Azithromycin as an Adjunct to Subgingival Professional Mechanical Plaque Removal in the Treatment of Grade C Periodontitis: A Systematic Review and Meta-analysis

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Abstract

Background: Although uncommon, Grade C Periodontitis (previously termed aggressive periodontitis) has significant implications for those affected. The use of antimicrobials as an adjunct to sub gingival professional mechanical plaque removal (PMPR) has been found to be beneficial in such cases. There are a number of guidelines in which the recommended first choice adjunctive antimicrobial regime is a combination of amoxicillin and metronidazole; however, this regime is inappropriate for patients with a perceived or true allergy to penicillin and due to the number of tablets, requires increased patient compliance. The macrolide, azithromycin, is used more infrequently but has been proposed as the second-choice antimicrobial regime, with advantages of improved tissue penetration and longer half-life contributing to a potential increase in patient compliance due to the reduced number of tablets required.

Objectives: The aim of this systematic review was to evaluate the use of azithromycin as an adjunct to non-surgical sub gingival PMPR in the treatment of rapidly progressing periodontal disease.

Search Strategy: Online database searches using high-level MeSH terms in a PICO structure were conducted for the Cochrane Library, Web of Science, SCOPUS, Medline via Ovid, CINAHL and OpenGrey. Supplementary searches of ongoing clinical trials were also conducted. Hand searching of relevant journals included Periodontology 2000, Journal of Clinical Periodontology and Journal of Periodontology.

Eligibility criteria: Studies selected for review included those where participants were diagnosed with Grade C Periodontitis (alternative terms included rapidly progressing periodontitis, aggressive periodontitis, early onset periodontitis and juvenile periodontitis) and underwent non-surgical periodontal therapy (involving subgingival PMPR) with adjunctive azithromycin, compared to those who had an alternative antibiotic regime or a placebo. Outcome measures were surrogate markers of periodontal disease including probing pocket depths, clinical attachment levels and bleeding on probing. Microbiological outcomes, adverse events and patient reported outcomes measures were to be looked at if available.

Study Appraisal and Data Analysis: Titles and abstracts of all studies identified through conducted searches were independently reviewed by the two authors. The full texts were

then independently reviewed of the studies appearing to meet the inclusion criteria. Any disagreements were resolved through discussion between the two authors.

Results: 122 studies were identified from electronic and hand searching (after duplicate removal). Of these, 6 were included for qualitative analysis and 4 of which were then included for quantitative analysis in a meta-analysis. For studies included in the meta-analysis, three were deemed at low risk of bias and one at serious risk of bias. There were conflicting results on whether azithromycin reduced the number of subgingival pathogens or detectable subgingival *A. actinomycetemcomitans* between included studies. The meta-analysis revealed statistically significant probing depth reduction difference in favour of azithromycin to the control at 3 (WMD=-0.39mm, 95% CI [-0.66, -0.13], I^2=0%) and 12 months (WMD=-1.32mm, 95% CI [-1.71, -0.93], I^2=0%). Clinical attachment level change was also statistically significant in favour of azithromycin to the control at 3 (WMD=-0.61mm, 95% CI [-1.13, -0.10], I^2=71%) and 12 months (WMD=-0.88mm, 95% CI [-1.32, -0.44], I^2=0%).

Conclusions: Based upon the results of this meta-analysis, azithromycin offers additional benefits in improvement in certain clinical parameters for subgingival debridement in patients with aggressive periodontitis over the control groups. These results appear to be maintained for up to 12 months after treatment completion. However, due to a lack of well-designed studies, and the limited number of studies included within the meta-analysis, the conclusions that can be drawn from the available evidence are limited. Further well-designed trials are required.

Systematic Review Registration: This systematic review was registered on the International Prospective Register of Systematic Reviews (PROSPERO) (ID:CRD42020168195).

1 Introduction

The terminology used to describe periodontal diseases which are faster in progression and affect a younger cohort of patients has changed frequently throughout the late 20th, and early 21st century. The 1989 World Workshop in Clinical Periodontics classification used the terms 'juvenile periodontitis', 'rapidly progressive periodontitis' and 'pre-pubertal periodontitis', all under the broader category of 'early-onset periodontitis' (American Academy of Periodontology, 1989).

This terminology was then removed all together in the 1999 International Workshop for a Classification of Periodontal Diseases and Conditions (Armitage, 1999) and replaced with the term 'aggressive periodontitis', subcategorised into localised and generalised forms of the disease.

Primary diagnostic features common to the disease included (Lang et al, 1999):

- Patients that are systemically healthy
- Rapid periodontal attachment loss and bone destruction
- Familial aggregation

Secondary diagnostic characteristics that were not always present included (Lang et al, 1999):

- Inconsistent levels of biofilm deposits for the destruction that was present
- Elevated levels of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*
- Phagocyte abnormalities
- Hyperresponsive macrophages

Proposed features common to localised aggressive periodontitis included onset around puberty, localised attachment loss to the first molar and incisor and not affecting more than two other teeth. For generalised aggressive periodontitis, proposed defining features included presentation in patients below 30 years of age, generalised interproximal attachment loss affecting at least 3 permanent teeth other than the first molars and incisors, and an episodic nature of destruction (Albandar, 2014).

Aggressive periodontitis has since been used as the accepted terminology, until the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions.

This most recent classification (Caton et al., 2018) removed the term 'aggressive periodontitis' as it was not believed there was sufficient evidence to support that chronic periodontitis and aggressive periodontitis had different aetiologies and pathophysiologies (Papanou et al., 2018) to be considered as separate disease entities. It is still recognised however that certain forms of periodontitis affect a younger cohort of patients, progress at a faster rate and can present as a molar-incisor pattern (Papanou et al., 2018, Fine et al, 2018). For the purposes of this paper, Grade C Periodontitis will be the terminology used, unless another term is explicitly stated within the referenced article.

Prevalence of Grade C Periodontitis has been shown to vary between geographical location as well as between different ethnicities (Susin et al, 2014). The prevalence is estimated to be, in African populations between 1-5%, for Caucasians in north and mid-

Europe 0.1% and in southern European populations 0.5%. For Caucasians in North America the disease affects 0.1-0.2% but up to 2.6% of the black population (Susin et al, 2014). These prevalence estimates should be interpreted carefully given the wide case definitions used within the studies (Susin et al, 2014). Although Grade C Periodontitis is less prevalent than other plaque induced periodontal disease, previously termed chronic periodontitis (reported to affect 46% of the adult population in the United States (Eke et al, 2015)), it can have profound implications for those affected. Sequalae include tooth loss at an earlier age, leading to prosthetic implications for tooth replacement and an increased restorative burden for patients. The severity of the bone loss associated with Grade C Periodontitis can also complicate and even limit future tooth replacement options.

Alongside effective patient performed plaque control, non-surgical professional mechanical plaque removal therapy (supra and sub gingival) is used as a first-line treatment for all biofilm induced periodontal diseases in order to reduce probing pocket depths and inflammation (Sanz et al, 2020). Systemic antimicrobials were first used as an adjunct to non-surgical treatment in Grade C periodontitis when it was discovered that one of the principle, culturable microbes, *Aggregatibacter actinomycetemcomitans,* could penetrate pocket epithelium, rendering it beyond the effects of mechanical debridement (Deas & Mealey, 2010). In the treatment of Grade C Periodontitis, the use of adjunctive systemic antimicrobials along with non-surgical sub-gingival professional mechanical plaque removal has been shown to improve surrogate periodontal markers including probing pocket depths and clinical attachment levels when compared to non-surgical therapy alone in a number of systematic reviews (Herrera et al, 2002, Rabelo et al, 2015, Keestra et al, 2015, Teughels et al, 2020).

