

# A systematic review of methods used to sample and analyse periradicular tissue fluid during root canal treatment

Virdee, S S; Butt, K; Grant, M; Camilleri, J; Cooper, P R; Tomson, P L

DOI:  
[10.1111/iej.13104](https://doi.org/10.1111/iej.13104)

License:  
Other (please specify with Rights Statement)

Document Version  
Peer reviewed version

Citation for published version (Harvard):  
Virdee, SS, Butt, K, Grant, M, Camilleri, J, Cooper, PR & Tomson, PL 2019, 'A systematic review of methods used to sample and analyse periradicular tissue fluid during root canal treatment', *International Endodontic Journal*, vol. 52, no. 8, pp. 1108-1127. <https://doi.org/10.1111/iej.13104>

[Link to publication on Research at Birmingham portal](#)

**Publisher Rights Statement:**  
Checked for eligibility: 04/03/2019

This is the peer reviewed version of the following article: Virdee, S. S., Butt, K., Grant, M., Camilleri, J., Cooper, P. R. and Tomson, P. L. (2019), A systematic review of methods used to sample and analyse periradicular tissue fluid during root canal treatment. *Int Endod J.*, which has been published in final form at: <https://doi.org/10.1111/iej.13104>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.

## General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

## Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

**A systematic review of methods used to sample and analyse  
periradicular tissue fluid during root canal treatment**

Journal:	<i>International Endodontic Journal</i>
Manuscript ID	IEJ-18-00770.R3
Manuscript Type:	Review
Keywords:	Endodontics, Periradicular Tissue Fluid, Inflammation, Cytokine, Sampling, Systematic Review, Apical Periodontitis

SCHOLARONE™  
Manuscripts

1  
2  
3 **A systematic review of methods used to sample and analyse periradicular tissue fluid during**  
4  
5 **root canal treatment**  
6  
7

8  
9 SS Virdee, K Butt, M Grant, J Camilleri, PR Cooper, PL Tomson  
10

11  
12 Department of Restorative Dentistry, School of Dentistry & Birmingham Dental Hospital, University of  
13  
14 Birmingham, Birmingham, United Kingdom  
15  
16

17  
18 **Running Title:** Periradicular Tissue Fluid  
19

20  
21  
22 **Key Words:** Endodontics; periradicular tissue fluid; cytokine; inflammation; sampling; systematic  
23  
24 review  
25  
26

27  
28 **Corresponding Author**  
29

30 Satnam Singh Virdee  
31

32 244 Yardley Wood Road, Moseley, Birmingham, B13 9JN  
33

34 Email: s.s.virdee.1@bham.ac.uk / satnamsinghvirdee@gmail.com  
35

36 Tel: +447852363615  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## Abstract

**Aim** The primary aim was to identify techniques used to sample and analyse Periradicular Tissue Fluid (PTF) in permanent teeth diagnosed with apical disease during root canal treatment. Secondly, to identify the types of inflammatory mediators studied using this approach.

## Methodology

**DATA SOURCES:** PubMed, EMBASE, Cochrane Library, Science Direct, Web of Science and OpenGrey

**ELIGIBILITY CRITERIA:** Clinical studies published until 1<sup>st</sup> June 2018 which utilised orthograde techniques to sample and analyse PTF were included. Cell culture, laboratory or animal studies and those concerned with investigating inflammatory mediator activity from within healthy or diseased pulp tissue, and not periradicular tissues, were excluded

**STUDY APPRASIAL & METHODS:** In accordance with PRISMA guidelines, data was extracted on study characteristics, target mediators, sampling and assay techniques and the parameters associated with the PTF sampling and eluting protocol. A qualitative synthesis was conducted and studies were critically appraised using a modified version of the Cochrane risk of bias tool.

## Results

**STUDY CHARACTERISTICS:** From 251 studies, 33 were eligible for inclusion. Sampling techniques included the use of paper points (n=27), fine needle aspiration (n=4) and filter strips (n=2). Assay techniques included Enzyme-linked-immunosorbant-assay (n=18), quantitative polymerase chain reaction (n=9), radioimmunoassay (n=4), colorimetric-assay (n=2), immunofluorometric-assay (n=1) and cytometric-bead-array (n=1). Forty-five different inflammatory mediators were targeted at the proteomic/metabolomic (n=25) or transcriptomic level (n=9).

**LIMITATIONS:** Significant heterogeneity exists within the methodology and only 5 studies disclosed unambiguous information about their PTF sampling and eluting protocols.

**Conclusions** Paper points and proteomic/metabolomic analysis are currently the preferred methods for studying and analysing PTF during NSRCT. The most studied analytes were IL-1 $\beta$  and TNF- $\alpha$ .

**IMPLICATIONS:** Further research is required to develop an optimised PTF sampling and eluting protocol to overcome methodological heterogeneity and future studies are advised to follow a standardised approach to reporting data.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Peer Review

## Introduction

Apical periodontitis is an inflammatory reaction of the periradicular tissues induced predominantly by complex interactions between the host's immune system and pathogenic bacterial communities of endodontic origin (Nair 1997). Although this process is initiated by invading microorganisms and their by-products, the destructive effects are the result of a localised inflammatory response (Yamasaki *et al.* 1994, Stashenko *et al.* 1995). This reaction consists of various leukocytes (i.e. macrophages and lymphocytes) which in turn produce a myriad of soluble inflammatory mediators (Graunaite *et al.* 2011). These host-derived auto- and para-crine signalling molecules coordinate overlapping destructive and regenerative inflammatory processes (i.e. apical bone resorption and deposition respectively) to facilitate the formation of a granuloma (Márton & Kiss 2014). This organised collection of leukocytes is a defensive response which acts to restrain endodontic pathogens inside the infected root canal (Metzger 2000, Márton & Kiss 2014). Additionally, its vascular nature results in production of an inflammatory exudate, which becomes enriched with key components of the immune response as the disease progresses (Nair 2004). In the literature this fluid is commonly referred to as the Periradicular Tissue Fluid (PTF) or periapical exudate.

The concentrations of known inflammatory mediators found within periradicular lesions have been linked to specific states of disease activity (Kawashima & Stashenko 1999). For example, Interleukin [IL]1- $\alpha$ , IL-1 $\beta$ , IL-2, Prostaglandin [PGE]-2, Tumour Necrosis Factor [TNF]- $\alpha$ , Interferon [IFN]- $\gamma$  and Macrophage Inflammatory Protein [MIP]-1 $\beta$  are considered potent stimulators of osteoclastic activity (Márton & Kiss 2000, Metzger 2000) whereas high concentrations of IL-4, IL-5, IL-6, IL-10 and IL-13 are reported to antagonistically suppress apical bone resorption (Stashenko *et al.* 1987, Fukada *et al.* 2009, Popovska *et al.* 2017). Furthermore, increased levels of IL-17A have been associated with the development of radicular cysts and abscesses (Ajuz *et al.* 2014, Ferreira *et al.* 2016). As described above, it is evident these molecular changes orchestrate the inflammatory process once it has been initiated and precede the presentation of clinical symptoms. It would therefore be highly informative to have the ability to study levels of these mediators within infected periradicular tissues. The precise information attained from a simple, non-invasive and accurate sampling procedure could help clinicians determine disease states, inform prognosis and establish a point at which treatment should be concluded to enable predictable outcomes. It could also provide researchers with more objective tools

1  
2  
3 to investigate the biological processes involved in periradicular disease, and their response to novel  
4 therapeutic interventions.  
5

6  
7 Unfortunately, traditional methods used to sample these mediators (i.e. direct surgical access) are  
8  
9 invasive, technique sensitive and do not permit longitudinal analyses (Torabinejad *et al.* 1992, Ajuz *et*  
10 *al.* 2014, Popovska *et al.* 2017). More recently, less invasive orthograde approaches have been  
11 developed. Consequently, an increasing number of clinical studies are sampling PTF via the root canal,  
12 during root canal treatment, and subsequently analysing levels of inflammatory mediators to inform  
13 clinicians of best-practice approaches (Matsuo *et al.* 1994, Shimauchi *et al.* 1996, Kuo *et al.* 1998a).  
14 Although this demonstrates proof of concept, very little is known about these techniques or whether  
15 they have been optimised, through methodology work-up experiments, to serve this important function.  
16 This contrasts with intricate sampling procedures in other areas of dentistry (i.e. collection of periodontal  
17 pathogens from subgingival plaque) where the influences of several basic parameters have been  
18 investigated in depth (Hartroth *et al.* 1999). Additionally, conflicting findings are often reported from  
19 studies with similar objectives and designs, which further warrants the need for investigating how these  
20 methods are currently being employed. For instance, Alptekin *et al.* (2005a) found no difference in PTF  
21 levels of PGE<sub>2</sub> in patients with acute apical periodontitis following endodontic treatment whereas Liu *et*  
22 *al.* (2003) identified a significant reduction. Therefore, the relevance of this review is that it would for  
23 the first time clarify the overall picture of how inflammatory mediators are currently being sampled and  
24 analysed from PTF during root canal treatment, as well as highlighting areas where strategies can be  
25 improved and informing the methodologies of future studies investigating molecular activity in diseased  
26 apical tissues.  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42

#### 43 - Objectives

44  
45 The primary aim of this study is to systematically review the literature to identify qualitative evidence to  
46 answer the following question: “what techniques have been used to sample and analyse inflammatory  
47 mediator activity from the PTF of permanent teeth diagnosed with apical disease during root canal  
48 treatment?” Secondly, this review aims to identify “what types of periradicular inflammatory mediators  
49 had been studied using these methodologies”.

#### 56 **Review**

#### 58 **Methodology**

1  
2  
3 This systematic review was conducted in accordance with the Preferred Reporting Items for  
4 Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

5  
6  
7 - Pre-Registration

8  
9 A protocol was pre-registered with the International Prospective Register of Systematic Reviews  
10 (PROSPERO) on 17<sup>th</sup> of July 2018 (record number: CRD42018100351).

11  
12  
13 - Eligibility Criteria

14  
15 A comprehensive search was carried out on all *in vivo* studies investigating the expression of  
16 inflammatory mediators from within periradicular tissues in human permanent teeth diagnosed with  
17 symptomatic/acute or asymptomatic/chronic apical periodontitis, acute or chronic apical abscess or  
18 condensing osteitis with or without vital pulp tissue (Glickman 2009). Permanent teeth with normal  
19 apical tissues, undergoing elective root canal treatment, were also included if mediators were being  
20 sampled from within the periradicular tissues. Only studies utilising orthograde sampling techniques to  
21 retrieve PTF through the root canal during root canal treatment were included whilst those utilising  
22 retrograde surgical approaches were not. Cell culture, laboratory or animal studies and those concerned  
23 with investigating inflammatory mediator activity from within healthy or diseased pulp tissue only, and  
24 not periradicular tissues, were also excluded. To prevent errors when interpreting data, searches were  
25 limited to articles available in languages that could be translated by the research team (i.e. English or  
26 Chinese). Furthermore, all studies published prior to the commencement date of the searches (i.e. 1<sup>st</sup>  
27 June 2018), as well as grey literature, were included in this review.

28  
29  
30 - Information Sources

31  
32 From the 1<sup>st</sup> of June 2018, six electronic databases were searched independently by SSV & KB. These  
33 included PubMed, EMBASE, Cochrane Library, Science Direct, Web of Science and OpenGrey.  
34 Supplemental search methods included reference list follow-up at the full text evaluation stage, expert  
35 contact and hand searched the contents pages and abstracts of articles published in the 2016-2018  
36 editions of the International Endodontic Journal and Journal of Endodontics as per Liberati *et al.* (2009).

37  
38  
39 - Search Strategy

40  
41 An electronic search strategy was developed based on the primary research questions of this review  
42 which was constructed using the Population, Intervention, Comparison and Outcome (PICO)  
43 framework. The strategy comprised of key terms relevant to the research topic and included both British  
44 and American spellings with subject headings of “apical periodontitis”, “sampling”, “periradicular tissue  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



1  
2  
3 fluid” and “cytokine expression”. These headings were expanded upon through synonyms, key phrases  
4 and indexed terms (i.e. MeSH) identified using the knowledge of the authors, existing literature and  
5 indexed databases. A search strategy was then developed using truncations and Boolean operators  
6  
7 (‘OR’, ‘AND’) and adapted for each database. Once completed, it was modified based on  
8  
9 recommendations from an independent clinical lecturer who peer-reviewed it against PRESS guidelines  
10  
11 for quality assurance purposes (Sampson *et al.* 2009). An example of the PubMed search can be found  
12  
13 in Table 1.  
14

15  
16 - Study selection

17  
18 After duplicates were removed, screening of titles/abstracts and full-text evaluation was performed  
19  
20 independently by SSV & KB using the above eligibility criteria. Any disagreements were resolved  
21  
22 through discussion with a third reviewer (PLT). Once selected for inclusion, the same reviewers used a  
23  
24 standardized pre-piloted form to extract data items for evidence synthesis and quality assessment.  
25

26 - Data items

27  
28 Pre-determined information was extracted from published studies and organised as follows: 1. Study  
29  
30 objectives, 2. Sample characteristics, 3. Mediators studied, 4. PTF sampling method, 5. Laboratory  
31  
32 assay technique and 6. Results. For each sampling method, information around the parameters used  
33  
34 to retrieve PTF (i.e. brand of device, size, insertion depth, sampling duration, how PTF volume was  
35  
36 measured and how a dry, bleeding or suppurative canal was managed) and the sampling regime (i.e.  
37  
38 samples per tooth and when baseline and subsequent samples were taken) was recorded. Additionally,  
39  
40 information around the parameters used to prepare samples for laboratory analysis was collected with  
41  
42 studies grouped according to whether proteomic/metabolomic or transcriptomic level analysis was  
43  
44 performed. When data was unattainable it was coded as being not reported.

