EMPIRICAL OR MICROBIOLOGICALLY-GUIDED SYSTEMIC ANTIMICROBIALS AS ADJUNCTS TO NON-SURGICAL PERIODONTAL THERAPY? A SYSTEMATIC REVIEW

Running title: Antibiotics based on baseline microbiota

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ABSTRACT

Background: It is unclear if patients with specific subgingival microbiological profiles benefit more from adjunctive systemic antibiotics.

Aims: To answer the question: 'What is the clinical benefit in periodontitis patients taking adjunctive systemic antimicrobials to non-surgical therapy, depending on pre-treatment detection of periodontopathogenic bacteria?'

Materials and Methods: A search was conducted in 4 electronic databases for randomised controlled trials reporting clinical outcomes following adjunctive antibiotic therapy for patients divided by baseline microbiological profiles.

Results: The initial search resulted in 643 papers, reduced to 5 after screening and author contact. Four of these studies were suitable for a fixed effects two-stage individual participant data meta-analysis adjusted for baseline data. Collectively, adjunctive Amoxicillin and Metronidazole yielded superior clinical results (measured as reduction of PPDs) compared to placebo. No significant differences were detected for the effect of adjunctive antibiotics by detection of *A. actinomycetemcomitans* on PPDs $\geq$ 5 mm (WMD=1.16, 95% CI[-5.37, 7.68], I^2=37.8%) or other clinical outcomes . All included studies had low risk of bias.

Conclusion: There is no evidence to suggest that baseline detection of periodontopathogenic bacteria should be used as criterion for prescribing adjunctive antibiotics, although only limited information on microbial data and specific antimicrobials were available for analysis.

CLINICAL RELEVANCE

Scientific rationale for study: It is not clear if adjunctive systemic antimicrobials are more effective in patients with specific periodontopathogenic bacteria. Principal findings: adjunctive Amoxicillin and Metronidazole were effective at improving periodontal clinical outcomes irrespective of subgingival detection of *A. actinomycetemcomitans*. Practical implications: based on current evidence, it is hard to justify microbiologically-guided adjunctive antibiotic use. More studies on this topic are required to provide guidelines for the use of personalised antimicrobial therapy.

BACKGROUND

The use of antibiotic treatment as adjunctive to non-surgical periodontal therapy was initially introduced to tackle the microbial aetiology of periodontitis (Baer & Socransky 1979) and later on more specifically with the main aim to eliminate periodontopathogenic bacteria (such as *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*) which are otherwise likely to persist following mechanical biofilm removal only (van Winkelhoff et al., 1989). Adjunctive antibiotic treatment was initially restricted to patients with juvenile periodontitis (Lindhe & Liljenberg, 1984), but then sometimes also extended to patients with chronic, adult forms of periodontitis (Winkel et al., 2001) and/or to diabetic patients with periodontitis (Grellmann et al., 2001). The adjunctive use of systemic antibiotics has shown to be beneficial, especially but not exclusively in patients with aggressive periodontitis, in terms of improvements in clinical parameters (probing pocket depths, PPD, and clinical attachment levels, CAL) compared with placebo (Sgolastra et al., 2012; Smiley et al., 2015; Zandbergen et al., 2001; Herrera et al., 2002; Haffajee et al., 2003; Jepsen & Jepsen, 2016), although the long-term stability of such improvements is probably highly influenced by the supportive periodontal therapy regime adopted (Ramberg et al., 2001). However, conflicting evidence has been reported on whether the baseline subgingival detection of the specific bacteria that the antibiotics have been introduced to target, affects the clinical improvements when antibiotics are given or not (Cionca et al., 2010; Guerrero et al., 2014). A recent retrospective study suggested that microbiological analysis could inform a clinically-based decision for the adjunctive use of systemic antibiotics (Eick et al., 2018). Overall, it is unclear whether subjects harboring specific pathogens subgingivally before treatment may benefit more from the adjunctive antibiotics compared to subjects not harboring them. The reliability of antimicrobial testing prior to treatment has also been questioned (Mellado et al., 2001; Salkin et al., 2003). Therefore, empirical antibiotic use often takes place, without prior knowledge of the subgingival microbial composition.