The choice of systemic antimicrobial regimes used as an adjunct have changed over time. Initially, tetracyclines were used as the antimicrobial of choice as they were found to offer improvements in both clinical and microbiological parameters (Slots & Rosling, 1983). Metronidazole, which has proven efficacy against anaerobic microbes, was then tested due to concerns raised around antimicrobial resistance to tetracyclines. In a randomised control trial by Saxén and Asikainen (1993), systemically delivered metronidazole demonstrated improved efficacy in suppression of *Aggregatibacter actinomycetemcomitans (A.actinomycetemcomitans)* in patients with localised aggressive periodontitis, which also appeared to correlate to improved clinical results. Further to this, in 2005, Guerrero et al undertook a randomised placebo-controlled trial to assess the effect of a 7-day adjunctive course of 500mg amoxicillin and 500mg metronidazole (each three times/day) in the treatment of generalised aggressive periodontitis. It was found that the group receiving adjunctive antimicrobials demonstrated statistically significantly improvements in full mouth probing pocket depths at both moderate (4-6mm) (0.4mm, 95% CI 0.1-0.7mm) and deep (≥7mm) (1.4mm, 95% CI 0.8-2mm) sites at 6 months in comparison to the placebo group.

In the United Kingdom, the standard of treatment for Grade C Periodontitis has been non-surgical sub-gingival professional mechanical plaque removal in combination with adjunctive antimicrobials where indicated (British Society of Periodontology and Implant Dentistry, 2016 & Faculty of General Dental Practice (FGDP) (UK)/Faculty of Dental Surgery (FDS), 2020). The choice of antimicrobials is most often 500mg amoxicillin three times/day for 7 days along with 200mg metronidazole three times/day for 7 days as this combination of antimicrobials has been documented most frequently to be the most

effective adjunct (Keestra et al, 2015). For patients allergic to penicillin, 100mg doxycycline once daily for 21days with a 200mg loading dose has been recommended (British Society of Periodontology and Implant Dentistry, 2016).

Although amoxicillin and metronidazole offer a wide range of antimicrobial activity, they are not without issue. Over half of the patients in the test group in the Guerrero et al (2005) study reported some degree of adverse event. Additionally, for the combination therapy of amoxicillin and metronidazole, a total of 42 capsules are required to be taken by the patient. Guerrero et al (2007) later demonstrated that incomplete adherence to this antibiotic regime resulted in poorer clinical outcomes for patients with aggressive periodontal disease compared to those who fully complied with the antimicrobial regime.

It is for these reasons that azithromycin has been suggested as a suitable alternative. The accepted regime for this antimicrobial is 500mg once daily for three days (FGDP UK/FDS, 2020), therefore only requiring the patient to take 3 tablets, theoretically making compliance easier. A retrospective cohort study that reviewed over 16,000 patient case notes from three clinical settings (inpatient clinics, outpatient clinics and orthopedic clinics) found a recorded antibiotic sensitivity prevalence of 9.89%. Penicillins accounted for 42.11% of these, whereas macrolides only accounted for 3.5% (Jourdan et al, 2020). This study was unable to classify the severity of the reactions for each antibiotic due to specific reaction data not being documented in 61.4% of patient records and did not mention the method of delivery for the antibiotics.

Azithromycin is a macrolide antimicrobial providing a broad spectrum of activity against both gram-positive and gram-negative organisms (commonly associated with periodontal disease) (Foulds et al, 1990). As well as its antimicrobial properties, azithromycin has also been shown to demonstrate anti-inflammatory activity (Ianaro et al, 2000). It reaches high levels of tissue concentration quickly and remains at this level for longer due to its extended half-life, hence the need for less frequent dosing (Foulds et al, 1993). Azithromycin is able to penetrate inflamed periodontal tissues (Blandizzi et al, 1999, Gomi et al, 2007) which is thought to be of added benefit when used as an adjunct in the treatment of Grade C Periodontitis due to *A.actinomycetemcomitans*' ability to penetrate periodontal tissues (Deas & Mealey, 2010). It is for these properties that azithromycin has been suggested as a suitable adjunct in the treatment of patients with more rapidly progressing periodontal diseases.

With the term aggressive periodontitis removed from the most recent classification of periodontal and peri-implant diseases and conditions (Caton et al, 2018), clinicians may be unsure in which circumstances systemic antimicrobials can offer additional benefit in conjunction with non-surgical treatment. As mentioned previously, diagnostic criteria for aggressive periodontitis have been an early age of onset, a high rate of disease progression, with a specific pattern affecting the teeth in a systemically healthy individual (Albandar, 2014). Although the new classification (Caton, 2018) does not specifically use the term aggressive periodontitis, these diagnostic criteria are still embedded within the staging and grading based diagnosis.

Staging has been used to classify the extent and severity of the disease at that current time point based upon the measurable extent of destroyed tissue that can be attributed to periodontal disease. Grading is used to give an indication of a patient's susceptibility to periodontal disease, taking into account the cumulative risk factors the patient has been

exposed to throughout their life course. Primary criteria for Grade C disease are a percentage bone loss/age ratio exceeding 1, destruction exceeding expectation for local biofilm presence and specific clinical patterns, for example, molar incisor pattern. One can therefore see that a Grade C (rapidly progressing) disease may closely represent previously termed aggressive periodontitis. The EFP S3 level clinical practice guideline for the treatment of Stage I to III periodontitis, only recommends the adjunctive use of specific systemic antimicrobials in specific patient groups only (Generalised periodontitis Grade C in younger adults) (Sanz et al, 2020) in the second step of periodontal therapy.

Recent guidance from the FGDP and FDS (FGDP UK/FDS, 2020), also recognise this and have recommended to UK based practitioners that "Systemic antimicrobials are only recommended as an adjunct to effective mechanical debridement, oral hygiene instruction and management of modifiable risk factors in patients aged <40-45 years with rapidly progressing periodontal disease". First line antibiotics are recommended as amoxicillin 500mg three times a day and metronidazole 400mg three times a day, both up to 5 days with azithromycin 500mg once a day for 3 days as a second line. This is for Stage 3 or 4 Grade C disease.

The judicious prescribing of antimicrobials is a fundamental requirement of antimicrobial stewardship, and therefore should be based on best available evidence.

Whilst recommendations have been made regarding the use of azithromycin in recent guidelines (FGDP UK/FDS, 2020), there have been no systematic reviews and meta-analysis, to the authors knowledge, looking specifically at treatment outcomes following the use of adjunctive azithromycin in the treatment of Grade C Periodontitis. Therefore, the aim of this systematic review was to investigate whether the adjunctive use of systemic azithromycin improves surrogate outcome measures and microbiological outcomes in the non-surgical treatment of Grade C Periodontitis, in comparison to either no alternative, a placebo or an alternative antimicrobial.

2 Methodology

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement (Moher, 2009). The methodology for the review was registered and reviewed with the International Prospective Register of Systematic Reviews (PROSPERO) (ID:CRD42020168195).

2.1 *Eligibility Criteria*

Participants – Studies were included where participants had an explicit diagnosis of Grade C Periodontitis. Alternative terms included aggressive periodontitis, rapidly progressing periodontitis, early onset periodontitis and juvenile periodontitis. No restrictions were placed on the number of participants, the sex of the participants, the age of the participants or the pattern of disease (either localised or generalised). Studies were excluded where participants had a diagnosis of other forms of periodontal disease or of peri-implantitis.

Interventions – Studies were included where participants' treatment regime included non-surgical periodontal therapy in combination with adjunctive systemic azithromycin.