45 - Data synthesis & outcome measures

46  
47 A qualitative synthesis was conducted on all studies that met the inclusion criteria. Briefly, key  
48  
49 characteristics of each study were initially summarised and presented in text and table format. These  
50  
51 data were then explored to determine the number and frequency of the different types of: 1. PTF  
52  
53 sampling techniques, 2. Laboratory assay techniques, and 3. Target inflammatory mediators. These  
54  
55 categories acted as the outcome measures in this review and were descriptive in nature.

56 - Risk of bias assessment

1  
2  
3 Bias was assessed independently by SSV & KB using the risk of bias tool proposed by Viswanathan *et*  
4 *al.* (2012), which accounted for different designs of clinical studies. Briefly, the design of each study  
5 was initially determined and then design-specific criteria for randomised and non-randomised controlled  
6 trials, cohort studies and cross sectional studies were applied respectively to assess for bias in 5  
7 domains. These included selection, performance, attrition, detection and reporting bias (Appendix S2).  
8 For each criterion, the risk of bias was deemed as being “low” when details were mentioned with no  
9 ambiguity, “high” if no evidence was presented or “unclear” if insufficient information was provided. If  
10 there were several criterions for an individual bias domain (i.e. selection, performance and detection  
11 bias), the total risk for that individual domain was considered high if 2 or more criterion were scored as  
12 being “high” or “unclear”. Upon completion, the overall risk of bias for each study was then considered  
13 as being low, medium or high if  $\leq 2$ , 3 - 4 or  $\geq 5$  bias domains respectively were deemed as having  
14 “high” or “unclear” risks of bias. Disputes were resolved through discussion with a third author (PLT)  
15 and outcomes were presented textually and graphically. Studies were not excluded based on the bias  
16 assessment as it would have no impact on the descriptive outcome measures of this review however,  
17 this information was used to highlight areas of improvement in studies investigating periradicular  
18 inflammatory mediator activity.

#### 33 - Inter-rater Reliability

34 Cohen’s Kappa statistical analysis was performed to evaluate the extent of inter-rater reliability in the  
35 process of extracting data from studies that met the inclusion criteria. The SPSS (V25) software was  
36 used to conduct this analysis and a score of 0.76 was achieved, which demonstrates “excellent” inter-  
37 rater agreement according to Cicchetti (1994).  
38  
39  
40  
41  
42  
43  
44

## 45 Results

#### 46 - Study selection

47 In total, 251 citations were identified from the initial database search. Eighty-four publications were  
48 eliminated due to duplications and the remaining 167 were reviewed against the inclusion criteria.  
49 Following title and abstract screening, 60 citations were eligible for full-text evaluation of which 33  
50 qualified for inclusion in the qualitative synthesis, all of which were published between 1991 and 2016  
51 (Figure 1). Reasons for rejecting the 27 studies at the full-text evaluation stage are provided in the  
52 Appendix S1.  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 - Study characteristics & qualitative synthesis  
4

5 *Study objectives*

6  
7 Of the 33 evaluated studies, 11 sampled PTF from teeth with apically infected lesions with the sole aim  
8  
9 to determine the concentration of specific mediators. Four studies compared the analyte concentrations  
10  
11 of diseased and normal apical tissues in medically fit patients and 2 studies compared the PTF cytokine  
12  
13 profiles in patients with co-morbidities such as HIV and Sickle Cell Anaemia (SCA) to that of healthy  
14  
15 controls. Ten studies explored correlations between the concentration of specific inflammatory  
16  
17 mediators and clinical/radiographic signs of apical disease, 8 studies monitored changes in cytokine  
18  
19 levels at different stages of root canal treatment and 6 evaluated the impact of clinical interventions on  
20  
21 periradicular inflammatory mediator activity. Twenty-seven studies were observational in their design  
22  
23 and 6 were interventional clinical trials, of which 4 were randomised. The objectives, design and results  
24  
25 for individual studies can be found in Table 2.

26 *Teeth sampled & disease state studied*

27  
28 Sample size ranged from 16 to 77 mature permanent teeth. Ten studies sampled from single-rooted  
29  
30 teeth, 12 studies sampled from single/multi-rooted teeth, and 11 studies did not disclose this  
31  
32 information. Chronic apical periodontitis was the most frequently studied diagnosis (n=14) followed by  
33  
34 acute apical periodontitis (n=6), acute apical abscess (n=1) and normal apical tissues (n=1). Four  
35  
36 studies collected PTF from teeth with both chronic and acute apical periodontitis and 9 did not declare  
37  
38 a specific diagnosis (Table 2).

39 *PTF sampling methods*

40  
41 It was determined that a wide range of sampling techniques were used, namely:

42  
43 i) Paper points (n=27): Four brands were used including Kerr (Kerr Manufacturing Co., Romulus, MI,  
44  
45 USA [n=10]), Dentsply-Maillefer (Dentsply-Maillefer Co., Ballaigues, Switzerland [n=3]), Ariadent  
46  
47 (Ariadent Co., Tehran, Iran [n=1]) and Orbis Dental (Orbis Dental Co., Münster, Germany [n=1]). The  
48  
49 size used included ISO size 40 (n=11), 30 (n=3) and 15 (n=2) and these were inserted into the canal to  
50  
51 working length (n=16) or 2 mm past the apex (n=11). Sampling time lasted for 30 (n=14), 60 (n=12) or  
52  
53 120 (n=1) seconds. Four studies used 1 point per sample (Wahlgren *et al.* 2002, Liu *et al.* 2003, Pezelj-  
54  
55 Ribarić *et al.* 2007, Shahriari *et al.* 2011) whereas five used 2 (Ataoğlu *et al.* 2002, Alptekin *et al.* 2005a,  
56  
57 2005b, Ehsani *et al.* 2012, Grga *et al.* 2012), eleven used 3 (Henriques *et al.* 2011, de Brito *et al.* 2012,  
58  
59 Tavares *et al.* 2012, 2013, Rechenberg *et al.* 2014, Bambirra *et al.* 2015, de Brito *et al.* 2015, Ferreira  
60

1  
2  
3 *et al.* 2015, *Martinho et al.* 2015, 2016, *Sette-Dias et al.* 2016), four used  $\leq 5$  (*Takayama et al.* 1996,  
4 *Shimauchi et al.* 1997, 1998, 2001) and two continued sampling until canals were dry (*Safavi &*  
5 *Rossomando* 1991, *Shimauchi et al.* 1996). In thirteen studies, modifications were made to paper points  
6  
7 either before or after sampling which included pre-coating with an eluting buffer (*Safavi & Rossomando*  
8 1991) or cutting the tip from the wet portion (*Liu et al.* 2003, *Zhi et al.* 2017) or a fixed 3 – 4 mms  
9  
10 (*Shahriair et al.* 2011, *de Brito et al.* 2012, *Tavares et al.* 2012, 2013, *Bambirra et al.* 2015, *de Brito et*  
11 *al.* 2015, *Ferreira et al.* 2015, *Martinho et al.* 2015, 2016, *Sette-Dias et al.* 2016). If PTF volume was  
12  
13 measured, only a wetted length (mm): volume ( $\mu$ l) calibration curve was used to determine this. Twelve  
14  
15 studies did not disclose details on brand of paper point, eleven studies did not disclose details on size,  
16  
17 one study did not indicate the sampling duration and number of points per sample and fourteen studies  
18  
19 did not report on how the PTF volume was measured (Table 3).

20  
21  
22  
23  
24 ii) Fine needle aspiration (n=4): Two studies used a NeoDental syringe (Neo Dental Chemical Products  
25  
26 Co. Ltd, Tokyo, Japan), one used a Hamilton microsyringe (Hamilton Co., NE, USA) and one study  
27  
28 used Drummond Scientific microdispenser replacement tubes (Drummond Scientific Co., Broomall, PA,  
29  
30 USA). Information regarding needle gauge, length, insertion depth, sampling duration or PTF volume  
31  
32 measurements were not disclosed (Table 3).

33  
34 iii) Methylcellulose filter paper strips (n=2): Two strips of the Interstate brand (Interstate Drug Exchange,  
35  
36 Amityville, NY, USA) were used per sample for a “few seconds” and a Periotron (Harco Electronics,  
37  
38 Tustin, CA, USA) subsequently measured PTF volume. No further information was disclosed (Table 3).

#### 39 *PTF sampling regime*

40  
41 Throughout treatment, 16 studies sampled PTF only once from each tooth, twelve sampled twice, one  
42  
43 sampled 3 times, two studies sampled 6 times and another two studies repeated sampling “7-14” times.  
44  
45 Baseline samples were taken either before (n=5) or after (n=25) root canal instrumentation. Of those  
46  
47 sampling longitudinally (n=17), the timing of subsequent samples ranged from 3 minutes to 15 day  
48  
49 intervals after baseline. Three studies did not disclose details on when the baseline sample was taken  
50  
51 (Table 3).

#### 52 *Managing dry, bleeding and suppurative canals*

53  
54 Where the sampling protocol resulted in no retrieval of PTF (i.e. dry canal), one study (*Safavi &*  
55  
56 *Rossomando* 1991) excluded samples whereas four others stated they used patency filing to draw PTF  
57  
58 into the canal and then proceed with re-sampling (*Ataoglu et al.* 2002, *Alptekin et al.* 2005a, 2005b,  
59  
60

1  
2  
3 Ehsani *et al.* 2012). Seven studies excluded samples when “more than a small amount of blood” was  
4 retrieved (Kuo *et al.* 1998a, 1998b, Ataoğlu *et al.* 2002, Alptekin *et al.* 2005a, 2005b, Ehsani *et al.* 2012,  
5 Rechenberg *et al.* 2014). Samples from discharging canals were included following drainage in five  
6 studies (Matsuo *et al.* 1994, 1995, Ataoğlu *et al.* 2002, Alptekin *et al.* 2005a, 2005b) but excluded in  
7 two other studies (Ehsani *et al.* 2012, Rechenberg *et al.* 2014). Twenty-eight, 26 and a further 26 studies  
8 did not disclose information on how they managed dry, bleeding or suppurative canals, respectively.

#### 14 *Laboratory assay techniques*

15  
16 i) Proteomic/Metabolomic level (n=25): Enzyme-linked-immunosorbant assay (ELISA) (n=18),  
17 radioimmunoassay (RIA) (n=4), colorimetric assay (CA) (n=2), immunofluorometric assay (IMFA) (n=1)  
18 and cytometric bead array (CBA) (n=1) were all techniques used to quantify the inflammatory mediators  
19 found within PTF, at the proteomic/metabolomic level (Table 3). In preparing samples, 50 to 300 µl of  
20 the elution buffers of phosphate buffered saline [PBS] (n=11), PBS-bovine serum albumin [PBS-BSA]  
21 (n=2), PBS-tween 20 (n=4), PBS-tween 20 + foetal calf serum (n=1) and trisaminomethane-hydrochloric  
22 acid [Tris-HCl] (n=1) were used. Two studies incubated samples for 60-180 minutes, five vortexed  
23 samples for 60 seconds, five centrifuged samples for 10–30 minutes at 4–15 000 x g, seven vortexed  
24 samples for 30 seconds and then centrifuged for 10 minutes at 5 000 x g and one incubated samples  
25 for 300 minutes, vortexed for 30 seconds and then centrifuged for 10 minutes at an unspecified gravity  
26 force. Further details on how samples were processed, including unspecified information, can be found  
27 in Table 3.

28  
29 ii) Transcriptomic level (n=9): Quantitative polymerase chain reaction (qPCR) was the only technique  
30 used to quantify the inflammatory mediators found within PTF at the transcriptomic level. All the samples  
31 were prepared in a consistent manner using Trizol reagent for RNA isolation. Briefly, samples  
32 underwent phase separation centrifugation for 15 minutes at 12 000 x g, precipitation centrifugation for  
33 10 minutes at 12 000 x g and then incubation for 10 minutes at 55°C.

