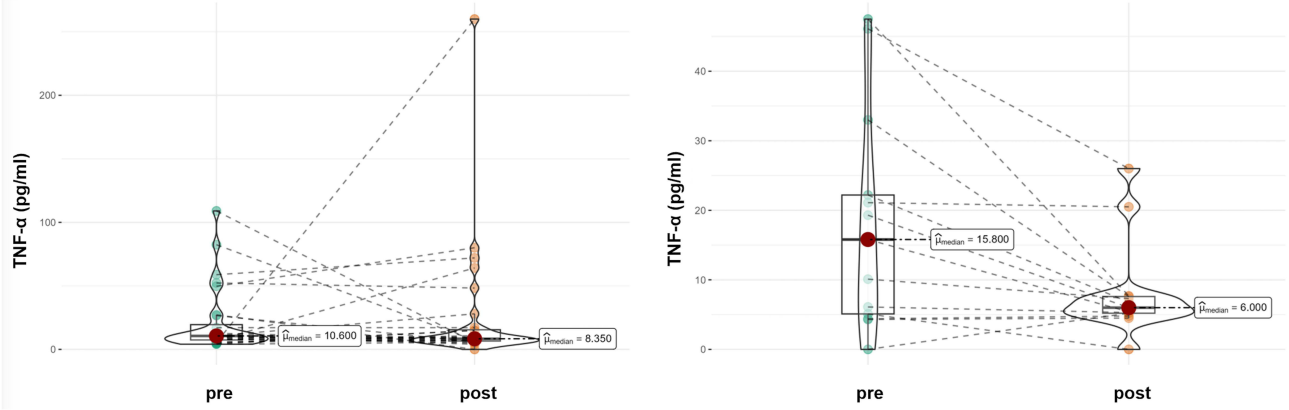

Figure 3 Evolution of TNF-α without intervention (left) and after tooth extraction (right).

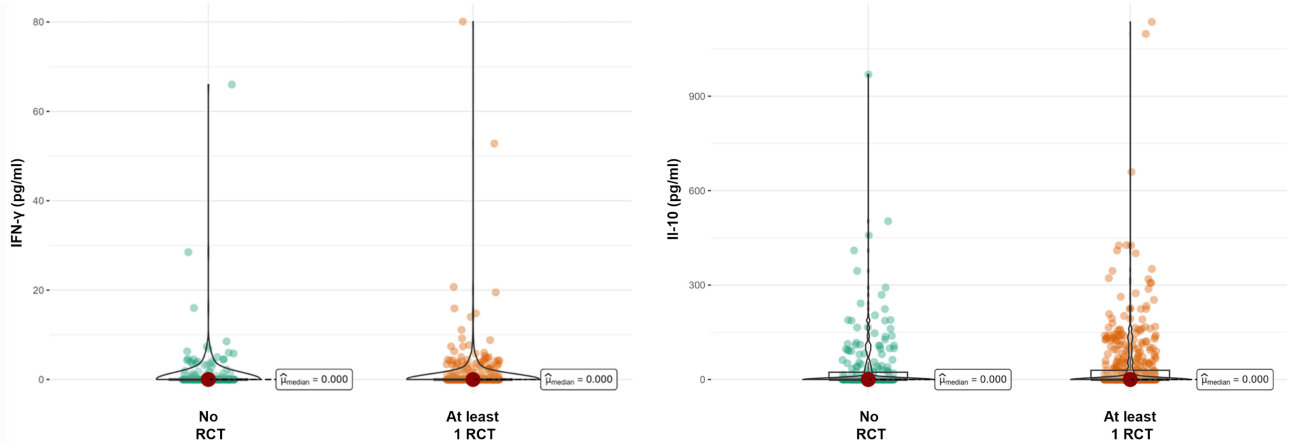

Figure 4 IFN-γ and IL-10 in patients without (left) and with (right) root canal treatment.