Studies were excluded where surgical periodontal therapy was undertaken, supragingival scaling alone was undertaken, any antimicrobial other than azithromycin was used as the intervention and where local delivery of azithromycin was given.

Comparators – Studies were included where the aforementioned interventions were compared with a group of participants who received non-surgical periodontal therapy in conjunction with either an adjunctive placebo or alternative antimicrobial, or no adjunct. Again, exclusions were placed on surgical periodontal therapy and local delivery of comparator.

Outcomes – The main outcome measures for studies were surrogate markers of periodontal disease including probing pocket depths, clinical attachment levels and bleeding on probing (specifically, mean changes between baseline and review), microbiological outcomes if available and adverse events. Secondary outcome measures included patient reported outcomes if included within the study. Studies were excluded where that was an absence of pre-operative and post-operative measurements for comparison.

Study Design – Randomised controlled trials, non-randomised controlled trials, cohort studies, case controls and case series undertaken on human subjects were all included. The study required a minimum of 3 months follow up after intervention to be included. Only studies published in English were considered for review.

2.2 *Search Strategy*

Electronic database searches were completed for the Cochrane Library, Web of Science, Scopus, MEDLINE (Medical Literature Analysis and Retrieval System Online, via Ovid) and CINAHL (Cumulative Index to Nursing and Allied Health Literature). The grey literature database opengrey.eu was searched, as well as clinicaltrials.gov to look for ongoing clinical trials. MeSH terms were created using the PICO framework and were adapted for each database individually. An example of the search strategy used for Scopus is summarised in *Table 1*. The searches were completed from the inception of the database through to May 2020.

Hand searching was completed for the following periodontal journals: *Periodontology 2000*, *Journal of Clinical Periodontology* and *Journal of Periodontology* for the same timeframe. Reference lists of included studies were screened for further eligible studies.

Example Search Terms (Scopus)	(TITLE-ABS-KEY (aggressive AND periodontitis) OR TITLE-ABS-KEY (aggressive AND periodontal AND disease) OR TITLE-ABS-KEY (juvenile AND periodontitis) OR TITLE-ABS-KEY (juvenile AND periodontal AND disease) OR TITLE-ABS-KEY (rapidly AND progressing AND periodontitis) OR TITLE-ABS-KEY (rapidly AND progressing AND periodontal AND disease) OR TITLE-

	ABS-KEY (early AND onset AND periodontitis) OR TITLE-ABS-KEY (early AND onset AND periodontal AND disease) OR TITLE-ABS-KEY (grade AND c AND periodontitis) OR TITLE-ABS-KEY (grade AND c AND periodontal AND disease) AND TITLE-ABS-KEY (azithromycin))

Table 1: search strategy using MeSH terms for Scopus database.

2.3 *Study Selection*

The titles and abstracts of all identified studies were screened independently by each of the authors (OJ, PH). For studies appearing to meet the inclusion criteria, or for studies where there was a lack of information to make a decision, the full texts were obtained and reviewed. Full texts were again reviewed independently by each of the authors (OJ, PH) to assess whether the inclusion criteria for the systematic review were met. Disagreements were resolved through discussion and re-evaluation of the article in question. Inter-reviewer agreement was assessed through Cohen's kappa score at both the screening and full text review stages of study selection.

2.4 *Data Collection*

The data collection for included texts was compiled within a computer-based data capture form independently by each reviewer (OJ, PH) and then compared. The data capture form was piloted prior to use to ensure all relevant information was captured for the studies. The following data was collected where available:

- Authors, year of publication and country/setting
- Study design
- Demographics of participants including average age, Male:Female ratio and comorbidities
- The diagnosis given to participants
- Interventions: details of treatment provided, azithromycin regime
- Comparators: details of treatment, regime of comparator if used
- Outcome measures: pocket probing depths (mm), clinical attachment levels (mm), bleeding on probing (%), microbiological outcomes, reported adverse events, patient reported outcomes
- Patients lost to follow up

2.5 *Risk of Bias Assessment*

Validated tools that were appropriate for the study design were used to carry out risk of bias assessments on all included studies. Risk of Bias In Non-randomised Studies-of Interventions (ROBINS-I) tool (Sterne et al, 2016) was used to assess non randomised controlled trial design studies. The Revised Cochrane Risk of Bias tool for randomised

controlled studies (RoB2) (Sterne et al, 2019), was used to assess randomised controlled trials.

The authors (OJ, PH) independently assessed the included studies using the appropriate tool and any differences in outcome were discussed.

Each of these tools looks at specific domains from which bias may arise, ultimately culminating in an overall risk of bias given to the study. The risk of bias was categorised as either low, high or some concerns for the Revised Cochrane risk of bias tool or low, moderate, serious or critical for the ROBINS-I tool, both at study level.

If sufficient studies were eligible within the review (at least 10), publication bias would be assessed visually through the use of a funnel plot (Page et al, 2021).

2.6 *Quality Assessment*

The certainty of any evidence found was assessed using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) Pro Guideline Development Tool (GRADEpro, G. D. T. (2020)).

2.7 *Statistical Analysis*

Included studies would be all be subject to qualitative review, where appropriate studies with sufficient methodological homogeneity would be subject to quantitative review, through a meta-analysis with the use of Review Manager (RevMan, 2014) version 5.3 computer programme.

3 Results

3.1 *Literature Search*

The study selection process is outlined in *Figure* 1, along with reasons for exclusion at the full text review stage. A total of 122 records were identified through online and hand searching after duplicates were removed. A total of 86 articles were excluded upon screening of titles and abstracts due to not meeting the inclusion criteria, leaving 36 articles included for full text review. Full text review identified 6 articles (Emingil et al, 2012, Ercan et al, 2015, Fujii et al, 2004, Haas et al, 2008, Haas et al, 2012, Martande et al, 2016) eligible for inclusion in the final review. Four studies were subsequently included for quantitative meta-analysis after evaluation of their clinical and methodological homogeneity was deemed sufficient. A decision was made by the authors to include the Martande et al, (2016) paper within this review. Although the paper does not specifically state one of the specific PICO population terms defined in the inclusion criteria, the population investigated were patients with “Aggregatibacter actinomycetemcomitans associated periodontitis (AAAP)”. It was stated within the paper that AAAP is a rapidly progressive disease that has certain microbiologic and immunologic characteristics which

influence the course and progression of periodontal destruction. The authors concluded that this classification was sufficiently similar to previous classifications of Grade C periodontitis that it should be included within the review.

The inter-reviewer agreement for the inclusion of articles for full text review was calculated using the Cohen's kappa coefficient which gave a result of 0.86. The Cohen's kappa coefficient for the inter-assessor agreement at the final stage for articles to be included for the systematic review was 0.81, indicating a good degree of inter-assessor agreement at both stages.