#### 34 *Inflammatory mediators analysed*

35  
36 Forty-five different mediators were reported as being studied with IL-1β being the most frequent analyte  
37 (n=17). Interleukin-1α, IL-1β, IL-6, IL-8, IL-17A, TNF-α, IFN-γ and Receptor Activator of Nuclear Factor  
38 Kappa-B Ligand [RANKL] were studied independently at a proteomic/metabolomic and transcriptomic  
39 level. Interleukin-1 receptor antagonist [IL-1ra], IL-2, IL-4, IL-5, IL-13, Immunoglobulin [Ig]-A, IgG, IgM,  
40 PGE<sub>2</sub>, Matrix Metalloproteinases [MMP]-1, MMP-2, MMP-8, MMP-9, Tissue Inhibitor of  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 Metalloproteinase [TIMP]-1, TIMP-2, MMP-1, 2, 9/TIMP-1, 2 complexes, Osteoprotegrin [OPG],  
4 Neutrophil Elastase [NE], Nitrous Oxide [NO] and  $\beta$ -glucuronidase [ $\beta$ G] were studied exclusively at a  
5 proteomic/metabolomic level. Interleukin-10, Monocyte Chemoattractant Protein [MCP]-1, MIP-1 $\beta$ ,  
6 Regulated on Activation Normal T cell Expressed and Secreted [RANTES], Chemokine Receptor  
7 [CXCR]-4, CCR5, Transforming growth factor [TGF]- $\beta$ , Osteopontin [OPN], alpha-2-integrin [ITGA2],  
8 Heat Shock Protein [HSP]-47 and Focal Adhesion Kinase [FAK] were studied exclusively at a  
9 transcriptomic level. Only 1 study assayed the same analytes at both proteomic/metabolomic and  
10 transcriptomic levels (Takeichi *et al.* 1996). The types and frequency of targeted mediators analysed  
11 are presented in Table 2 & Figure 2.  
12  
13  
14  
15  
16  
17  
18  
19

20 - Bias assessment:

21  
22 An overall risk of bias was deemed “high” in 4 studies, “medium” in 23 and “low” in 6. A “high” or “unclear”  
23 risk of bias was found in 12 studies for the selection domain, 15 for the performance domain, 3 for the  
24 attrition domain, 24 for the detection domain and 32 for the reporting domain. Only 11 studies disclosed  
25 information on all the parameters associated with sampling and eluting periradicular inflammatory  
26 mediators (Shimauchi *et al.* 1996, 1997, 1998, 2001, Takayama *et al.* 1996, Ataoğlu *et al.* 2002, Alptekin  
27 *et al.* 2005a, 2005b, Shahriari *et al.* 2011, Ehsani *et al.* 2012, Grga *et al.* 2013). However, only 5 of  
28 these studies reported unambiguous and precise information (Alptekin *et al.* 2005a, 2005b, Shahriari  
29 *et al.* 2011, Ehsani *et al.* 2012, Grga *et al.* 2013). The results of the bias assessment for individual  
30 studies are presented in Appendix S3.  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40

## 41 Discussion

42 - Summary of findings

43  
44 Thirty-three studies met the inclusion criteria and were generally deemed to have a “medium” risk of  
45 bias due to lack of reporting and heterogeneous methodology (Appendix S3). However, the studies  
46 suggest paper points and proteomic/metabolomic analyses are the most common approaches used to  
47 sample and quantify analytes respectively from diseased apical tissues during root canal treatment  
48 (Table 3). Furthermore, a broad range of inflammatory mediators have been subjected to analysis with  
49 IL-1 $\beta$  and TNF- $\alpha$  being the most studied (Figure 2). These findings are discussed in more detail below.  
50  
51  
52  
53  
54  
55  
56

### 57 *PTF sampling method*

1  
2  
3 According to the outcomes from this review, Safavi & Rossomando (1991) were the first identified study  
4 to sample PTF and determine expression of inflammatory mediators when sampled through the root  
5 canal using paper points. Whilst several other methods such as fine needle aspiration and absorption  
6 with methylcellulose filter paper strips have since been explored, paper points remain the most  
7 commonly used approach. This could be because, unlike filter strips, their length, shape and taper  
8 readily conform to the shape of the root canal and therefore allow for more accurate and controlled  
9 sampling within the periradicular region. Clinical operators would also be familiar with their use. This  
10 was acknowledged by Kuo *et al.* (1998a), who highlighted the need for filter strips to be made longer  
11 as well as their limited absorbance capacity. Furthermore, paper points are also very efficient at  
12 absorbing small volumes of fluid, as evident by their application in other disciplines within (Hartroth *et*  
13 *al.* 1999) and outside dentistry (Lima *et al.* 2015). This property is particularly favourable for longitudinal  
14 sampling of PTF, as tissue fluid volume decreases over the course of root canal treatment due to healing  
15 (Matsuo *et al.* 1994). Conversely, syringes are not well adapted for retrieving such small volumes of  
16 fluid as was reported by Matsuo *et al.* (1995), who experienced challenges in attaining adequate  
17 amounts of PTF in the latter stages of treatment. Additionally, small amounts of fluid will also be lost in  
18 the lumen of the syringe and needle. Therefore, it appears paper points are the most well established  
19 approach for sampling PTF and subsequently analysing the concentration of single or multiple analytes  
20 during root canal treatment. Nevertheless, this method is not without its limitations as reliable  
21 periradicular sampling requires a patent root canal, which is not always predictably achievable due to  
22 calcifications, curvatures or procedural errors. Furthermore, the paper point could become  
23 contaminated with blood or pus, originating from the infected periradicular tissues, which may eventually  
24 interfere with the assay procedure.

#### 25 *PTF sampling protocol*

26 The findings of this review confirm that within any given approach to sampling PTF (i.e. paper points,  
27 fine needle aspiration and filter strips), there is an absence of standardisation within the protocol (Table  
28 3). This variation in basic parameters (i.e. brand, duration of sampling, insertion depth and size of  
29 device) can explain the conflicting outcomes reported by some studies. For example, Liu *et al.* (2003)  
30 found PTF levels of PGE<sub>2</sub> significantly reduced in patients with acute apical periodontitis following root  
31 canal treatment, however, Alptekin *et al.* (2005a) found no difference. Both studied a population with  
32 similar characteristics and used paper points to retrieve periapical exudate however, their sampling  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 protocol varied in that different brands, sizes and number of points per sample were used (Table 3).  
4 This contrasts sampling procedures in other areas of dentistry (i.e. collection of periodontal pathogens  
5 from subgingival plaque) where these parameters have been investigated in depth and an optimised  
6 protocol developed (Hartroth *et al.* 1999). On the other hand, it is currently not known how these factors  
7 would influence PTF sampling.  
8  
9  
10  
11

### 12 *Eluting protocol*

13  
14 This review highlights a significant variation in how samples are being prepared for  
15 proteomic/metabolomic analysis. Several elution methods including vortex, centrifugation and  
16 incubation have been used alongside numerous buffers to elute inflammatory mediators from paper  
17 points (Table 3). However, it is not known how these differing strategies would influence the percentage  
18 recovery of analytes. Such an *in vitro* investigation has been carried out in the field of ophthalmology  
19 where sponges were spiked with known concentrations of 25 different recombinant pro-inflammatory  
20 analytes, and then eluted using various buffers and techniques prior to being assayed (Inic-Kanada *et*  
21 *al.* 2012). Significant variation in the percentage recovery was noted between different eluting buffers  
22 and inflammatory mediators, which are not isolated findings (VanDerMeid *et al.* 2011). Conversely,  
23 Shimauchi *et al.* (1996) was the only article identified in this review to carry out a similar experiment  
24 however, this assayed only IL-1 $\beta$  and the influence of various eluting buffers and techniques on the  
25 recovery of periradicular inflammatory analytes has not yet been investigated.  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36

### 37 *Laboratory assay techniques*

38  
39 A wide group of periradicular inflammatory mediators have been studied at either the  
40 proteomic/metabolomic or transcriptomic level (Figure 2). Assay techniques for the latter use  
41 transcribed mRNA sequences as biomarkers whereas the former target the actual secreted  
42 protein/metabolite (Vogel & Marcotte 2012). As mRNA is translated into its respective protein it is  
43 assumed there should be a strong correlation between the two and therefore, both can be used to  
44 quantify the presence of a specific mediator. However in human cells, a weak correlation between  
45 concentrations of protein and its respective mRNA abundances has been observed (Vogel *et al.* 2010),  
46 which could be attributed to various post-transcriptional or translational mechanisms (i.e.  
47 controls/checkpoints) (Maier *et al.* 2009). These findings are further supported by Takeichi *et al.* (1996),  
48 which provided the only study in this review to assay the same biomarkers at both the gene and protein  
49 level. They reported that although the mRNA for IL-6 was not detected, a significant amount of its  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



1  
2  
3 respective protein was present in the sampled PTF. These data imply that evaluating protein/metabolite  
4 expression is likely to be more representative of actual periradicular inflammatory mediator activity than  
5 mRNA expression, and potentially explains why it is the preferred approach amongst the studies in this  
6  
7 review.  
8  
9

### 10 *Target analytes*

11 Interleukin-1 $\beta$  and TNF- $\alpha$  were the most frequently studied analytes according to this review (Figure 4).  
12  
13 This may be for several reasons, firstly, their role in the pathophysiology of periradicular disease has  
14 been previously well reviewed (Nair 2004), secondly, they are considered the most relevant to human  
15 osteoclastic activity (Stashenko *et al.* 1987) and thirdly, their presence in apical lesions have been  
16 repeatedly demonstrated with their concentrations being proportionate to the size of lesions (Safavi &  
17 Rossomando 1991, Matsuo *et al.* 1994). This currently makes them ideal biomarker for periradicular  
18 disease activity however, growing research into the role of other analytes is likely to give rise to  
19 alternative targets.  
20  
21  
22  
23  
24  
25  
26  
27

#### 28 - Quality of included studies

29  
30 The studies included in this review were generally of medium to low quality according to the  
31 aforementioned risk of bias tool (Appendix S3). The source of bias in interventional studies (i.e. RCTs  
32 & CCTs) originated from the lack of clarity on the randomisation and concealment process, and absence  
33 of any power calculations. No study reported on using a blinded assessor or analytical techniques (i.e.  
34 stratification or multivariate analysis) to control confounding factors and only one study (Ehsani *et al.*  
35 2012) referenced a pre-registered protocol in their text. Furthermore, this review confirms a lack of  
36 reporting and high levels of heterogeneity in the sampling and eluting protocols, which would make it  
37 difficult to pursue any quantitative synthesis of data. This lack of standardisation could be attributed to  
38 the absence of any existing evidence based guidelines on how to apply these techniques in the context  
39 of root canal treatment. For these reasons, there is a degree of uncertainty around the conclusions  
40 drawn from these studies, which should be taken with caution when applying them to a clinical setting.  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50

### 51 **Recommendations for future studies**

52  
53 In terms of paper point sampling, basic parameters such as i) manufacturer (Pumarola-Suñé *et al.*  
54 1998), ii) ISO size (Hartroth *et al.* 1999), iii) duration of sampling (Hartroth *et al.* 1999) and iv) insertion  
55 depth need to be studied to develop an optimised protocol that allows for maximum PTF absorbance.  
56  
57 To attain maximum mediator recovery, factors such as different i) buffer types (Inic-Kanada *et al.* 2012)  
58  
59  
60

1  
2  
3 and ii) elution methods (i.e. incubation, vortex, centrifugation and combinations) needs to be  
4 investigated to develop an optimised elution protocol.  
5

6  
7 Finally, key information needs to be explicitly and unambiguously provided in the methodology of  
8 studies investigating periradicular inflammatory mediator activity so that a meta-analysis can be  
9 pursued in the future. This includes i) sample characteristics: an explicit diagnosis and tooth type, ii)  
10 parameters of PTF sampling: method, number of operators, manufacturer, ISO size, insertion depth,  
11 sampling duration, number of points per sample, if any modifications were made to the point, how PTF  
12 volume was measured, number of samples per tooth, timing of the baseline and subsequent samples  
13 in relation, and the management of a dry, bleeding and suppurative canal and iii) parameters of PTF  
14 elution: assay technique, buffer type and volume, duration and temperature of incubation if used,  
15 duration of vortex if used and duration, force and temperature of centrifugation if used.  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25

## 26 **Conclusions**

27  
28 Within the limitations of the studies included in this review, which were of medium to low quality, two  
29 main conclusions can be drawn regarding how periradicular inflammatory mediators are currently being  
30 studied during root canal treatment:  
31  
32

- 33 1. Paper points and proteomic/metabolomic level assays are currently the most commonly used  
34 methods to sample and analyse inflammatory mediators within PTF respectively.  
35
- 36 2. The most targeted analytes are currently IL-1 $\beta$  and TNF- $\alpha$ .  
37  
38

39 This review also highlights the need for the development an optimised sampling and eluting protocol  
40 and a standardised approach to reporting by future studies.  
41  
42  
43  
44  
45  
46

## 47 **Conflicts of interest**

48  
49 The authors have stated explicitly that there is no conflict of interest in connection with this article  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## References

- Ajuz NC, Antunes H, Mendonça TA, Pires FR, Siqueira JF, Armada L (2014) Immunoexpression of interleukin 17 in apical periodontitis lesions. *Journal of Endodontics*, **40**, 1400-3.
- Alfenas CF, Mendes TA, Ramos HJ, et al. (2017) Human Exoproteome in Acute Apical Abscesses. *Journal of Endodontics*, **43**, 1479-85.
- Alptekin, NO, Ari H, Haliloglu S, Alptekin T, Serpek B, Ataoglu T (2005a). The effect of endodontic therapy on periapical exudate neutrophil elastase and prostaglandin-E2 levels. *Journal of Endodontics*, **31**, 791-5.
- Alptekin NO, Ari H, Ataoglu T, Haliloglu S, Alptekin T, Serpek B (2005b) Neutrophil elastase levels in periapical exudates of symptomatic and asymptomatic teeth. *Journal of Endodontics*, **31**, 350-3.
- Amaya MP, Criado L, Blanco B, et al. (2013). Polymorphisms of pro-inflammatory cytokine genes and the risk for acute suppurative or chronic nonsuppurative apical periodontitis in a Colombian population. *International Endodontic Journal*, **46**, 71-8.
- Araujo-Pires AC, Francisconi CF, Bigueti CC. et al. (2014) Simultaneous analysis of T helper subsets (Th1, Th2, Th9, Th17, Th22, Tfh, Tr1 and Tregs) markers expression in periapical lesions reveals multiple cytokine clusters accountable for lesions activity and inactivity status. *Journal of Applied Oral Science*, **22**, 336-6.
- Ataoğlu T, Üngör M, Serpek B, Haliloğlu S, Ataoğlu H, Ari H (2002) Interleukin-1 $\beta$  and tumour necrosis factor- $\alpha$  levels in periapical exudates. *International Endodontic Journal*, **35**, 181-5.
- Baeza M, Garrido M, Hernández-Ríos P, et al. (2016) Diagnostic accuracy for apical and chronic periodontitis biomarkers in gingival crevicular fluid: an exploratory study. *Journal of Clinical Periodontology*, **43**, 34-45.
- Bambirra W, Maciel KF, Thebit MM, de Brito LCN, Vieira LQ, Sobrinho APR (2015) Assessment of apical expression of alpha-2 integrin, heat shock protein, and proinflammatory and immunoregulatory cytokines in response to endodontic infection. *Journal of Endodontics*, **41**, 1085-90.
- Barkhordar RA, Hayashi C, Hussain MZ (1999) Detection of interleukin-6 in human dental pulp and periapical lesions. *Dental Traumatology*, **15**, 26-7.
- Carvalho AS, Oliveira, LDD, Cardoso FGDR, Oliveira FED, Valera MC, Carvalho CAT (2016). Limewater and Polymyxin B Associated with NaOCl for Endotoxin Detoxification in Root Canal with Necrotic Pulp. *Brazilian Dental Journal*, **27**, 573-7.