A recent survey of U.S. periodontists revealed that prescription, initiation, and duration of antibiotics varied considerably in many of the treatments, suggesting that guidelines and protocols are needed for antibiotic use (Froum & Weinberg, 2015). Given the widespread concerns about antibiotic resistance and consequent need to try and reduce antibiotic prescriptions, there is an urge to develop guidelines for antibiotic prescription in periodontal therapy (Rams et al., 2014). In this respect, a personalised approach to treatment, where baseline microbial composition informs decisions on adjunctive antibiotic therapy, may be

beneficial. However, it is not clear whether such approach could be justified based on existing evidence.

The aim of this systematic review was to assess if the baseline presence of periodontopathogenic bacteria (including but not restricted to *A. actinomycetemcomitans, P. gingivalis* and *Tannerella forsythia*) influence the clinical benefits of a course of systemic antimicrobials (including but not restricted to amoxicillin plus metronidazole) used as adjuncts to non-surgical periodontal therapy. The PICO question outline was:

- Population: subjects with periodontitis receiving systemic antimicrobials or placebo as adjunct to non-surgical periodontal therapy
- Intervention: non-surgical periodontal therapy + systemic antimicrobials (or placebo)
- Comparisons: pre-treatment detection or not of specific subgingival bacteria
- Outcomes: changes in PPD and CAL at 6 months follow-up

MATERIALS AND METHODS

A systematic review protocol was written in the planning stages and the PRISMA checklist (Moher et al., 2009) was followed both in the planning and reporting of the review (checklist attached as supplemental material 1). The protocol was registered with PROSPERO (number CRD42018107899).

Focused question

What is the clinical benefit (measured as PPD and CAL reduction) in periodontitis patients taking adjunctive systemic antimicrobials to non-surgical therapy, depending on pre-treatment detection of periodontopathogenic bacteria?

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Eligibility criteria

The inclusion criteria for studies in the systematic review were:

o Study design: randomised controlled trials (RCT) investigating the use of systemic antimicrobials as adjuncts to non-surgical periodontal therapy

o Reporting clinical outcome of treatment in subjects divided by subgingival bacteria detection pre-treatment

o At least 6-months follow-up

Choice of main outcomes

The outcome for the review was the change in PPD and CAL 6 months after treatment in patients divided by treatment (antimicrobial vs. placebo) and by baseline detection of subgingival bacteria.

Information sources

The search was conducted through the electronic databases MEDLINE, EMBASE and Cochrane library up to 24th July 2018 and was complemented by a search through the reference lists of included studies. No language restriction was included in the initial search. Among published literature, peer-reviewed studies, reports, book chapters and conference abstracts were screened. Narrative or systematic reviews on the topic were searched in order to identify suitable papers. The search was complemented by searches on the Open Grey database and by a hand search in Journal of Dental Research, Journal of Periodontology, Journal of Clinical Periodontology and Journal of Periodontal Research from 2000 until 2018 and by contacting editors of the journals above to enquire about any potentially relevant papers soon to be published. An attempt was also made to contact authors of potentially-relevant papers in order to obtain raw data and to clarify potential inclusion.