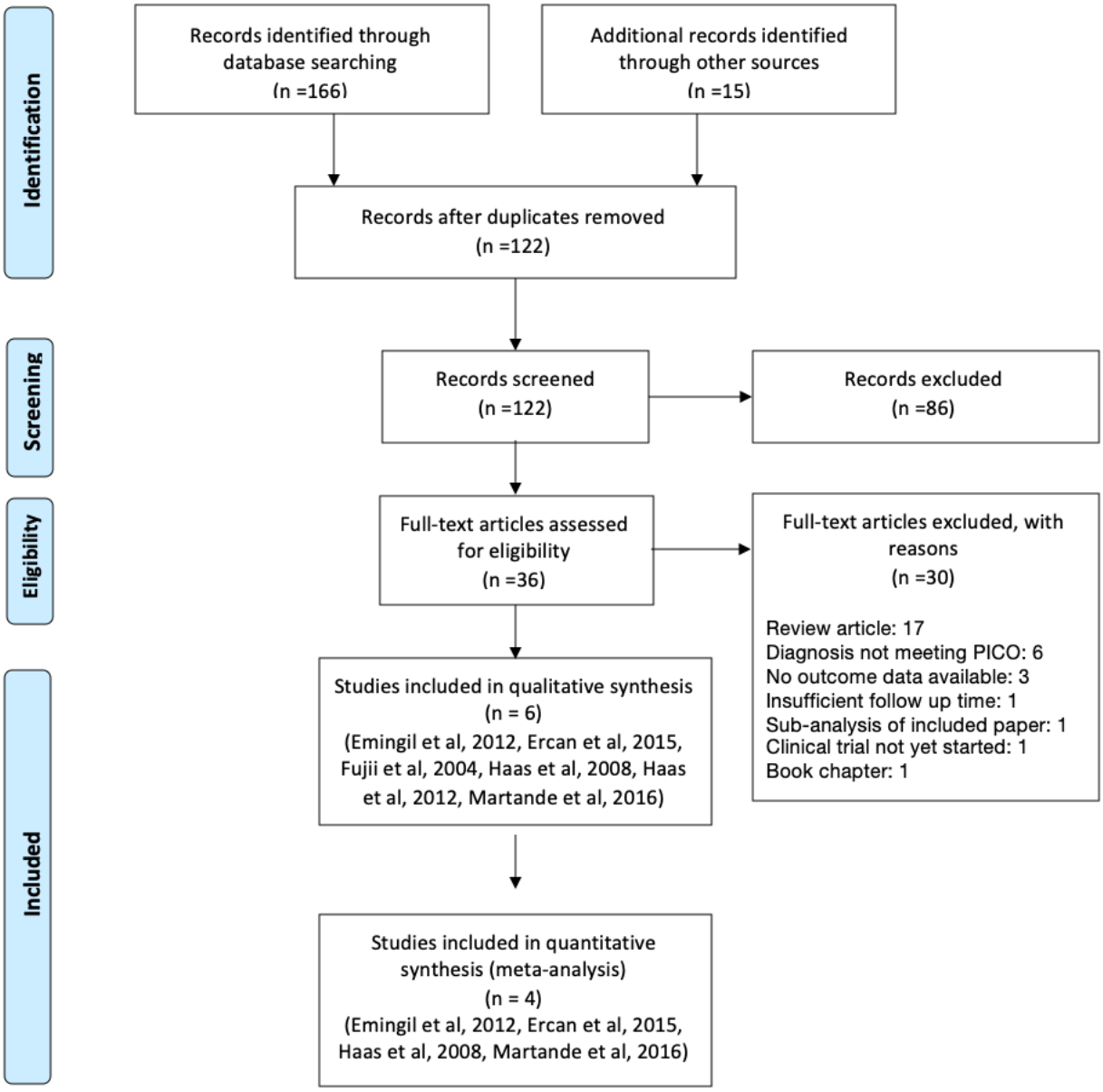

Figure 1: PRISMA study selection flow diagram

3.2 *Description of the Studies*

Table 2 outlines the data captured from each article included for full text review. Four randomised control trials (Emingil et al, 2012, Haas et al, 2008, Haas et al, 2012, Martande et al, 2016), one non-randomised control trial (Fujii et al, 2004) and one retrospective analysis (Ercan et al, 2015) were included for qualitative analysis. Haas et al (2012) was included for narrative qualitative review of microbiological findings. Quantitative synthesis of microbiological findings was not undertaken due to heterogeneity in sampling methods between the studies reporting on microbiological outcome measures. Fujii et al (2004), although providing outcome data for the azithromycin group, did not include outcome data for the comparison group or the time points at which data was collected. Therefore, their results are only discussed narratively and not included as part of the meta-analysis.

Table 2: Overview of Study Characteristics (see table attached)

3.3 *Risk of Bias Assessment*

The risk of bias assessments were completed independently by the two review authors, and then compared, with disagreements resolved through discussion. Outlined in *Table 3* are the risk of bias outcomes for each of the 6 included studies. The four included randomised controlled trials (Haas et al, 2008, Emingil et al, 2012, Haas et al 2012 Martande et al, 2016) were all deemed to be of low risk of bias due to robust methodology within each. The retrospective analysis (Ercan et al, 2015) was deemed to be of serious risk of bias due to a lack of information surrounding confounding factors at baseline. The non-randomised controlled trial (Fujii et al, 2004) demonstrated a critical risk of bias due to a lack of information about baseline characteristics of participants as well as incomplete reporting of outcomes. Each individual study was assessed using the appropriate risk of bias tool depending upon study design, and the tool guided the authors as to the overall risk of bias based upon that of each individual domain (outlined within *Table* 3).

Paper	**Method**	**Overall Risk of Bias**	**Domain**
Emingil et al, 2012	Cochrane RoB V2, (Sterne et al, 2019)	**Low**	Risk of bias arising from the randomization process: **LOW** Risk of bias due to deviations from the intended interventions: **LOW** Missing outcome data: **LOW** Risk of Bias in measurement of the outcome: **LOW** Risk of bias in selection of the reported result: **LOW**
Haas et al, 2008	Cochrane RoB V2, (Sterne et al, 2019)	**Low**	Risk of bias arising from the randomization process: **LOW** Risk of bias due to deviations from the intended interventions: **LOW** Missing outcome data: **LOW** Risk of Bias in measurement of the outcome: **LOW** Risk of bias in selection of the reported result: **LOW**

Haas et al, 2012	Cochrane RoB V2, (Sterne et al, 2019)	**Low**	Risk of bias arising from the randomization process: **LOW** Risk of bias due to deviations from the intended interventions: **LOW** Missing outcome data: **LOW** Risk of Bias in measurement of the outcome: **LOW** Risk of bias in selection of the reported result: **LOW**
Martande et al, 2014	Cochrane RoB V2, (Sterne et al, 2019)	**Low**	Risk of bias arising from the randomization process: **LOW** Risk of bias due to deviations from the intended interventions: **LOW** Missing outcome data: **LOW** Risk of Bias in measurement of the outcome: **LOW** Risk of bias in selection of the reported result: **LOW**
Fujii et al, 2004	Robins-I (Sterne et al, 2016)	**Critical**	Bias due to confounding: **CRITICAL** Bias in selection of participants into the study: **LOW** Bias in classification of interventions: **LOW** Bias due to deviations from intended interventions: **MODERATE** Bias due to missing data: **CRITICAL** Bias in measurement of outcomes: **MODERATE** Bias in selection of the reported result: **MODERATE**
Ercan et al, 2015	Robins-I (Sterne et al, 2016)	**Serious**	Bias due to confounding: **SERIOUS** Bias in selection of participants into the study: **SERIOUS** Bias in classification of interventions: **LOW** Bias due to deviations from intended interventions: **LOW** Bias due to missing data: **LOW** Bias in measurement of outcomes: **MODERATE** Bias in selection of the reported result: **LOW**

Table 3: Risk of bias assessment for all included studies.

3.4 *Statistical analysis*

Available data from each of the studies were divided into comparative timepoints (3, 6 and 12 months) for each of the outcome measurements to aid the clinical relevance of the results. Time point means were used to calculate the mean changes in periodontal parameter from baseline which would be used as the summary measures for meta-analysis. Standard deviations were then calculated for any studies where they were not provided in preparation for data synthesis.