- 1  
2  
3 Cicchetti DV (1994) Guidelines, criteria and rules of thumb for evaluating normed and standardized  
4 assessment instruments in psychology. *Psychological Assessment*, **6**, 284-290.
- 5  
6  
7 de Brito LCN, Teles FR, Teles RP, Nogueira PM, Vieira LQ, Ribeiro Sobrinho AP (2015) Immunological  
8 profile of periapical endodontic infections from HIV- and HIV+ patients. *International Endodontic*  
9 *Journal*, **48**, 533-41.
- 10  
11  
12 de Brito LCN, Teles FRF, Teles RP, Totola AH, Vieira LQ, Sobrinho APR (2012) T-lymphocyte and  
13 cytokine expression in human inflammatory periapical lesions. *Journal of Endodontics*, **38**, 481-85.
- 14  
15  
16 Dezerega A, Osorio C, Mardones J, *et al.* (2010) Monocyte chemotactic protein-3: possible involvement  
17 in apical periodontitis chemotaxis. *International Endodontic Journal*, **43**, 902-8.
- 18  
19  
20 Ehsani M, Moghadamnia AA, Zahedpasha S, *et al.* (2012). The role of prophylactic ibuprofen and N-  
21 acetylcysteine on the level of cytokines in periapical exudates and the post-treatment pain. *DARU*  
22 *Journal of Pharmaceutical Sciences*, **20**, 30.
- 23  
24  
25  
26 Ferreira DC, Paiva, SS, Carmo FL, *et al.* (2011) Identification of herpesviruses types 1 to 8 and human  
27 papillomavirus in acute apical abscesses. *Journal of Endodontics*, **37**, 10-6.
- 28  
29  
30 Ferreira LGV, Rosin FCP, Correa L (2016) Analysis of Interleukin 17A in periapical abscess and  
31 granuloma lesions. *Brazilian Oral Research*, **30**, e34.
- 32  
33  
34 Ferreira SBP, de Brito LCN, Oliveira MP, *et al.* (2015) Periapical cytokine expression in sickle cell  
35 disease. *Journal of Endodontics*, **41**, 358-62.
- 36  
37  
38 Fukada SY, Silva TA, Garlet GP, Rosa, AL, Da Silva JS Cunha FQ (2009) Factors involved in the T  
39 helper type 1 and type 2 cell commitment and osteoclast regulation in inflammatory apical  
40 diseases. *Molecular Oral Microbiology*, **24**, 25-31.
- 41  
42  
43 Gazivoda D, Dzopalic T, Bozic B, Tatomirovic Z, Brkic Z, Colic M (2009) Production of proinflammatory  
44 and immunoregulatory cytokines by inflammatory cells from periapical lesions in culture. *Journal of Oral*  
45 *Pathology & Medicine*, **38**, 605-11.
- 46  
47  
48 Glickman GN (2009) AAE Consensus Conference on Diagnostic Terminology: background and  
49 perspectives. *Journal of Endodontics*, **35**, 1619-20
- 50  
51  
52 Graunaite I, Lodiene G, Maciulskiene V (2011) Pathogenesis of apical periodontitis: a literature  
53 review. *Journal of Oral & Maxillofacial Research*, **2**, e1.
- 54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 Grga Đ, Dželetović B, Damjanov M, Hajduković-Dragojlović L (2013) Prostaglandin E2 in apical tissue  
4 fluid and postoperative pain in intact and teeth with large restorations in two endodontic treatment  
5 visits. *Srpski arhiv za celokupno lekarstvo*, **141**, 17-21.  
6  
7  
8 Hartroth B, Seyfahrt I, Conrads G (1999) Sampling of periodontal pathogens by paper points: evaluation  
9 of basic parameters. *Oral Microbiology & Immunology*, **14**, 326-30.  
10  
11  
12 Henriques LCF, de Brito LCN, Tavares WLF, Vieira LQ, Sobrinho APR (2011). Cytokine analysis in  
13 lesions refractory to endodontic treatment. *Journal of Endodontics*, **37**, 1659-62.  
14  
15  
16 Hernádi K, Gyöngyösi E, Mészáros B, *et al.* (2013) Elevated tumor necrosis factor-alpha expression in  
17 periapical lesions infected by Epstein-Barr virus. *Journal of Endodontics*, **39**, 456-60.  
18  
19  
20 Inic-Kanada A, Nussbaumer A, Montanaro J, *et al.* (2012) Comparison of ophthalmic sponges and  
21 extraction buffers for quantifying cytokine profiles in tears using Luminex technology. *Molecular Vision* ,  
22  
23  
24 **18**, 2717-25.  
25  
26 Jakovljevic A, Knezevic A, Karalic D, *et al.* (2015) Pro-inflammatory cytokine levels in human apical  
27 periodontitis: Correlation with clinical and histological findings. *Australian Endodontic Journal*, **41**, 72-  
28  
29  
30 7.  
31  
32 Kawashima N, Stashenko P (1999) Expression of bone-resorptive and regulatory cytokines in murine  
33 periapical inflammation. *Archives of Oral Biology*, **44**, 55-66.  
34  
35 Keleş A, Alçın H (2015) Use of EndoVac system for aspiration of exudates from a large periapical lesion:  
36 a case report. *Journal of Endodontics*, **41**, 1735-7.  
37  
38  
39 Kuo ML, Lamster IB, Hasselgren G (1998a) Host mediators in endodontic exudates: I. Indicators of  
40 inflammation and humoral immunity. *Journal of Endodontics*, **24**, 598-603.  
41  
42  
43 Kuo ML, Lamster IB, Hasselgren G (1998b) Host mediators in endodontic exudates: II. Changes in  
44 concentration with sequential sampling. *Journal of Endodontics*, **24**, 636-40.  
45  
46  
47 Liberati A, Altman DG, Tetzlaff J, *et al.* (2009) The PRISMA statement for reporting systematic reviews  
48 and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration.  
49  
50  
51 *British Medical Journal*, **339**, b2700.  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 Lima L, Lange RR, Turner-Giannico A, Montiani-Ferreira F (2015) Evaluation of standardized  
4 endodontic paper point tear test in New Zealand white rabbits and comparison between corneal  
5 sensitivity followed tear tests. *Veterinary Ophthalmology*, **18**, 119-24.

6  
7  
8  
9 Liu W, Yu J, Zhou H (2003) Changes of prostaglandin E2 levels in periapical exudates after root canal  
10 treatment. *West China Journal of Stomatology*, **21**, 39-40.

11  
12 Machado de Oliveira JC, Siqueira JF, Rôças, IN, *et al.* (2007) Bacterial community profiles of  
13 endodontic abscesses from Brazilian and USA subjects as compared by denaturing gradient gel  
14 electrophoresis analysis. *Molecular Oral Microbiology*, **22**, 14-8.

15  
16  
17  
18 Maier T, Guell M, Serrano L (2009) Correlation of mRNA and protein in complex biological  
19 samples. *FEBS Letters*, **583**, 3966–73.

20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Martinho FC, Chiesa WM, Leite FR, Cirelli JA, Gomes BP (2012) Correlation between  
clinical/radiographic features and inflammatory cytokine networks produced by macrophages  
stimulated with endodontic content. *Journal of Endodontics*, **38**, 740-5.

Martinho FC, Nascimento GG, Leite FR, Gomes AP, Freitas LF, Camões IC (2015) Clinical influence  
of different intracanal medications on Th1-type and Th2-type cytokine responses in apical  
periodontitis. *Journal of Endodontics*, **41**, 169-75.

Martinho FC, Teixeira FF, Cardoso FG, *et al.* (2016) Clinical investigation of matrix metalloproteinases,  
tissue inhibitors of matrix metalloproteinases, and matrix metalloproteinase/tissue inhibitors of matrix  
metalloproteinase complexes and their networks in apical periodontitis. *Journal of Endodontics*, **42**,  
1082-8.

Márton IJ, Kiss C (2014) Overlapping protective and destructive regulatory pathways in apical  
periodontitis. *Journal of Endodontics*, **40**, 155-63.

Márton, IJ, Kiss C (2000) Protective and destructive immune reactions in apical periodontitis. *Molecular  
Oral Microbiology*, **15**, 139-50.

Matsuo T, Ebisu S, Nakanishi T, Yonemura K, Harada Y, Okada H (1994) Interleukin-1 $\alpha$  and interleukin-  
1 $\beta$  in periapical exudates of infected root canals: correlations with the clinical findings of the involved  
teeth. *Journal of Endodontics*, **20**, 432-5.

Matsuo T, Nakanishi T, Ebisu S (1995) Immunoglobulins in periapical exudates of infected root canals:  
correlations with the clinical findings of the involved teeth. *Dental Traumatology*, **11**, 95-9.

Metzger Z (2000) Macrophages in periapical lesions, *Endodontics & Dental Traumatology*, **16**, 1-8.

1  
2  
3 Metzger Z, Abramovitz I & Bergenholtz G (2003) *Textbook of Endodontology*. 2<sup>nd</sup> Ed, Oxford, England:  
4 Blackwell, 113-26.

5  
6  
7 Nair PN (1997) Apical periodontitis: a dynamic encounter between root canal infection and host  
8 response. *Periodontology 2000*, **13**, 121-48.

9  
10 Nair PN (2004) Pathogenesis of apical periodontitis and the causes of endodontic failures. *Critical*  
11 *Reviews in Oral Biology & Medicine*, **15**, 348-81.

12  
13  
14 Noda M, Komatsu H, Inoue S, Sano H (2000) Antibiotic susceptibility of bacteria detected from the root  
15 canal exudate of persistent apical periodontitis. *Journal of Endodontics*, **26**, 221-4.

16  
17  
18 Nonaka CFW, Maia AP, do Nascimento GJF, *et al.* (2008) Immunoexpression of vascular endothelial  
19 growth factor in periapical granulomas, radicular cysts, and residual radicular cysts. *Oral Surgery, Oral*  
20 *Medicine, Oral Pathology, Oral Radiology and Endodontics*, **106**, 896-902.

21  
22  
23 Pezelj-Ribarić S, Magašić K, Prpic J, Miletić I, Karlović, Z (2007) Tumor necrosis factor-alpha in peripical  
24 tissue exudates of teeth with apical periodontitis. *Mediators of Inflammation*, **2007**, e69416

25  
26  
27 Popovska L, Dimova C, Evrosimovska B, *et al.* (2017) Relationship between IL-1 $\beta$  production and  
28 endodontic status of human periapical lesions. *Vojnosanitiski Pregled*, **74**, 1134-9.

29  
30  
31 Pourhajibagher M, Ghorbanzadeh R, Parker S, Chiniforush N, Bahador A (2017) The evaluation of  
32 cultivable microbiota profile in patients with secondary endodontic infection before and after photo-  
33 activated disinfection. *Photodiagnosis & Photodynamic Therapy*, **18**, 198-3.

34  
35  
36 Provenzano JC, Siqueira JF, Rôças IN, Domingues RR, Leme AFP, Silva MR (2013) Metaproteome  
37 analysis of endodontic infections in association with different clinical conditions. *PLoS One*, **8**, 76108.

38  
39  
40 Pumarola-Suñé J, Solá-Vicens L, Sentís-Vilalta J, Canalda-Sahli C, Brau-Aguadé E (1998)  
41 Absorbency properties of different brands of standardized endodontic paper points. *Journal of*  
42 *Endodontics*, **24**, 796-8.

43  
44  
45 Rechenburg DK, Bostanci N, Zehnder M, Belibasakis GN (2014) Periapical fluid RANKL and IL-8 are  
46 differentially regulated in pulpitis and apical periodontitis. *Cytokine*, **69**, 116-9.

47  
48  
49 Rôças IN, Siqueira JF (2018) Frequency and levels of candidate endodontic pathogens in acute apical  
50 abscesses as compared to asymptomatic apical periodontitis. *PLoS one*, **13**, e0190469.