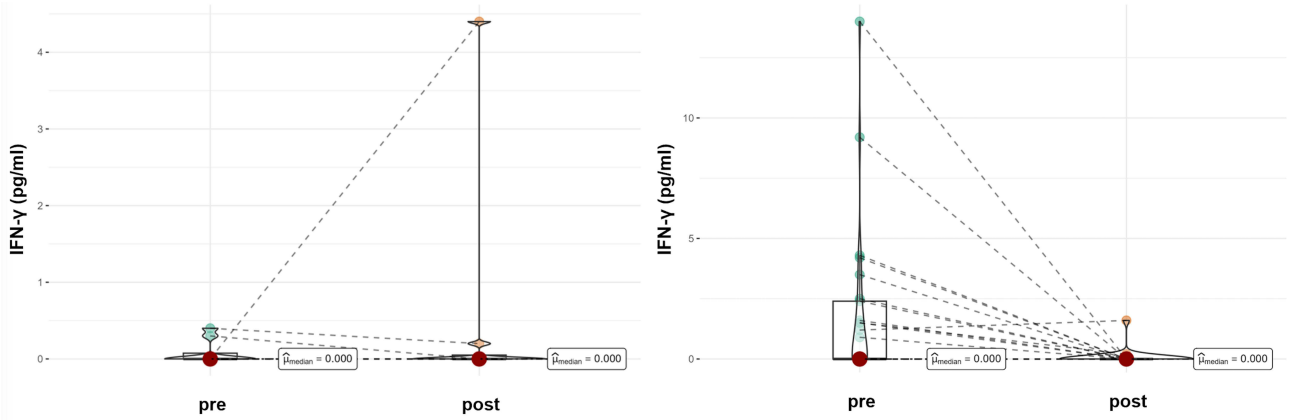

Figure 5 Evolution of IFN-γ without intervention and after tooth extraction.

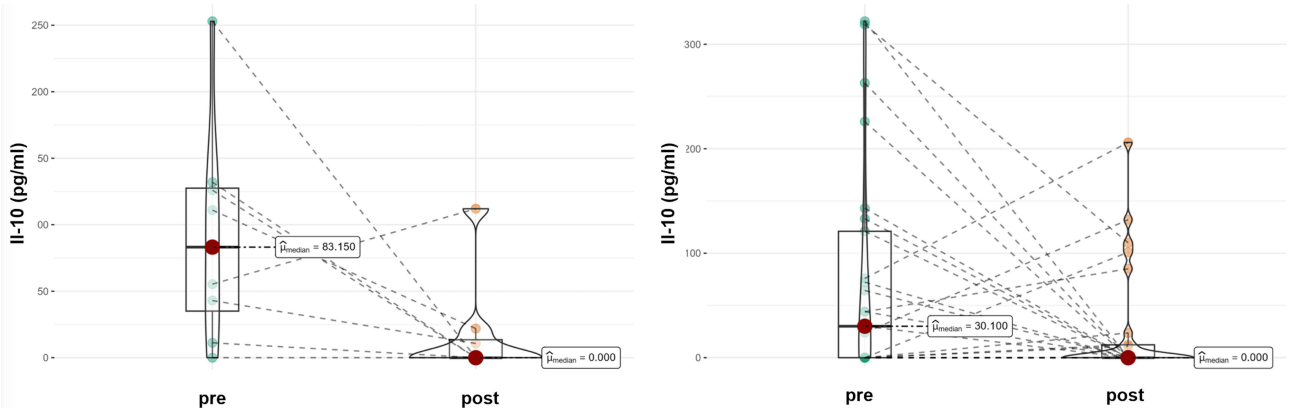

Figure 6 Evolution of IL-10 without intervention and after tooth extraction.

Lymphocyte Transformation Test

In a subcohort of RCT patients (n = 147), the lymphocyte transformation test was performed to assess a possible allergic sensitization to root filling materials. A total of 39.46% (n = 58) had a positive reaction to at least one of the materials (two patients responded to two materials), with raw gutta percha (56%) and eugenol (19%) being the most frequent triggers (Figure 7). These results did not correlate with the inflammation parameters.

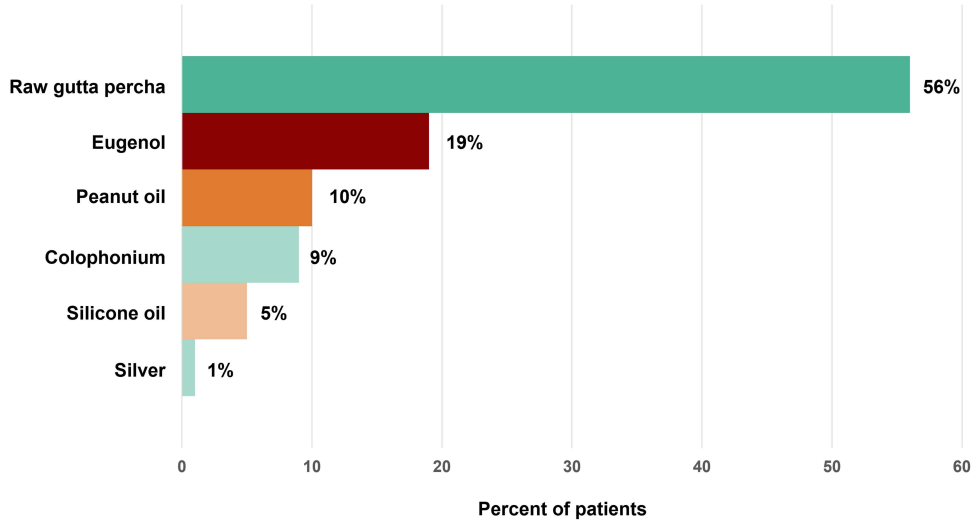

Figure 7 Distribution of sensitization to root canal filling materials.