Methodological and clinical heterogeneity were looked at descriptively for the included studies by comparing study characteristics (*Table 2*) from the data collection proforma. Four studies, (Emingil et al, 2012, Ercan et al, 2015, Haas et al, 2008, Martande et al, 2016) were deemed to have sufficient methodological homogeneity particularly at the comparative timepoints for a meta-analysis to be undertaken using the Review Manager (RevMan, 2014) version 5.3 computer programme.

3.5 *Clinical Outcomes*

Of the six studies included for qualitative review, four studies were included for quantitative analysis after assessment of heterogeneity (Emingil et al, 2012, Ercan et al, 2015, Haas et al, 2008, Martande et al, 2016) and two were not (Fujii et al, 2004 & Haas et al, 2012). There was sufficient clinical and methodological homogeneity between these studies in terms of the population, intervention, comparator and outcomes assessed to proceed with quantitative analysis (*Table 2*). Statistical heterogeneity was assessed using Chi^2 and I^2 in the Review Manager (RevMan, 2014) version 5.3 computer programe which found significant heterogeneity for CAL (Chi^2=6.92, p=0.03, I^2=71%) at the 3-month time point and PPD (Chi^2=18.45, p<0.0001, I^2=95%) and CAL (Chi^2=4.19, p=0.04, I^2=77%) at the 6-month time point. No heterogeneity was found for any measurements at the 12-month time points. It is difficult to interpret heterogeneity results with few studies to compare; therefore, a random-effects model was used for statistical analyses.

3.5.1 *Qualitative summary of individual included studies*

Overall, there was full compliance from all participants with the recommended intervention/control regimes in all studies except for one patient in the Haas et al (2008) who did not comply with the full recommended regime. After unblinding it was noted that this patient was in the placebo group and they were excluded from the final analysis, as they were lost to follow up after three months. Three out of the six studies (Emingil et al, 2012, Haas et al, 2008 and Haas et al, 2012) included smokers as a stratified variable within treatment groups, two studies (Ercan et al, 2015 and Martande et al, 2016) excluded smokers and one study did not discuss this potential confounder (Fujii et al, 2004). Three of the studies also excluded patients with comorbidities such as diabetes (Emingil et al, 2012, Ercan et al, 2015 and Martande et al, 2016), and three studies did not record whether patients with comorbidities were accepted as part of the trial (Fujii et al, 2004, Haas et al, 2008 and Haas et al, 2012). All of the included randomised control trials (Emingil et al, 2012, Haas et al, 2008 and Martande et al 2016) were powered to 80% with an alpha of 0.05 and were deemed to be of low risk of bias (*Table* 3).

Emingil et al (2012) reported statistically significant improvements in periodontal parameters (probing depths, clinical attachment loss and bleeding upon probing) for both the azithromycin and placebo group ($p<0.05$) that were similar between the two groups at all time points ($p>0.05$). At 1 month, a statistically significant difference was noted in the mean percentage of pockets ≥7mm, that demonstrated a probing depth reduction of ≥3mm from baseline, favouring the azithromycin group. This finding was not noted at the subsequent 3- and 6-month time points.

Ercan et al (2015) reported significant reductions in probing depths (azithromycin $p<0.01$, metronidazole and amoxicillin $p<0.001$ and control $p<0.001$), clinical attachment level (azithromycin $p<0.01$, metronidazole and amoxicillin $p<0.001$ and control $p<0.01$) and bleeding on probing (azithromycin $p<0.001$, metronidazole and amoxicillin $p<0.001$ and control $p<0.001$) from baseline to 3 months. The differences in these parameters between the groups was found not to be statistically significant, concluding that all treatment protocols reduced clinical periodontal parameters.

Fujii et al (2004) will only be discussed narratively and was not included as part of the meta-analysis. Within the azithromycin group of this study, significant reductions were noted in probing depths (p<0.01), change in percentage of pockets ≥4mm (p<0.01) and bleeding on probing (p<0.05). However, the authors did not record the timepoints at which these statistics were undertaken and there was no comparative outcome data for the control group. Therefore, the results from this study should be interpreted with caution due to the critical risk of bias associated with the paper.

Haas et al (2008) reported statistically significant reduction in probing pocket depths ≥4mm at 12 months from baseline between the azithromycin (2.88mm ± 0.23) and placebo (1.85mm ± 0.36) group (P=0.025). Gain in clinical attachment level was also found to be statistically significant between azithromycin (1.68mm ± 0.20) and placebo (0.97mm ± 0.29) groups (P=0.05). Bleeding on probing difference was not found to be statistically significant (P=0.91).

Haas et al (2012) look solely at the microbiological outcomes of the patients first reported in the Haas et al (2008) study. These findings are described in section 3.6, microbiological outcomes.

Martande et al (2016) reported statistically significant differences in mean reduction of probing depths favouring the azithromycin group at all time points from baseline (1 month (P=0.022), 3 months (P=0.002), 6 months (P<0.001) and 12 months (P<0.001)). This was also seen for clinical attachment level gain at 3, 6 and 12 months favouring the azithromycin group (3 months (P<0.001), 6 months (P<0.001) and 12 months (P<0.001). There was only a significant difference noted for difference between bleeding on probing percent at the 6-month timepoint (P=0.027).

3.5.2 *Quantitative review of follow up timepoints and meta-analysis*

At 3 months, based upon 3 studies (Emingil et al, 2012, Ercan et al, 2015 and Martande et al, 2016) and 132 participants (Test F:M- 35:31, mean age 30.2 yrs and Control F:M - 33:33 control, mean age 31.5 yrs) a statistically significant difference in favour of azithromycin was found for probing pocket depth reduction (*Figure* 2) (WMD=-0.39mm, 95% CI [-0.66, -0.13], I^2=0%) and clinical attachment level (*Figure* 3) (WMD=-0.61mm, 95% CI [-1.13, -0.10], I^2=71%) but not for bleeding on probing (*Figure* 4) (WMD=-2.91%, 95% CI [-6.82, 0.99], I^2=0%) when compared to a placebo or no adjuncts alongside professional mechanical plaque removal. For the 3-month outcomes, the certainty of the evidence was deemed to be low as assessed by using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) Pro Guideline Development Tool (GRADEpro, G. D. T. (2020)) due to risk of bias and imprecision of results. (*Table 4*)