51  
52  
53 Rocca JP, Peloux Y, Marcilly A (1987) Sampling, isolation and identification of bacteria implicated in  
54 periapical granulomas complicated by pulp gangrene. *Revue française d'endodontie: publication*  
55 *officielle de la Société française d'endodontie*, **6**, 11-26.

- 1  
2  
3 Safavi KE, Rossomando EF (1991) Tumor necrosis factor identified in periapical tissue exudates of  
4 teeth with apical periodontitis. *Journal of Endodontics*, **17**, 12-4.  
5  
6  
7 Sampson M, McGowan J, Cogo E, Grimshaw J, Moher D, Lefebvre C (2009) An evidence-based  
8 practice guideline for the peer review of electronic search strategies. *Journal of Clinical*  
9 *Epidemiology*, **62**, 944-52.  
10  
11  
12 Sette-Dias AC, Maciel KF, Abdo EN, *et al.* (2016) Cytokine Expression in Patients Hospitalized for  
13 Severe Odontogenic Infection in Brazil. *Journal of Endodontics*, **42**, 706-10.  
14  
15  
16 Shahriari S, Rezaei A, Jalalzadeh SM, Mani K, Zamani A (2011) Effect of Ibuprofen on IL-1 [Beta], TNF-  
17 [alpha] and PGE2 Levels in Periapical Exudates: A Double Blinded Clinical Trial. *Iranian Journal of*  
18 *Immunology*, **8**, 176-82.  
19  
20  
21  
22 Shimauchi H, Miki Y, Takayama SI, Imai T, Okada H (1996) Development of a quantitative sampling  
23 method for periapical exudates from human root canals. *Journal of Endodontics*, **22**, 612-5.  
24  
25  
26 Shimauchi H, Takayama S, Imai-Tanaka T, Okada H (1998). Balance of interleukin-1 beta and  
27 interleukin-1 receptor antagonist in human periapical lesions. *Journal of Endodontics*, **24**, 116–9  
28  
29  
30 Shimauchi H, Takayama SI, Miki Y, Okada H (1997) The change of periapical exudate prostaglandin  
31 E2 levels during root canal treatment. *Journal of Endodontics*, **23**, 755-8.  
32  
33  
34 Shimauchi H, Takayama SI, Narikawa-Kiji M, Shimabukuro Y, Okada H (2001) Production of  
35 Interleukin–8 and Nitric Oxide in Human Periapical Lesions. *Journal of Endodontics*, **27**, 749-52.  
36  
37  
38 Silva TA, Garlet GP, Lara VS, Martins W, Silva JS, Cunha FQ (2005) Differential expression of  
39 chemokines and chemokine receptors in inflammatory periapical diseases. *Molecular Oral*  
40 *Microbiology*, **20**, 310-6.  
41  
42  
43 Siqueira JF, Rôças IN (2003) Detection of Filifactor alocis in endodontic infections associated with  
44 different forms of periradicular diseases. *Molecular Oral Microbiology*, **18**, 263-5.  
45  
46  
47 Siqueira JF, Rôças IN (2004) Treponema species associated with abscesses of endodontic  
48 origin. *Molecular Oral Microbiology*, **19**, 336-9.  
49  
50  
51 Soares JA, Brito-Júnior M, Silveira FF, Nunes E, Santos SM (2008) Favourable response of an  
52 extensive periapical lesion to root canal treatment. *Journal of Oral Science*, **50**, 107-11.  
53  
54  
55 Sousa TO, Haiter-Neto F, Nascimento EHL, Peroni LV, Freitas DQ, Hassan B (2017) Diagnostic  
56 accuracy of periapical radiography and cone-beam computed tomography in identifying root canal  
57 configuration of human premolars. *Journal of Endodontics*, **43**, 1176-9.  
58  
59  
60



1  
2  
3 Sousa, EL, Martinho FC, Nascimento GG, Leite FR, Gomes BP (2014) Quantification of endotoxins in  
4 infected root canals and acute apical abscess exudates: monitoring the effectiveness of root canal  
5 procedures in the reduction of endotoxins. *Journal of Endodontics*, **40**, 177-81.  
6

7  
8 Stashenko P, Dewhirst FE, Peros WJ, Kent RL, Ago JM (1987) Synergistic interactions between  
9 interleukin 1, tumor necrosis factor, and lymphotoxin in bone resorption. *The Journal of*  
10 *Immunology*, **138**, 1464-8.  
11

12  
13 Stashenko P, Wang CY, Riley E, *et al.* (1995) Reduction of infection-stimulated periapical bone  
14 resorption by the biological response modifier PGG glucan, *Journal of Dental Research*, **74**, 323-30.  
15

16  
17 Takayama SI, Miki Y, Shimauchi H, Okada H (1996) Relationship between prostaglandin E2  
18 concentrations in periapical exudates from root canals and clinical findings of periapical  
19 periodontitis. *Journal of Endodontics*, **22**, 677-80.  
20

21  
22 Takeichi O, Saito I, Okamoto Y, Tsurumachi T, Saito T (1998) Cytokine regulation on the synthesis of  
23 nitric oxide in vivo by chronically infected human polymorphonuclear leucocytes. *Immunology*, **93**, 275-  
24 80.  
25

26  
27 Takeichi O, Saito I, Tsurumachi T, Moro I, Saito T (1996) Expression of inflammatory cytokine genes in  
28 vivo by human alveolar bone-derived polymorphonuclear leukocytes isolated from chronically inflamed  
29 sites of bone resorption. *Calcified Tissue International*, **58**, 244-8.  
30

31  
32 Tavares WLF, de Brito LCN, Henriques LCF, *et al.* (2013) The impact of chlorhexidine-based  
33 endodontic treatment on periapical cytokine expression in teeth. *Journal of Endodontics*, **39**, 889-92.  
34

35  
36 Tavares WLF, de Brito LCN, Henriques LCF, *et al.* (2012) Effects of calcium hydroxide on cytokine  
37 expression in endodontic infections. *Journal of Endodontics*, **38**, 1368-71.  
38

39  
40 Torabinejad M, Cotti E, Jung T (1992) Concentrations of leukotriene B4 in symptomatic and  
41 asymptomatic periapical lesions. *Journal of Endodontics*, **18**, 205-8.  
42

43  
44 VanDerMeid KR, Su SP, Krenzer KL, Ward KW, Zhang JZ (2011). A method to extract cytokines and  
45 matrix metalloproteinases from Schirmer strips and analyze using Luminex. *Molecular Vision*, **17**,  
46 1056-63.  
47

48  
49 Vernal R, Dezerega A, Dutzan N, *et al.* (2006) RANKL in human periapical granuloma: possible  
50 involvement in periapical bone destruction. *Oral Diseases*, **12**, 283-9.  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 Viswanathan M, Ansari MT, Berkman ND, *et al.* (2012) Assessing the risk of bias of individual studies  
4 in systematic reviews of health care interventions. *AHRQ Methods for Effective Health Care*. AHRQ  
5 Publication No. 12-EHC047-EF  
6  
7  
8 Vogel C, Marcotte EM (2012). Insights into the regulation of protein abundance from proteomic and  
9 transcriptomic analyses. *Nature Reviews:Genetics*, **13**, 227–32.  
10  
11 Vogel C, de Sousa Abreu R, Ko D, *et al.* (2010). Sequence signatures and mRNA concentration can  
12 explain two-thirds of protein abundance variation in a human cell line. *Molecular Systems Biology*, **6**,  
13 400.  
14  
15  
16  
17  
18 Wahlgren J, Salo T, Teronen O, Luoto H, Sorsa T, Tjäderhane L (2002) Matrix metalloproteinase-8  
19 (MMP-8) in pulpal and periapical inflammation and periapical root-canal exudates. *International*  
20 *Endodontic Journal*, **35**, 897-904.  
21  
22  
23  
24 Wang Q, Zhou XD, Zheng QH, Wang Y, Tang L, Huang DM (2010) Distribution of *Porphyromonas*  
25 *gingivalis* fimA genotypes in chronic apical periodontitis associated with symptoms. *Journal of*  
26 *Endodontics*, **36**, 1790-5.  
27  
28  
29  
30 Yamasaki M, Kumazawa M, Kohsaka T, Nakamura H, Kameyama Y (1994) Pulpal and periapical tissue  
31 reactions after experimental pulpal exposure in rats. *Journal of Endodontics*, **20**, 13-7.  
32  
33  
34 Yan PF, Liang JP, Chen WM, Gu SS (2007). Quantitive study of interleukin 1beta in periapical exudates  
35 of chronic periapical periodontitis in the course of root canal therapy. *Shanghai Journal of*  
36 *Stomatology*, **16**, 229-31.  
37  
38  
39  
40 Yu JH, Yu WY, Liu WH, Ye Y (2002) The clinical evaluation of tinidazole-iodoform-phenocamphor paste  
41 as an intra-canal sterilization medication for acute periapical periodontitis. *Shanghai Journal of*  
42 *Stomatology*, **11**, .7-9.  
43  
44  
45  
46 Zhi J, Yu D, Yuan D, Chen J (2017) Interleukin-17 in apical exudates of periapical periodontitis treated  
47 with minocycline controlled-release formulation, *Chinese Journal of Tissue Engineering Research*, **21**,  
48 1508-13.  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## PubMed Search Strategy

Search	Input Query	Items
<b>#1</b>	(((((("apical periodontitis"[Title/Abstract] OR "periapical periodontitis"[MeSH Terms]) OR "acute apical periodontitis"[Title/Abstract]) OR "chronic apical periodontitis"[Title/Abstract]) OR "apical abscess*"[Title/Abstract]) OR "acute apical abscess*"[Title/Abstract]) OR "chronic apical abscess*"[Title/Abstract]) OR "periradicular abscess*"[Title/Abstract]) OR "periapical granuloma"[MeSH Terms]) OR "periapical granuloma*"[Title/Abstract]) OR "apical granuloma*"[Title/Abstract]) OR "periapical lesion*"[Title/Abstract]) OR "apical lesion*"[Title/Abstract]) OR "endodontic lesion*"[Title/Abstract]) OR "endodontic infection*"[Title/Abstract]) OR "periradicular disease*"[Title/Abstract]) OR "infected root canal*"[Title/Abstract] AND "humans"[MeSH Terms]	
<b>#2</b>	("sampling"[Title/Abstract] OR "sampling studies"[MeSH Terms]) OR "sampling studies/methods"[MeSH Terms] AND "humans"[MeSH Terms]	
<b>#3</b>	(((((periradicular[All Fields] AND tissue fluid[Title/Abstract]) OR (periapical[All Fields] AND (tissue exudate[Title/Abstract] OR tissue exudates[Title/Abstract]))) OR "periapical exudate*"[Title/Abstract]) OR "periapical exudate samples"[Title/Abstract]) OR "inflammatory exudate"[Title/Abstract]) OR "exudate"[Title/Abstract]) OR "exudates and transudates"[MeSH Terms] AND "humans"[MeSH Terms]	
<b>#4</b>	(((((("cytokines"[MeSH Terms] OR "cytokine expression"[Title/Abstract]) OR "cytokine profile"[Title/Abstract]) OR "cytokine analysis"[Title/Abstract]) OR "chemokine"[Title/Abstract]) OR "inflammation mediators"[MeSH Terms]) OR "interleukin 1"[MeSH Terms]) OR "interleukin*"[Title/Abstract]) OR "il 1 alpha"[Title/Abstract]) OR "il 1 beta"[Title/Abstract]) OR "il 2"[Title/Abstract]) OR "il 4"[Title/Abstract]) OR "il 6"[Title/Abstract]) OR "il 8"[Title/Abstract]) OR "il 10"[Title/Abstract]) OR "il 12"[Title/Abstract]) OR "il 13"[Title/Abstract]) OR "il 17"[Title/Abstract]) OR "il 23"[Title/Abstract]) OR "tumour necrosis factor"[Title/Abstract]) OR "tnf alpha"[Title/Abstract]) OR "interferon gamma"[MeSH Terms]) OR "ifn gamma"[Title/Abstract]) OR "prostaglandins"[MeSH Terms]) OR "pge2"[Title/Abstract]) OR "leukotriene b4"[MeSH Terms]) OR "ltb4"[Title/Abstract] AND "humans"[MeSH Terms]	
<b>#5</b>	(#1) AND #2	
<b>#6</b>	(#1) AND #3	
<b>#7</b>	(#1) AND #4	
<b>#8</b>	(#2) AND #3	
<b>#9</b>	(#2) AND #4	
<b>#10</b>	(#3) AND #4	
<b>#11</b>	((#1) AND #2) AND #3	
<b>#12</b>	((#1) AND #2) AND #4	
<b>#13</b>	((#1) AND #3) AND #4	
<b>#14</b>	((#2) AND #3) AND #4	
<b>#15</b>	((#1) AND #2) AND #3) AND #4	