Discussion

In this article, we showed the involvement of root-treated teeth in inflammatory processes, as extraction of these teeth or renewal of the root filling could significantly reduce the levels of individual cytokine markers. While levels of RANTES/CCL5 were significantly suppressed by both treatments, TNF-α levels were only reduced by tooth extraction, whereas CRP levels were not significantly altered by any treatment. Likewise, tooth extractions significantly decreased pro-inflammatory IFN-γ levels but not anti-inflammatory IL-10 levels. As root canal filling material renewal was considered as an option for some patients, we investigated the allergic potential of individual filling materials. We found that allergic sensitization to root filling materials is common among RCT patients, with raw gutta percha and eugenol being frequent triggers of allergen-specific T-cell response.

Cytokines such as RANTES/CCL5 have been identified at sites of chronic inflammation such as periodontitis[19] and bone marrow defects of the jawbone.[20] While other publications reported increased RANTES/CCL5 in tissue samples and in the gingival crevicular fluid,[19,20] we assessed RANTES/CCL5 levels in the blood of patients, supporting the link between dental and systemic health.[21] The elevated IFN-γ levels are indicative of an active type 1 T-helper cell response, whereas the anti-inflammatory IL-10 expression is predominantly based on regulatory T-cells.[9,10] This IFN-γ/IL-10 response has been attributed to bacteria in root-treated teeth, initiating tissue degradation by producing toxic hydrogen sulfide compounds such as methyl mercaptans and thioether compounds such as dimethyl sulfides and diethyl sulfides,[7] as these sulfide compounds can be detected in the gingival fluid of periodontal pockets.[22] Furthermore, toxic sulfur compounds produced by periodontal bacteria have been shown to potentially contribute to the development of mitochondriopathies.[23]

Elevated levels of pro-inflammatory molecules after oral surgical procedures may induce a systemic inflammatory response, resulting in a transiently increased cardiovascular risk and endothelial dysfunction.[24–26] Nevertheless, endodontic treatment has been demonstrated to provide long-term benefits on the levels of inflammatory mediators and endothelial dysfunction, as an improvement of endothelial dysfunction has been shown to be associated with reduced inflammatory mediators following primary RCT.[25] The substantial decrease in inflammation after RCT observed by others[27] is in line with our current study as well as previous results.[3]

We recognized the following limitations of our study. The small sample size of interventional groups of patients along with a high variability of individual cytokine levels limited the generalizability and significance of some results. Due to their systemic relevance, elevated cytokines cannot always be clearly attributed to root canal infections alone, but might be a reaction to other underlying conditions, such as inflammation processes or infections. Nevertheless, the results on the reduction of certain cytokine levels by tooth extraction and renewed dental RCT were significant and consistent with published data. Although the impact on clinical symptoms has not been illustrated in this study, clinical improvement was frequently observed in the intervention group.

Conclusion

In summary, the objective of our work is to contribute to the in-depth characterization of the inflammatory response following root canal treatments and the subsequent course of inflammatory molecule levels following appropriate treatment. This characterization might facilitate the identification of appropriate biomarkers for an in vivo monitoring of treatment success in daily routine. Furthermore, our findings confirmed the critical role of root filling material in influencing the immunological response after RCT.

Abbreviations

Cpm, counts per minute; CRP, C-reactive protein; IFN-γ, interferon-γ; IL-10, interleukin-10; PBMC, peripheral blood mononuclear cells; RANTES, regulated on expression, T-cell expressed and secreted, CCL5, chemotactic cytokine ligand 5; RCT, root canal treatment; Th1 cells, type 1 T-helper cells; TNF-α, tumor necrosis factor-α.

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Author Contributions

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

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Disclosure

Dr Kurt Müller reports personal fees from Dresden International University, personal fees from Institut für Funktionelle Medizin, personal fees from Biovis Laboratory Limburg, outside the submitted work. The authors report no other conflicts of interest in this work.

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