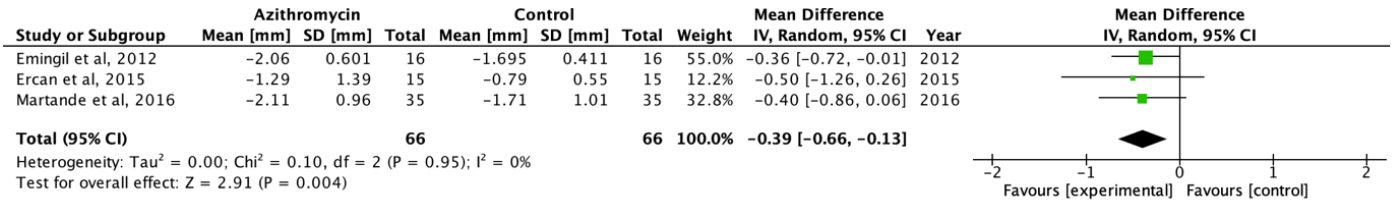

Figure 2: PPD (mm) Changes at 3 months from baseline

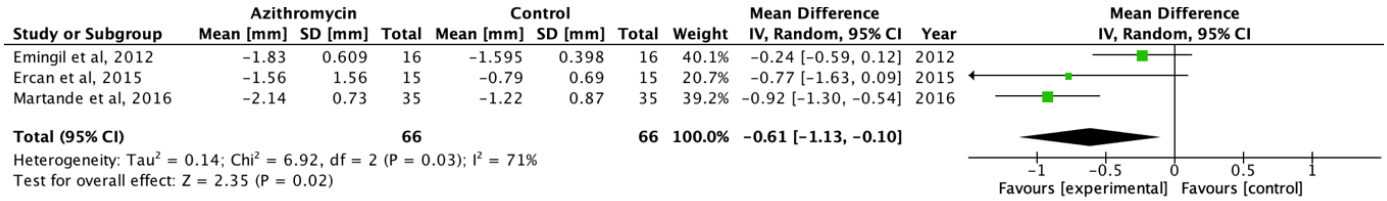

Figure 3: CAL (mm) Changes at 3 months from baseline

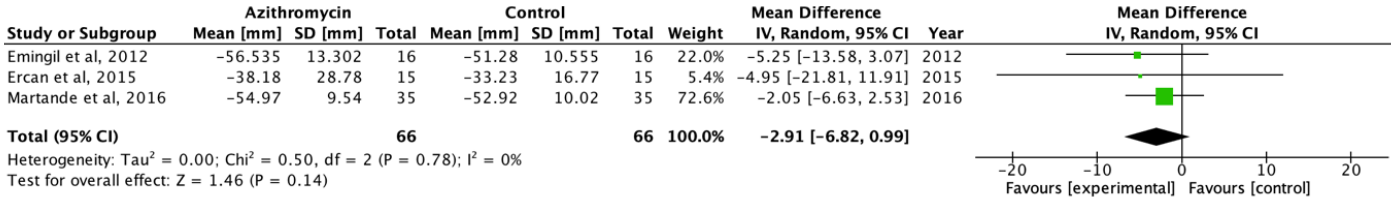

Figure 4: BoP (%) Changes at 3 months from baseline

At 6 months, based upon two studies (Emingil et al, 2012 and Martande et al, 2016) and 102 participants (Test F:M- 23:28, mean age 30.7 yrs and Control F:M- 22:29, mean age 31.4 yrs), there was no difference noted for probing pocket depth reduction (*Figure* 5) (WMD=-0.88mm, 95% CI [-2.10, 0.34], I2=95%) or clinical attachment level (*Figure* 6) (WMD=-0.58mm, 95% CI [-1.26, 0.10], I^2=76%) between azithromycin and the placebo groups. Bleeding on probing was lower in the azithromycin group than the placebo group (*Figure* 7) (WMD=-4.89%, 95% CI [-8.85, -0.93], I^2=0%). The certainty of the evidence was deemed to be moderate for probing pocket depths and clinical attachment levels outcomes due to imprecision of results, and high for bleeding on probing (*Table 4*).

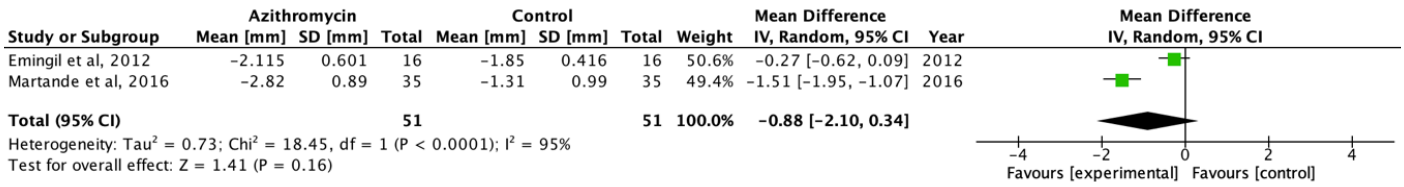

Figure 5: PPD (mm) Changes at 6 months from baseline

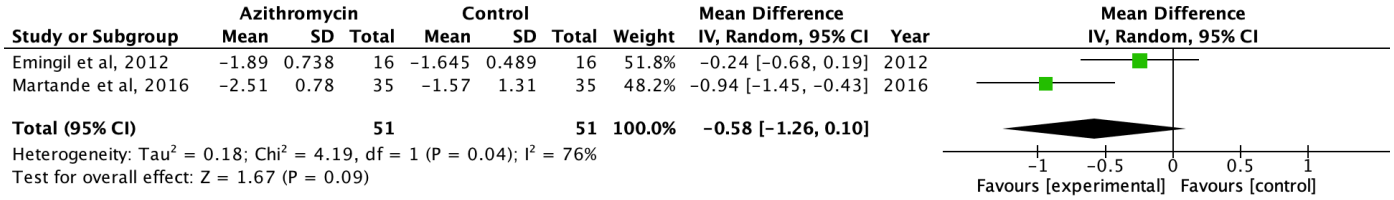

Figure 6: CAL (mm) Changes at 6 months from baseline

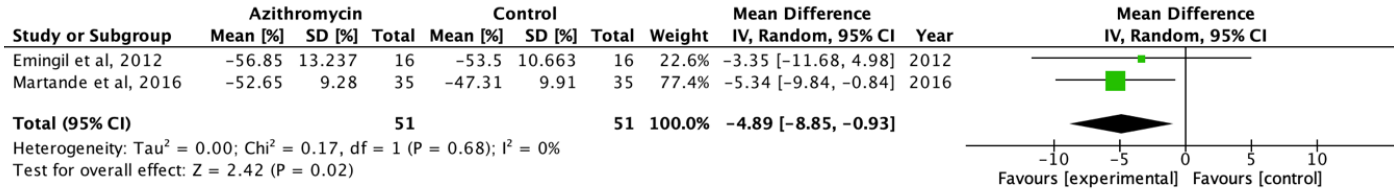

Figure 7: BoP (%) Changes at 6 months from baseline

At 12 months, based upon two studies (Haas et al, 2008 and Martande et al, 2016) and 94 participants (Test F:M- 23:24, mean age 27.6 yrs and Control F:M- 18:29, mean age 26.7 yrs), a statistically significant difference was detected in favour of azithromycin for probing pocket depth reduction (*Figure* 8) (WMD=-1.32mm, 95% CI [-1.71, -0.93], I^2=0%) and clinical attachment level (*Figure* 9) (WMD=-0.88mm, 95% CI [-1.32, -0.44], I^2=0%) in comparison to placebo groups when used in conjunction with professional mechanical plaque removal. No statistically significant difference was detected for bleeding on probing (*Figure* 10) (WMD=-3.36%, 95% CI [-7.47, 0.76], I^2=0%). The certainty of the evidence was deemed to be high for probing pocket depths and clinical attachment levels and moderate for bleeding on probing, rated down for imprecision (*Table 4*).