\* = truncation term

**Table 1:** PubMed search strategy

Study			Sample		Mediators Studied	Methodology		Results
Author	Objectives	Design	Diagnosis & Size (n)	Tooth Type		PTF Sampling	Assay Technique	
Safavi & Rossomando (1991)	Compare PTF TNF- $\alpha$ levels in teeth with & without apical periodontitis	Cross-sectional	CAP (n=6) NAT (n=5)	Single/multi rooted	TNF- $\alpha$	Paper Points	ELISA	Elevated TNF- $\alpha$ levels in teeth with apical lesions compared to those without.
Matsuo <i>et al.</i> (1994)	Explore correlations between PTF IL-1 $\alpha$ & IL-1 $\beta$ levels & clinical findings in teeth with apical lesions	Cohort	"Apical lesion" (n=69)	Single rooted	IL-1 $\alpha$ & IL-1 $\beta$	Fine Needle Aspiration	ELISA	Pus containing PTF had higher IL-1 $\alpha$ (p<0.01) & larger lesions showed higher IL-1 $\beta$ than IL-1 $\alpha$ (P<0.05). After treatment, IL-1 $\alpha$ levels increased whilst IL-1 $\beta$ decreased.
Matsuo <i>et al.</i> (1995)	Explore correlations between PTF IgG & IgA levels & clinical findings in teeth with apical lesions	Cohort	"Apical lesion" (n=69)	Single rooted	IgG & IgA	Fine Needle Aspiration	ELISA	IgG levels higher than IgA (p<0.01). Larger lesions showed higher IgG & IgA levels (p<0.01). Throughout treatment, IgG & IgA decreased.
Shimauchi <i>et al.</i> (1996)	Develop a quantitative method for collecting & quantifying biomarkers in PTF	Cross-sectional	CAP/AAP (n=29)	-	IL-1 $\beta$	Paper Points	ELISA	There is a curvilinear relationship between absorbed PTF & paper point wetted length (p<0.0001). There is a linear relationship between absorbed and eluted IL-1 $\beta$ (p<0.05).
Takayama <i>et al.</i> (1996)	Explore correlations between PTF PGE <sub>2</sub> levels & clinical findings of teeth with apical periodontitis	Cross-sectional	"Apical lesion" (n=77)	-	PGE <sub>2</sub>	Paper Points	RIA	Higher PGE <sub>2</sub> levels in teeth with radiolucent areas (p<0.05) & acute clinical symptoms (p<0.05). As lesion size increased, PGE <sub>2</sub> levels decreased (p<0.05).
Takeichi <i>et al.</i> (1996)	Quantify PTF levels of PMN derived inflammatory cytokines in teeth with apical periodontitis	Cross-sectional	CAP (n=16)	-	IL-1 $\alpha$ , IL-1 $\beta$ , IL-6 & TNF- $\alpha$	Fine Needle Aspiration	ELISA & qPCR	Purified PMNs from PTF expressed high levels of IL-1 $\alpha$ , IL-1 $\beta$ & TNF- $\alpha$ mRNA (p<0.05). Although IL-6 mRNA was not detected, high IL-6 levels of protein were present.
Shimauchi <i>et al.</i> (1997)	Monitor longitudinal changes in PTF PGE <sub>2</sub> levels in teeth with apical lesions during RCT	Cohort	"Apical lesion" (n=20)	-	PGE <sub>2</sub>	Paper Points	RIA	PGE <sub>2</sub> levels decreased after treatment (p<0.01). Remission of disease associated with reduction in PGE <sub>2</sub> levels (p<0.05).
Shimauchi <i>et al.</i> (1998)	Quantify PTF IL-1 $\beta$ & IL-1ra levels in teeth with apical lesions	Cross-sectional	"Apical lesion" (n=29)	-	IL-1 $\beta$ & IL-1ra	Paper Points	ELISA	High levels of IL-1ra, compared with IL-1 $\beta$ , and positive correlations between IL-1ra & IL-1 $\beta$ levels found (P<0.05). IL-1ra:IL-1 $\beta$ ratio from symptomatic lesions lower than asymptomatic lesions (p<0.05).

	Author (Year)	Objective	Design	Sample	Interventions	Measurements	Methods	Results	
1	Takeichi <i>et al.</i> (1998)	Quantify PTF NO levels in teeth with apical periodontitis	Cross-sectional	CAP/AAP (n=30)	-	NO	Fine Needle Aspiration	Colorimetric	PMNs can spontaneously produce NO at the site of chronic infection
2									
3	Kuo <i>et al.</i> (1998a)	Quantify PTF $\beta$ G, IL-1 $\beta$ , IgG, IgA & IgM levels in teeth with apical lesions and explore correlations to clinical findings	Cohort	"Apical lesion" (n=32)	Single/multi rooted	$\beta$ G, IL-1 $\beta$ , IgG, IgA & IgM	Methylcellulose filter paper strips	ELISA	Higher $\beta$ G & IL-1 $\beta$ levels in pus containing PTF (p<0.05) & larger lesions (p<0.05). Higher IgM in lesions with a sinus tract or swelling (IgM).
4									
5									
6									
7									
8	Kuo <i>et al.</i> (1998b)	Monitor longitudinal changes in PTF $\beta$ G, IL-1 $\beta$ , IgG, IgA & IgM levels when accessing the root canal of teeth with apical lesions	Cohort	"Apical lesion" (n=32)	Single/multi rooted	$\beta$ G, IL-1 $\beta$ , IgG, IgA & IgM	Methylcellulose filter paper strips	ELISA	Mediator activity in less involved teeth did not change after treatment however, $\beta$ G & IL-1 $\beta$ levels in teeth with pus containing PTF decreased after treatment whereas IgA & IgM increased (p<0.05).
9									
10									
11									
12									
13	Shimauchi <i>et al.</i> (2001)	Quantify PTF IL-8 & NO levels in teeth with apical lesions	Cross-sectional	"Apical lesion" (n=27)	-	IL-8 & NO	Paper Points	ELISA & Colorimetric	IL-8 levels higher in teeth with pus containing PTF (p<0.01) and clinical symptoms (p<0.05). A positive correlation was found between IL-8 and NO (p<0.001).
14									
15									
16									
17									
18	Ataoğlu <i>et al.</i> (2002)	Quantify PTF IL-1 $\beta$ & TNF- $\alpha$ levels in teeth with apical lesions and explore correlation to clinical findings	Cross-sectional	"Apical lesion" (n=35)	Single rooted	IL-1 $\beta$ & TNF- $\alpha$	Paper Points	ELISA	IL-1 $\beta$ levels 12-fold higher than TNF- $\alpha$ however no significant correlation found between these mediators (p>0.05). High IL-1 $\beta$ levels associated with large lesions (p<0.05).
19									
20									
21									
22									
23	Wahlgren <i>et al.</i> (2002)	Quantify PTF & pulp MMPP-8 levels in teeth with necrotic pulps & apical lesions & monitor longitudinal changes during RCT	Cohort	CAP/AAP (n=11) NAT (n=10)	Single rooted	MMP-8	Paper Points	IFMA	MMP-8 levels decreased after treatment (p<0.05)
24									
25									
26									
27	Liu <i>et al.</i> (2002)	Monitor longitudinal changes in PTF PGE <sub>2</sub> levels in teeth with AAP during RCT	Cohort	AAP (n=25)	Single/multi rooted	PGE <sub>2</sub>	Paper Points	RIA	PGE <sub>2</sub> levels decreased after treatment (p<0.0001)
28									
29									
30	Alptekin <i>et al.</i> (2005a)	Explore correlations between PTF NE & PGE <sub>2</sub> levels & clinical findings in teeth with AAP & monitor longitudinal changes during RCT	Cohort	AAP (n=31)	Single rooted	PGE <sub>2</sub> & NE	Paper Points	ELISA	PGE <sub>2</sub> & NE levels higher in teeth with clinical symptoms (p<0.05) however their levels did not change after treatment (p>0.05)
31									
32									
33									
34									
35	Alptekin <i>et al.</i> (2005b)	Explore correlations between PTF NE levels & clinical findings in teeth with AAP	Cross-sectional	AAP (n=31)	Single rooted	NE	Paper Points	ELISA	Higher NE levels in teeth with clinical symptoms (p<0.05)
36									
37									
38									
39	Pezelj-Ribarić <i>et al.</i> (2007)	Quantify PTF TNF- $\alpha$ levels in teeth with apical periodontitis & explore correlations to clinical findings	Cross-sectional	AAP (n=20) CAP (n=40)	Single rooted	TNF- $\alpha$	Paper Points	ELISA	Higher TNF- $\alpha$ levels in teeth with larger lesions (p<0.05)
40									
41									
42									
43									
44									
45									
46									

1	Henriques <i>et al.</i> (2011)	Compare PTF inflammatory cytokine levels in teeth with & without apical lesions refractory to endodontic treatment	Cross-sectional	CAP (n=20) NAT (n=20)	-	IL-1 $\beta$ , IL-17A, IFN- $\gamma$ , IL-10, TNF- $\alpha$ & MCP-1	Paper Points	qPCR	Higher IFN- $\gamma$ , TNF- $\alpha$ , IL-17A & MCP-1 mRNA expression in secondary lesions (p<0.05). No difference in IL-1 $\beta$ mRNA expression (p>0.05) between groups & IL-10 was insignificant in both groups (p>0.05).
2									
3									
4	Shahriari <i>et al.</i> (2011)	Evaluate impact of Ibuprofen on PTF IL-1 $\beta$ , TNF- $\alpha$ & PGE <sub>2</sub> levels in teeth with apical lesions undergoing RCT	Randomised controlled trial	CAP/AAP (n=30)	-	IL-1 $\beta$ , TNF- $\alpha$ & PGE <sub>2</sub>	Paper Points	ELISA	PGE <sub>2</sub> levels reduced after treatment in Ibuprofen group only (p<0.05). No significant difference in IL-1 $\beta$ & TNF- $\alpha$ reduction between groups before & after treatment (p>0.05).
5									
6									
7									
8	de Brito <i>et al.</i> (2012)	Quantify PTF CD4+CD28+ & CD8+ derived inflammatory cytokine mRNA expression in teeth with CAP & monitor longitudinal changes during RCT	Cohort	CAP (n=20)	Single/multi rooted	IL-1 $\beta$ , IL-10, IL-17A, TNF- $\alpha$ , IFN- $\gamma$ , MCP-1, MIP-1 $\beta$ , RANTES, RANKL, CXCR4, & CCR5	Paper Points	qPCR	IFN- $\gamma$ , IL-1 $\beta$ , RANKL & RANTES mRNA expression reduced (p<0.05) & increase in IL-10 & CxCR4 mRNA expression after treatment (p<0.05). No difference in TNF- $\alpha$ , IL-17A, MCP-1, MIP-1 $\beta$ & CCR5 levels after treatment.
9									
10									
11									
12									
13									
14	Ehsani <i>et al.</i> (2012)	Evaluate impact of Ibuprofen & N-acetylcysteine on PTF TNF- $\alpha$ , IL-6 and IL-17A levels in teeth with CAP & explore correlations to clinical findings	Randomised controlled trial	CAP (n=80)	Single/multi rooted	TNF- $\alpha$ , IL-6 & IL-17	Paper Points	ELISA	IL-6 levels reduced in Ibuprofen group (p<0.05) and IL-17A levels reduced in ibuprofen/NAC group (p<0.05). No difference in detected in TNF- $\alpha$
15									
16									
17									
18									
19	Tavares <i>et al.</i> (2012)	Evaluate impact of calcium hydroxide dressing on PTF inflammatory cytokine mRNA expression in teeth with CAP	Non-randomised controlled trial	CAP (n=20)	Single/multi rooted	IL-1 $\beta$ , IL-10 IL-17A, TNF- $\alpha$ , IFN- $\gamma$ & MCP-1	Paper Points	qPCR	IL-1 $\beta$ , IL-10, IFN- $\gamma$ mRNA expression in teeth receiving a calcium hydroxide dressing lower than in those that did not (p<0.05).
20									
21									
22									
23	Grga <i>et al.</i> (2013)	Monitor longitudinal changes in PTF PGE <sub>2</sub> levels during RCT in vital teeth with or without a large restorations	Cohort	NAT (n=47)	Single rooted	PGE <sub>2</sub>	Paper Points	RIA	PGE <sub>2</sub> levels in teeth with large restorations higher after treatment than in intact teeth (p<0.05)
24									
25									
26									
27	Tavares <i>et al.</i> (2013)	Evaluate impact of chlorhexidine dressing on PTF inflammatory cytokine mRNA expression in teeth with CAP	Non-randomised controlled trial	CAP (n=20)	Single/multi rooted	IL-1 $\beta$ , IL-17A, IL-10, TNF- $\alpha$ , IFN- $\gamma$ & MCP-1	Paper Points	qPCR	IL-1 $\beta$ , IL-10, MCP-1 & IFN- $\gamma$ mRNA expression increased after treatment in teeth with no dressing than in those which received a Chlorhexidine dressing (p<0.05).
28									
29									
30									
31									
32	Rechenberg <i>et al.</i> (2014)	Compare PTF RANKL, OPG & IL-8 levels in teeth with irreversible pulpitis to those with AAP	Cross-sectional	AAP (n=27) NAT (n=21)	Single/multi rooted	IL-8, RANKL & OPG	Paper Points	ELISA	Higher RANKL in irreversible pulpitis than apical periodontitis (p<0.05) and lower IL-8 levels in irreversible pulpitis than apical periodontitis (p<0.05)
33									
34									
35									
36									
37	Bambirra <i>et al.</i> (2015)	Monitor longitudinal changes in PTF inflammatory mediator mRNA expression in teeth with CAP during RCT	Cohort	CAP (n=20)	Single/multi rooted	IL-1 $\beta$ , IL-8, IL-10, IL-17A, TNF- $\alpha$ , IFN- $\gamma$ , MCP-1, MIP-1 $\beta$ , ITGA2, HSP47, OPN & FAK	Paper Points	qPCR	Reduction in mRNA expression of all inflammatory mediators after treatment (p<0.05)
38									
39									
40									
41									
42									
43									
44									
45									
46									