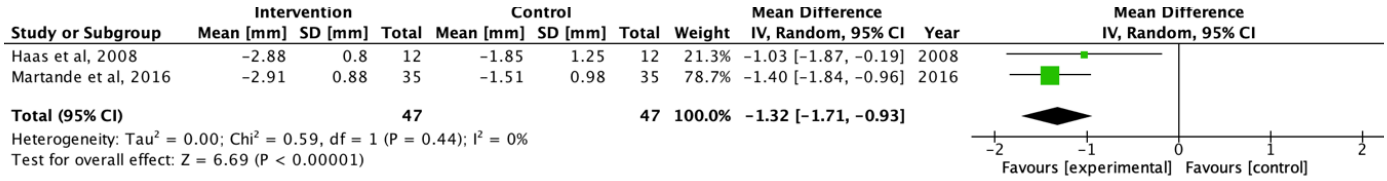

Figure 8: PPD (mm) Changes at 12 months from baseline

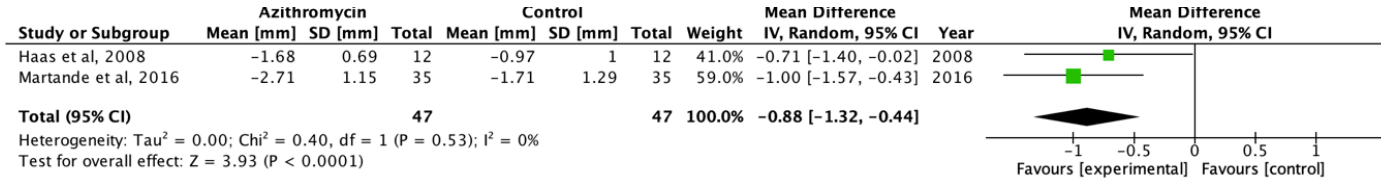

Figure 9: CAL (mm) Changes at 12 months from baseline

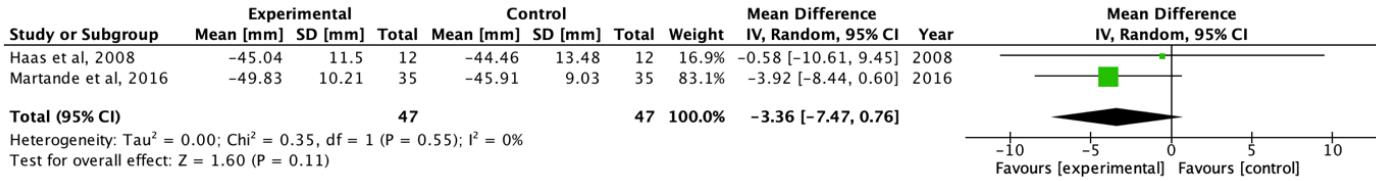

Figure 10: BoP (%) Changes at 12 months from baseline

3.6 *Microbiological Outcomes*

Three of the studies reported on microbiological outcomes (Haas et al, 2012, Emingil et al, 2012, Martande et al, 2014). These were not included for meta-analysis due to clinical

heterogeneity and differences in sampling and analysis methods, and therefore are discussed narratively.

Haas et al, 2012, took microbiological samples at baseline, 15 days after supragingival plaque control and then 3, 6 and 12 months post treatment. Three separate subgingival plaque pools were collected for each patient: one from healthy sites, one from diseased sites of the maxilla and one from diseased sites of the mandible. Samples were collected from the deepest site at each tooth using sterile paper points. There were no statistically significant differences noted in the levels of bacterial species at healthy sites at baseline between the two groups. This was also the case for diseased sites with the exception of *P. intermedia* which was found to be higher in the placebo group ($p<0.05$). When comparing between the intervention and control groups, the change between baseline and 15 days in the reduction of bacterial species was found to be similar. When all subjects were combined, diseased sites showed a significant reduction in *A. gerencseriae* ($p = 0.028$), *C. ochracea* ($p = 0.035$*), and T. denticola* ($p = 0.04$*)*, whereas healthy sites did not show a significant reduction in bacterial levels. Between baseline and 12 months post-treatment, the majority of bacterial species reduced in both groups without a significant difference. The authors concluded that no major microbiological differences were found between azithromycin and control groups after non-surgical periodontal therapy, although most species demonstrated significant reductions over the 12 months. Although azithromycin demonstrated beneficial clinical results when compared to a placebo and non-surgical periodontal treatment, it did not show significant effects on the subgingival microflora.

Emingil et al, 2012, undertook subgingival plaque sampling at baseline, immediately post treatment, 2 weeks, 1 month and 6 months for microbiological analysis. Plaque samples were obtained by using sterile paper points placed into ≥6mm pockets at the mesio-buccal site of two pre-selected teeth. Results were presented as detection frequency of periodontopathogens as the percentage of patients positive for the selected pathogen. *A. actinomycetemcomitans* was detected in 2/16 participants in the azithromycin group and 5/16 participants in the placebo group at baseline, reducing to 1/16 participants in the azithromycin group and 1/16 participants at 6 months. The authors explain due to the low prevalence of patients testing positive, statistical analysis was not possible. For *P. gingivalis, P. intermedia, T. forsythia and F. nucleatum*, the total amount of bacteria at the different time points was calculated from the subgingival plaque samples. At baseline, there was no significant difference in total amounts between the azithromycin and placebo group. For both groups, the total amount of *P. gingivalis, P. intermedia and T. forsythia* showed significant reductions between baseline and 6 months ($p<0.05$). Overall, both groups demonstrated similar levels of bacterial reduction, but over half of the sites sampled still remained positive for the species after treatment.

Martande et al, 2014, took subgingival plaque samples from the deepest site in each quadrant at baseline, 3-, 6- and 12-months to analyse levels of *A. actinomycetemcomitans*. Results were presented as the number of individuals with detection of subgingival *A. actinomycetemcomitans* at the time intervals. At baseline, all individuals in both test and control groups demonstrated detectable subgingival *A. actinomycetemcomitans*. For each of the time points at 3, 6 and 12 months, the number of individuals with detectable subgingival *A. actinomycetemcomitans* were significantly lower ($P<0.0001$) in the test group in comparison to the control group. At 3 months 2/35

participants (5.71%) in the test group and 25/25 participants (65.71%) in the control group demonstrated detectable *A. actinomycetemcomitans.* At 6 months, this was 3/35 (8.57%) and 27/35 participants (77.14%) respectively and at 12 months 5/35 (14.28%) and 28/35 participants (80%) respectively.

The reduction in overall numbers of periodontal pathogens was similar between azithromycin and placebo groups in both the Haas et al (2012) and Emingil et al (2012) studies. When looking at the specific reduction of detectable subgingival A. actinomycetemcomitans, there were conflicting results. Martande et al (2014) demonstrated a significant reduction in the test group whereas Emingil et al (2012) demonstrated similar reductions between groups (although numbers were small which precluded statistical analysis).

3.7 *Adverse Events*

Adverse events were reported upon in 3 of the studies (Haas et al, 2008, Emingil et al, 2012, Martande et al, 2014). No patients in the azithromycin groups experienced any side effects. One patient in the Haas et al (2008) study reported a headache after administration of the medicine, the patient later dropped out of the study and after unblinding, it was revealed the patient was allocated to the placebo group.

3.8 *Patient Reported Outcomes*

None of the 6 studies included for analysis included patient reported outcome measures within them.

Table 4: GRADE Summary of Findings Table for "Azithromycin compared to placebo/alternative adjunct in combination with non-surgical professional mechanical plaque removal in the treatment of Grade C Periodontitis" (see attached document for table)

4 Discussion

This systematic review identified four studies which were included in the meta-analysis with a follow-up of at least 3 months that looked at the effect of adjunctive systemic azithromycin in comparison to a control (either a placebo or no adjunct) for the non-surgical treatment of Grade C Periodontitis.

Overall, the meta-analysis demonstrated a statistically significant difference in favor of azithromycin in combination with non-surgical professional mechanical plaque removal over the control for probing pocket depth reductions at 3 (p=0.004) and 12 months (p<0.00001), clinical attachment level at 3 (p=0.03) and 12 (p<0.0001) months and bleeding on probing reduction at 6 months (p=0.02). No statistically significant differences were noted for bleeding upon probing at 3 months (p=0.14) and 12 months (p=0.11),

probing pocket depths at 6 months (p=0.16) or clinical attachment levels at 6 months (p=0.09) between intervention and control groups.

The certainty of the evidence for the 3-month outcomes was lower than that for the 6 and the 12-month outcomes. This was due to a retrospective cohort study that provided data for the 3 months outcomes (Ercan et al, 2015) being at serious risk of bias after using the ROBINS-I tool (Sterne et al, 2016). The remaining papers that were included in the meta-analysis were all randomised control trials deemed to be at low risk of bias after assessment using the Revised Cochrane risk of bias tool for randomised controlled studies (RoB2) (Sterne et al, 2019) and adequately powered to 80%. Steps were taken within the studies to control confounding factors such as smoking status and comorbidities by either stratifying them as a variable in the randomisation process or using excluding them from the trial. The GRADE summary of findings table (*Table 4*) also highlights those specific outcomes rated down for imprecision where there was a wide spread of data between the studies, resulting in the certainty of evidence for probing depth reduction and clinical attachment level at 6 months, and bleeding on probing at 12 months being deemed moderate. The study by Fujii et al (2004) was deemed to be at critical risk of bias (Sterne et al, 2016) and not included for meta-analysis due to missing outcome data.

There was significant methodological heterogeneity between studies which precluded meta-analysis on the microbiological outcomes between test and control groups. Narratively, there were conflicting results on whether azithromycin reduced the number of subgingival pathogens or detectable subgingival *A. actinomycetemcomitans.*

The three randomised controlled trials were all superiority trials rather than noninferiority trials and compared azithromycin against a placebo rather than the more commonly accepted regime of amoxicillin and metronidazole. It is therefore difficult to compare clinical outcomes between the two adjunctive antimicrobial regimes. Ercan et al (2015) in a retrospective cohort study did collect data for a regime of amoxicillin 500mg and metronidazole 500mg both 3 times a day but did not state the number of days of the regime. This paper concluded that all regimes (azithromycin, amoxicillin and metronidazole and periodontal treatment only) resulted in statistically significant reductions in outcome measures from baseline, but no statistically significant difference was found between the regimes.

When comparing the results from this systematic review to systematic reviews that have looked at the use of amoxicillin and metronidazole in the treatment of Grade C Periodontitis, the changes in PPD (mm) for the adjunctive use of azithromycin are comparable.

Keestra et al (2015) reported at 3 months, a combination of metronidazole and amoxicillin resulted in a statistically significant mean pocket probing depth reduction difference of 0.39 ± 0.16mm (8 studies, 248 patients) in comparison to the control. In this current review, a comparable statistically significant probing depth reduction difference in favour of azithromycin to the control at 3 months was also noted (WMD=-0.39mm, 95% CI [-0.66, -0.13], I^2=0%).

At 6 months, Teughels et al (2020) reported a statistically significant difference in PPD (mm) for the use of amoxicillin and metronidazole in the treatment of aggressive

periodontitis in comparison to a control (PMPR and placebo only) (WMD=0.505, 95% CI [0.356; 0.654]). Keestra et al (2015) reported metronidazole and amoxicillin resulted in a statistically significant mean pocket probing depth reduction difference of 0.51 ± 0.09mm (7 studies, 214 patients) when compared to the control. In this present review, there was no difference noted for probing pocket depth reduction (WMD=-0.88mm, 95% CI [-2.10, 0.34], I2=95%) between azithromycin and control groups at 6 months.

At 12 months, Teughels et al (2020) also reported a statistically significant difference in PPD (mm) for the use of amoxicillin and metronidazole in the treatment of aggressive periodontitis in comparison to a control (WMD = 0.519 (95% CI [0.230; 0.807]). Keestra et al (2015) reported a statistically significant mean pocket probing depth reduction difference of 0.51 ± 0.38mm (2 studies, 65 patients) in favour of amoxicillin and metronidazole over controls. In this present review, a larger statistically significant difference was detected in favour of azithromycin for probing pocket depth reduction over placebo-controlled groups (WMD=-1.32mm, 95% CI [-1.71, -0.93], I^2=0%), although this result is only based upon two studies (94 participants).

Rabelo et al (2015) specifically looked at the use of systemic antibiotics in the treatment of aggressive periodontitis in a systematic review. They reported a statistically significant gain in mean full mouth clinical attachment level (WMD: 0.51 mm [95% CI: 0.06, 0.96]; p=0.03; I^2 =77%) for scaling and root planing with azithromycin in comparison to a placebo and no difference for probing depth reduction (WMD: 0.67 mm [95% CI: 0.08, 1.41]; p = 0.08; I^2 = 91%), based upon two trials. The time points for these measurements were not noted and therefore comparison to results from this review are difficult.

The main limitation of this systematic review is the small number of studies that were eligible for meta-analysis at similar time points to address the PICO question. Although four studies were included for meta-analysis, only three were available to assess 3-month time points and two studies for each of the 6- and 12-month time points. These results should therefore be interpreted with caution due to the limited number of comparable clinical outcomes looking at the adjunctive use of azithromycin with subgingival professional mechanical plaque removal in patients with Grade C Periodontitis. A potential confounding factor identified in the Emingil et al (2012) randomised control trial was the early reassessment of periodontal parameters at 1-month post treatment which could have affected the healing of the periodontal tissues.

Although the results from this systematic review and meta-analysis have shown additional benefits of adjunctive azithromycin in the non-surgical periodontal treatment of patients with Grade C Periodontitis for certain clinical outcomes at certain timepoints, the prescription of antimicrobials has to be undertaken with care. One of the many roles of dental practitioners is to act as an antimicrobial steward, ensuring that antimicrobials are always prescribed in a judicious way to preserve their future effectiveness (NICE, 2015). In the United Kingdom, the National Institute for Health and Care Excellent (NICE) released antimicrobial stewardship guidelines (NICE, 2015) to prevent their over-prescribing in order to slow the emergence of antimicrobial resistance and ensure that they remain an effective treatment for infection.

One of the ways Dentists can act as antimicrobial stewards is by always following evidence-based prescribing and ensuring that they are only used where there are proven benefits to patients. In the UK, guidance from the Faculty of General Dental Practice

(FGDP) and Faculty of Dental Surgery (FDS) was released 2020 (FGDP & FDS, 2020) also highlighting the need for responsible and judicious prescribing in their "Good Practice Guidelines". This guideline highlights the available evidence behind different antimicrobial prescribing regimes as an adjunct to subgingival professional mechanical plaque removal and provides an evidence base for dentists to prescribe from, after weighing up the patient specific risks and benefits. Within the UK, the treatment of patients with Grade C or Stage IV periodontitis, cases which are described in the FGDP and FDS (2020) guidance as warranting adjunctive antimicrobials, are defined as Level 3 complexity treatment. In practicality, this means that patients with this staging and grading of periodontal disease can be referred to registered periodontal specialists or consultants within the National Health Service (NHS) system and may not be seen in general dental care.

Based upon the results of this meta-analysis, azithromycin offers additional benefits in improvement in certain clinical parameters for subgingival debridement in patients with aggressive periodontitis over the control groups. These results appear to be maintained for up to 12 months after treatment completion.

Conclusion

This review has found evidence that azithromycin as an adjunct to subgingival professional mechanical plaque removal improves clinical outcomes (PPD and CAL at 3 months, BoP at 6 months and PPD and CAL at 12 months) in comparison to a placebo or no alternative, when treating patient for Grade C Periodontitis, which appears to be maintained for up to 12 months with varying degrees of certainty. It should be noted that these conclusions are based on a small number of studies. Further, well-designed studies with longer follow-up times are required to investigate whether the adjunctive use of azithromycin with subgingival professional mechanical plaque removal offers clinical improvements over non-surgical treatment alone or alternative antimicrobial regimes in cases of Grade C Periodontitis. There is also opportunity to further investigate patient related outcomes measures surrounding treatment of Grade C Periodontitis with azithromycin in comparison to certain control groups.

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Declaration of Interest

None.

Registration

This systematic review was registered on the International Prospective Register of Systematic Reviews (PROSPERO) (ID:CRD42020168195).