	Author (Year)	Objective	Cohort	Sample Size	Study Design	Interventions	Outcome Measures	Assay	Findings
1	de Brito <i>et al.</i> (2015)	Compare PTF CD4+CD28+ & CD8+ derived cytokine mRNA expression in patients with & without HIV	Cohort	CAP (n=53)	Single/multi rooted	IL-1 $\beta$ , IL-10, IL-17A, TNF- $\alpha$ , IFN- $\gamma$ , MCP-1, MIP-1 $\beta$ , RANKL, RANTES, CXCR4 & CCR5	Paper Points	qPCR	Increased IL-10 & CXCR4 mRNA expression & reduced RANKL, IFN- $\gamma$ , IL-1 $\beta$ & RANTES after treatment in HIV negative patients (p<0.05). Increased RANKL, IFN- $\gamma$ , IL-1 $\beta$ , TNF- $\alpha$ , IL-17A, RANTES & CXCR4 mRNA expression after treatment in HIV positive patients (p<0.05).
5	Ferreira <i>et al.</i> (2015)	Compare PTF inflammatory cytokine mRNA expression in patients with & without SCA	Cross-sectional	CAP (n=36)	-	IL-1 $\beta$ , IL-10, IL-17A, TNF- $\alpha$ , IFN- $\gamma$ , MCP-1, MIP-1 $\beta$ & RANKL	Paper Points	qPCR	No significant difference observed in inflammatory mRNA expression in SCA positive & SCA negative patients after treatment (p>0.05)
10	Martinho <i>et al.</i> (2015)	Evaluate impact of different interappointment dressing materials on PTF levels of Th1-type and Th2-type cytokines in teeth with CAP	Randomised controlled trial	CAP (n=30)	Single rooted	TNF- $\alpha$ , IFN- $\gamma$ , IL-2, IL-4, IL-5 & IL-13	Paper Points	ELISA	Lower IL-2, TNF- $\alpha$ , IFN- $\gamma$ levels and higher IL-4, IL-5 & IL-13 levels after use of dressing (p<0.05). No difference observed between types of dressings (p>0.05).
15	Martinho <i>et al.</i> (2016)	Quantify PTF levels of MMPs, TIMPs and MMP/TIMP complexes in teeth with CAP and explore their correlation to clinical findings	Cross-sectional	CAP (n=20)	Single rooted	MMP1, MMP2, MMP9, TIMP1, TIMP2, MMP1,2,9/TIMP1,2	Paper Points	ELISA	Higher MMP1, -2 & -9 in teeth with larger lesions (p<0.05). Higher MMP-1 levels decreased chances of TTP, whereas MMP-9 increased chances of TTP (p<0.05).
21	Sette-Dias <i>et al.</i> (2016)	Compare PTF levels of inflammatory cytokines in teeth with AAA to those with NAT	Cross-sectional	AAA (n=12) NAT (n=12)	Single/multi rooted	IL-1 $\beta$ , IL-8, IL-10, IL-17A, TNF- $\alpha$ , IFN- $\gamma$ , MCP-1, MIP-1 $\beta$ & TGF- $\beta$	Paper Points	qPCR	Higher IFN- $\gamma$ , IL-1 $\beta$ , TNF- $\alpha$ , IL-17A, IL-8, MCP-1 mRNA expression in odontogenic infections (p<0.05)
26	Zhi <i>et al.</i> (2016)	Evaluate impact of minocycline interappointment dressing on PTF levels of IL-7A in teeth with AAP	Randomised controlled trial	AAP (n=16) NAT (n=16)	-	IL-17A	Paper Points	CBA	Lower IL-17A levels in calcium hydroxide and minocycline groups after treatment (p<0.05)

**Table 2:** Characteristics of all studies that met the inclusion criteria (AAP: acute apical periodontitis, CAP: chronic apical periodontitis, CBA: cytometric bead array, CCR5: chemokine receptor type 5, CXCR4: chemokine receptor type 4, ELISA: enzyme linked immunosorbant assay, FAK: focal adhesion kinase, HSP: heat shock protein, IFMA: immunofluorometric assay, IFN: interferon, Ig: immunoglobulin, IL: interleukin, IL-1ra: interleukin-1 receptor agonist, ITGA2: alpha-2-integrin, MCP: monocyte chemoattractant protein, MIP: macrophage inflammatory protein, MMP: matrix metalloproteinase, NAT: normal apical tissues, NE: neutrophil elastase, NO: nitrous oxide, OPG: osteoprotegerin, OPN: osteopontin, PGE<sub>2</sub>: prostaglandin-E2, PMN: polymorphonuclear cell, PTF: periradicular tissue fluid, qPCR: quantitative polymerase chain reaction, RANKL: receptor activator of nuclear factor kappa-B ligand, RANTES: regulated on activation normal T cell expressed and secreted, RCT: root canal treatment, RIA: radioimmunoassay, TGF: transforming growth factor, TIMP: tissue inhibitor of metalloproteinase, TNF: tumour necrosis factor,  $\beta$ G:  $\beta$ -glucuronidase & -: not reported )

Technique	Study	Parameters used to sample PTF via root canals				Timing of PTF samples				Parameters used to elute & prepare PTF samples			
		Brand	Size (ISO)	Insertion Depth	Time (s)	Samples/ Tooth	Baseline	Subsequent	Assay Tech.	Elution Buffer	Incubated (mins)	Vortexed (secs)	Centrifuged (mins)
1													
2													
3	Safavi & Rossomando (1991)	-	-	WL	-	1	Before Instrumenting	-	ELISA	PBS + Tween 20 & FCS (100 µl)	60	-	-
4	Shimauchi <i>et al.</i> (1996)	Kerr	40	WL	30	1	Before Instrumenting	-	ELISA	PBS + Tween 20 (?? µl)	-	-	10 @ 10 000 g
5	Takayama <i>et al.</i> (1996)	Kerr	40	WL	30	1	After Instrumenting	-	RAI	PBS (150 µl)	-	30	10 @ 5 000 g
6	Shimauchi <i>et al.</i> (1997)	Kerr	40	WL	30	2	After Instrumenting	7-10 days	RAI	PBS (150 µl)	-	30	10 @ 5 000 g
7	Shimauchi <i>et al.</i> (1998)	Kerr	40	WL	30	1	After Instrumenting	-	ELISA	PBS (150 µl)	-	30	10 @ 5 000 g
8	Shimauchi <i>et al.</i> (2001)	Kerr	40	WL	30	1	After Instrumenting	-	CA	PBS (150 µl)	-	30	10 @ 5 000 g
9	Ataoğlu <i>et al.</i> (2002)	Kerr	40	WL	30	1	After Instrumenting	-	ELISA	PBS (250 µl)	-	60	-
10	Wahlgren <i>et al.</i> (2002)	-	-	WL	120	3	After Instrumenting	14 days	IFMA	Tris + HCl (50 µl)	180	-	-
11	Liu <i>et al.</i> (2003)	Dentsply-Mai.	30	WL	30	2	After Instrumenting	10-12 days	RIA	-	-	-	30 @ 4 000 g
12	Alptekin <i>et al.</i> (2005a)	Kerr	40	WL	30	2	After Instrumenting	7-10 days	ELISA	PBS – BSA (250 µl)	-	60	-
13	Alptekin <i>et al.</i> (2005b)	Kerr	40	WL	30	1	After Instrumenting	-	ELISA	PBS – BSA (250 µl)	-	60	-
14	Pezelj-Ribarić <i>et al.</i> (2007)	-	-	WL	60	1	After Instrumenting	-	ELISA	PBS (?? µl)	-	-	-
15	Henriques <i>et al.</i> (2011)	-	40	2 mm past WL	60	1	After Instrumenting	-	qPCR	TRIZOL (?? µl)	10	-	25 @ 12 000 g
16	Shahriari <i>et al.</i> (2011)	Ariadent	30	WL	30	2	After Instrumenting	4 days	ELISA	PBS (250 µl)	-	60	-
17	de Brito <i>et al.</i> (2012)	-	-	2 mm past WL	60	2	After Instrumenting	7 days	qPCR	TRIZOL (?? µl)	-	-	-
18	Ehsani <i>et al.</i> (2012)	Kerr	40	WL	30	1	After Instrumenting	-	ELISA	PBS (300 µl)	-	60	-
19	Tavares <i>et al.</i> (2012)	-	-	2 mm past WL	60	2	After Instrumenting	15 days	qPCR	TRIZOL (?? µl)	10	-	25 @ 12 000 g
20	Grga <i>et al.</i> (2013)	Kerr	40	WL	30	2	After Instrumenting	3 days	RIA	PBS (150 µl)	-	30	10 @ 5 000 g
21	Tavares <i>et al.</i> (2013)	-	-	2 mm past WL	60	2	After Instrumenting	15 days	qPCR	TRIZOL (?? µl)	10	-	25 @ 12 000 g
22	Rechenberg <i>et al.</i> (2014)	Orbis	-	2 mm past WL	30	1	After Instrumenting	-	ELISA	PBS (300 µl)	300	30	10 @ ?? g
23	Bambirra <i>et al.</i> (2015)	-	-	2 mm past WL	60	2	After Instrumenting	7 days	qPCR	TRIZOL (?? µl)	10	-	25 @ 12 000 g
24	de Brito <i>et al.</i> (2015)	-	-	2 mm past WL	60	2	After Instrumenting	7 days	qPCR	TRIZOL (?? µl)	10	-	25 @ 12 000 g
25	Ferreira <i>et al.</i> (2015)	-	-	2 mm past WL	60	1	After Instrumenting	-	qPCR	TRIZOL (?? µl)	10	-	25 @ 12 000 g
26	Martinho <i>et al.</i> (2015)	Dentsply-Mai.	15	2 mm past WL	60	2	After Instrumenting	14 days	ELISA	-	-	-	-
27	Martinho <i>et al.</i> (2016)	Dentsply-Mai.	15	2 mm past WL	60	1	After Instrumenting	-	ELISA	-	-	-	-
28	Sette-Dias <i>et al.</i> (2016)	-	-	2 mm past WL	60	2	After Instrumenting	14 days	qPCR	TRIZOL (?? µl)	10	-	25 @ 12 000 g
29	Zhi <i>et al.</i> (2016)	-	30	WL	30	2	After Instrumenting	7 days	CBA	-	-	-	30 @ 4 000 g
30													
31													
32													
33													
34													
35													
36													
37													
38													
39													
40													
41													
42													
43													
44													
45													
46													

**Table 3:** Basic parameters of the sampling methods used to retrieve periradicular tissue fluid via the root canal. (-: not reported, WL: working length, BSA: bovine serum albumin, FCS: fetal calf



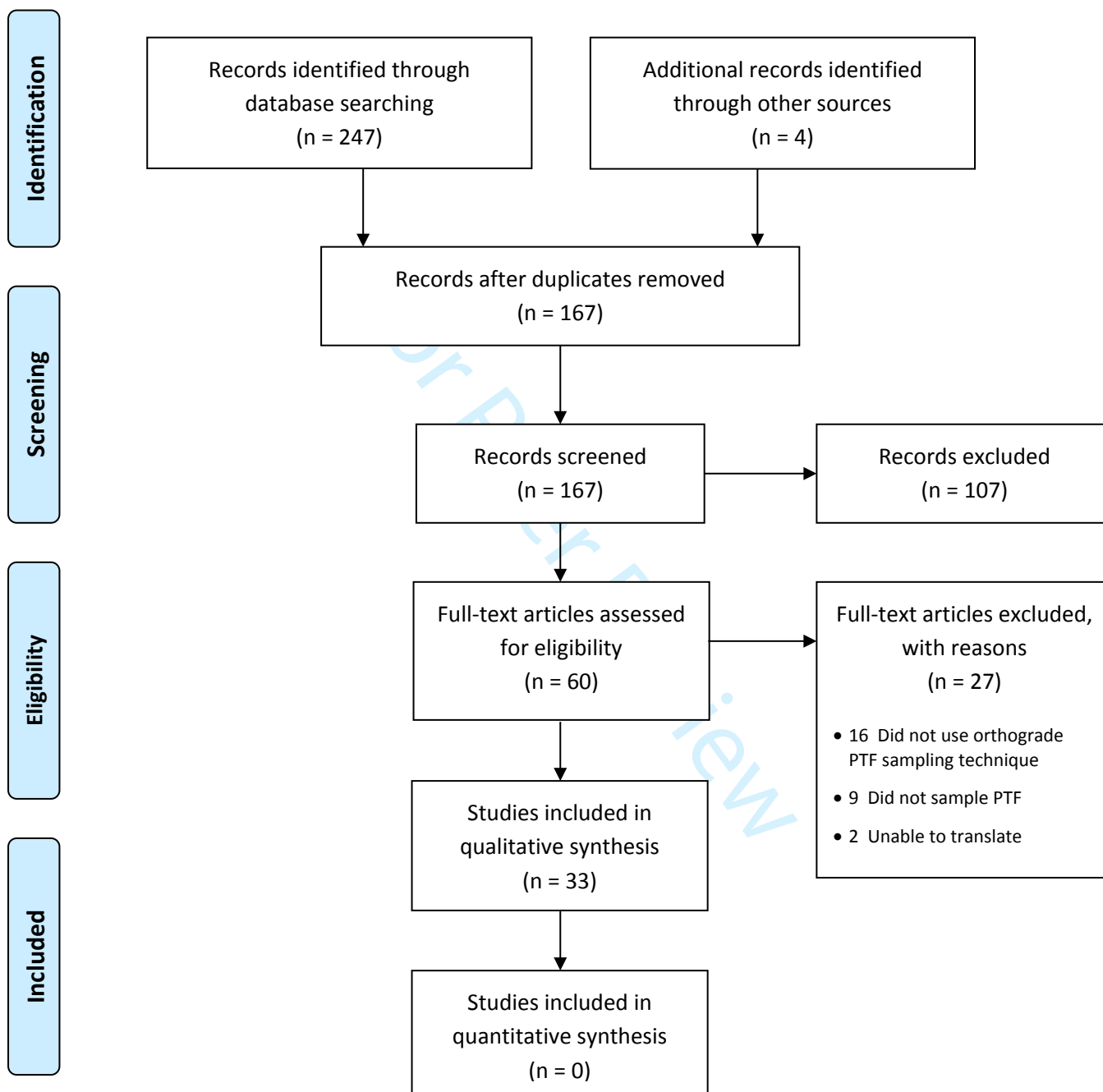
serum, HCl: hydrochloric acid, PBS: phosphate buffered solution, PTF: periradicular tissue fluid, Tris: trisaminomethane, g: gravity force, ELISA: enzyme-linked-immunosorbant assay, <sup>125</sup>I: radioimmunoassay, CA: colorimetric assay, IMFA: immunofluorometric assay, CBA: cytometric-bead-array, qPCR: quantitative polymerise chain reaction )

2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46

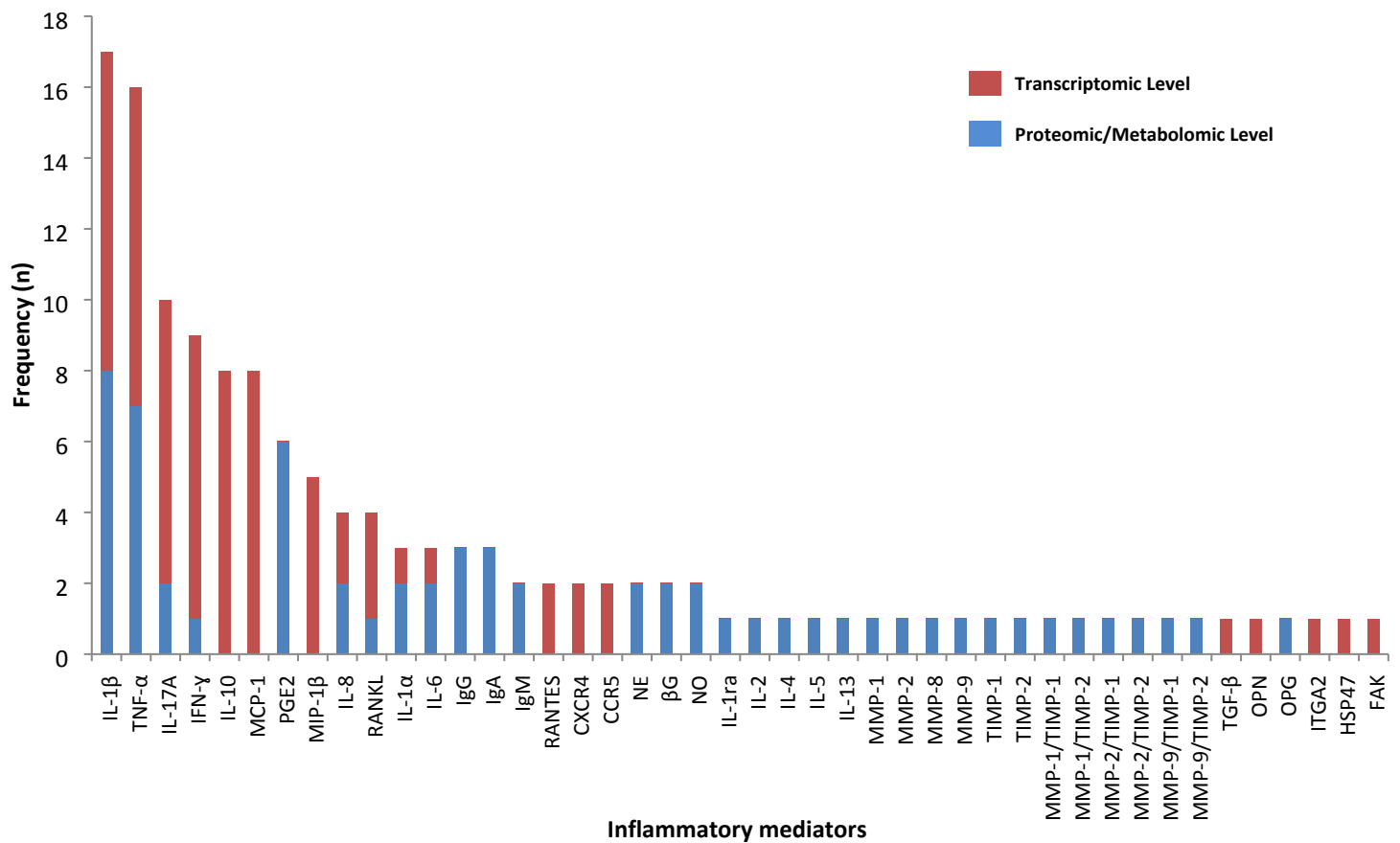
For Peer Review



## PRISMA 2009 Flow Diagram



**Figure 1:** PRISMA flow diagram depicting the flow of information through the phases of the systematic review



**Figure 2:** The types of inflammatory mediators targeted and the frequency at which they were studied at a proteomic/metabolomic and transcriptomic level (CCR5: chemokine receptor type 5, CXCR4: chemokine receptor type 4, FAK: focal adhesion kinase, HSP: heat shock protein, IFN: interferon, Ig: immunoglobulin, IL: interleukin, IL-1ra: interleukin-1 receptor agonist, ITGA2: alpha-2-integrin, MCP: monocyte chemoattractant protein, MIP: macrophage inflammatory protein, MMP: matrix metalloproteinase, NE: neutrophil elastase, NO: nitrous oxide, OPG: osteoprotegerin, OPN: osteopontin, PGE<sub>2</sub>: prostaglandin-E<sub>2</sub>, RANKL: receptor activator of nuclear factor kappa-B ligand, RANTES: regulated on activation normal T cell expressed and secreted, TGF: transforming growth factor, TIMP: tissue inhibitor of metalloproteinase, TNF: tumour necrosis factor, βG: β-glucuronidase & -: not reported)

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For Peer Review

	Study	Reason for Exclusion
1	Rocca <i>et al.</i> (1987)	Did not sample periradicular tissue fluid
2	Barkhordar <i>et al.</i> (1999)	Did not use orthograde sampling technique
3	Noda <i>et al.</i> (2000)	Did not sample periradicular tissue fluid
4	Yu <i>et al.</i> (2002)	Unable to attain access to full text
5	Siqueira & Rôças (2003)	Did not use orthograde sampling technique
6	Siqueira & Rôças (2004)	Did not use orthograde sampling technique
7	Silva <i>et al.</i> (2005)	Did not use orthograde sampling technique
8	Vernal <i>et al.</i> (2006)	Did not use orthograde sampling technique
9	Machado de Oliveira <i>et al.</i> (2007)	Did not use orthograde sampling technique
10	Yan <i>et al.</i> (2007)	Unable to attain access to full text
11	Nonaka <i>et al.</i> (2008)	Did not use orthograde sampling technique
12	Soares <i>et al.</i> (2008)	Did not sample periradicular tissue fluid
13	Gazivoda <i>et al.</i> (2009)	Did not use orthograde sampling technique
14	Dezerega <i>et al.</i> (2010)	Did not use orthograde sampling technique
15	Wang <i>et al.</i> (2010)	Did not sample periradicular tissue fluid
16	Ferreira <i>et al.</i> (2011)	Did not use orthograde sampling technique
17	Martinho <i>et al.</i> (2012)	Did not sample periradicular tissue fluid
18	Amaya <i>et al.</i> (2013)	Did not sample periradicular tissue fluid
19	Hernádi <i>et al.</i> (2013)	Did not use orthograde sampling technique
20	Provenzano <i>et al.</i> (2013)	Did not use orthograde sampling technique
21	Araujo-Pires <i>et al.</i> (2014)	Did not use orthograde sampling technique
22	Sousa <i>et al.</i> (2014)	Did not use orthograde sampling technique
23	Keleş & Alçin (2015)	Case report
24	Baeza <i>et al.</i> (2016)	Did not sample periradicular tissue fluid
25	Carvalho <i>et al.</i> (2016)	Did not sample periradicular tissue fluid
26	Alfenas <i>et al.</i> (2017)	Did not use orthograde sampling technique
27	Pourhajibagher <i>et al.</i> (2017)	Did not sample periradicular tissue fluid

### Appendix S1: Excluded articles at full-text evaluation with reason

Bias Domain	Criterion	RCTs	CCTs	Cohort	Cross-section
<b>Selection</b>	Was the allocation sequence generated adequately (e.g., random number table, computer-generated randomization)?	x			
	Was the allocation of treatment adequately concealed (e.g., pharmacy- controlled randomization or use of sequentially numbered sealed envelopes)?	x			
	Were participants analysed within the groups they were originally assigned to?	x	x		
	Did the study apply inclusion/exclusion criteria uniformly to all groups?	x	x	x	x
	Did the strategy for recruiting participants into the study differ across study groups?	x	x	x	x
	Does the design or analysis control account for important confounding and modifying variables through matching, stratification, multivariable analysis, or other approaches?	x	x	x	x
<b>Performance</b>	Did researchers rule out any impact from a concurrent intervention or an unintended exposure that might bias results?	x	x		
	Did the study maintain fidelity to the intervention protocol?	x	x	x	x
<b>Attrition</b>	If attrition (overall or differential nonresponse, dropout, loss to follow-up, or exclusion of participants) was a concern, were missing data handled appropriately (e.g., intention-to-treat analysis and imputation)?	x	x	x	x
<b>Detection</b>	Were interventions implemented consistently across all study participants?	x	x		
	In prospective studies, was the length of follow-up different between the groups?	x	x	x	
	Were the outcome assessors blinded to the intervention or exposure status of participants?	x	x	x	x
	Were outcomes assessed using valid measures and implemented consistently across all study participants?	x	x	x	x
<b>Reporting</b>	Were the potential outcomes pre-specified by the researchers? Are all pre-specified outcomes reported?	x	x	x	x

**Appendix S2** – Design specific criteria used for assessing the bias in studies which met the inclusion criteria in this systematic review. Table has been adapted and modified from Viswanathan *et al.* 2012. (RCT = randomised controlled trial, CCT = controlled clinical trial) overall

Study	Selection	Performance	Attrition	Detection	Reporting	Overall Bias
Safavi & Rossomando (1991)	+	+	+	-	?	Medium
Matsuo <i>et al.</i> (1994)	+	+	+	?	?	Medium
Matsuo <i>et al.</i> (1995)	+	+	+	?	?	Medium
Shimauchi <i>et al.</i> (1996)	?	?	?	-	?	High
Takayama <i>et al.</i> (1996)	+	+	+	-	?	Medium
Takeichi <i>et al.</i> (1996)	+	+	+	?	?	Medium
Shimauchi <i>et al.</i> (1997)	-	-	+	-	?	High
Shimauchi <i>et al.</i> (1998)	-	+	+	-	?	Medium
Takeichi <i>et al.</i> (1998)	+	?	+	?	?	Medium
Kuo <i>et al.</i> (1998a)	+	-	+	-	?	Medium
Kuo <i>et al.</i> (1998b)	+	-	+	-	?	Medium
Shimauchi <i>et al.</i> (2001)	-	+	+	-	?	Medium
Ataoglu <i>et al.</i> (2002)	+	-	?	-	?	High
Wahlgren <i>et al.</i> (2002)	+	+	?	+	?	Medium
Liu <i>et al.</i> (2002)	+	+	+	-	?	Medium
Alptekin <i>et al.</i> (2005a)	+	-	+	-	?	Medium
Alptekin <i>et al.</i> (2005b)	+	-	+	-	?	Medium
Pezelj-Ribarić <i>et al.</i> (2007)	?	+	+	-	?	Medium
Henriques <i>et al.</i> (2011)	+	+	+	+	?	Low
Shariar <i>et al.</i> (2011)	?	-	+	+	?	Medium
de Brito <i>et al.</i> (2012)	+	+	+	?	?	Medium
Ehsani <i>et al.</i> (2012)	?	-	+	+	+	Medium
Tavares <i>et al.</i> (2012)	+	+	+	+	?	Low
Grga <i>et al.</i> (2012)	+	+	+	+	?	Low
Tavares <i>et al.</i> (2013)	+	+	+	-	?	Medium
Rechenberg <i>et al.</i> (2014)	+	?	+	-	?	Medium
Bambirra <i>et al.</i> (2015)	+	?	+	-	?	Medium
de Brito <i>et al.</i> (2015)	-	?	+	-	?	Low
Ferreira <i>et al.</i> (2015)	-	?	+	-	?	Low
Martinho <i>et al.</i> (2015)	?	+	+	+	?	Medium
Martinho <i>et al.</i> (2016)	+	+	+	+	?	Low
Sette-Dias <i>et al.</i> (2016)	-	-	+	-	?	High
Zhi <i>et al.</i> (2017)	?	+	+	+	?	Medium