The MEDLINE search strategy (adapted to the other databases) is described below:
(‘‘Periodontitis’’[MeSH] OR ‘‘Chronic Periodontitis’’[MeSH] OR ‘‘Periodontal Diseases’’[MeSH] OR ‘‘Periodontal Pocket’’[MeSH] OR ‘‘Periodontal Attachment Loss’’[MeSHh] OR periodontitis OR periodontal disease* OR periodontal pocket* OR attachment loss OR pocket depth OR periodontal non surgical treatment OR periodontal non surgical therapy OR scaling root planing OR dental scaling OR periodontal treatment OR periodontal therapy OR ‘‘Dental Scaling’’[MeSH] OR ‘‘Root Planing’’[MeSH])
AND (‘‘Antibiotic’’[MeSH] OR ‘‘Antimicrobial’’)
AND (‘‘Microbial testing’’ OR ‘‘16s’’ ‘‘Microbial analysis’’ OR ‘‘PCR’’ OR ‘‘Culture’’ OR ‘‘Checkerboard’’ OR ‘‘Bacteria’’)
AND ("Treatment Outcome" OR "probing pocket depth" OR "PPD" OR "clinical attachment level" OR "CAL"). This search was modified and adapted to the other databases.

Study selection

Study selection was conducted by two independent reviewers (LN, VK) in the following stages:

1. Initial screening of potentially-suitable titles and abstracts against the inclusion criteria to identify potentially relevant papers
2. Screening of the full papers identified as possibly relevant in the initial screening

Studies were excluded if not meeting the inclusion criteria. Following the screening of titles and abstracts (step 1), the studies included by both reviewers were compared and a complete database for step 2 was formed joining all studies selected by at least one reviewer. Following step 2, in case of a disagreement between reviewers, the decision about study eligibility was made trying to reach a consensus between the two reviewers. Emails were sent to the authors of excluded papers, enquiring whether relevant unpublished data could be obtained. When this was possible, studies were included. The agreement value between reviewers was calculated after step 1 and after step 2 using Kappa statistics.

Data collection process/ data items

A standardized data extraction form was used to record data from each included study, encompassing number of patients, demographics, definition and diagnosis of periodontitis, clinical methods (assessment and treatment), antibiotic/placebo regime, length of follow-up, number of patients lost to follow-up, methods for bacterial detection, clinical outcomes (divided by treatment groups and by bacterial detection groups), microbial outcomes and patient-reported outcomes.

Risk of bias in individual studies

The quality of the included studies was assessed through risk of bias analysis as it could impact on the overall results and conclusions ('Systematic reviews, CRD's guidance for undertaking reviews in health care', University of York, 2008). The Cochrane Collaborations tool was used for assessing risk of bias (Higgins & Green, 2011) (see Appendix 1).

Summary measures/Synthesis of results/ Statistical methods

Study results were categorized according to periodontal treatment (antibiotic/placebo) and bacterial detection (yes/no). Two-stage, fixed effect individual participant data meta-analyses were performed for all outcomes using the Stata module *ipdmetan* (Fisher, 2015). The main outcome was reduction of PPD $\geq$ 5 mm at 6 months. Unbiased estimates of the weighted mean difference (WMD) of treatment effects between bacterial detection groups were obtained estimating the treatment-bacterial detection interaction within each study separately and subsequently pooling these estimates. Estimates of WMDs are presented with associated 95%

confidence intervals (95% CI) Forest plots were produced to graphically represent the differences in treatment effects between studies and the overall pooled estimate. Heterogeneity was assessed first using clinical judgement and then statistical heterogeneity is described using the p-value for the chi-squared test statistic Q, denoted p_Q, and the I^2 index (Higgins and Thompson, 2002). Funnel plots were used to explore the presence of publication bias.

RESULTS

Study selection

Figure 1 reports the flowchart representing study selection and inclusion. The initial search identified 611 PubMed, 4 Embase, and 85 Cochrane Library database articles, with a total of 643 papers after de-duplication. No additional papers were identified by the Open Grey search, while 3 were identified by hand search, resulting in a total of 646 papers. Following first-stage screening of titles and abstracts, 64 articles qualified for full-text screening (considered potentially suitable by at least one reviewer). After full text reading, 3 articles met the defined inclusion criteria and 61 were excluded (reported as supplemental material 2). The reasons for exclusion were as follows: 41 did not report clinical response divided by microbial detection, 7 had no control group, 3 were not RCTs, 3 had follow-up <6 months, 2 studies did not use antibiotics as adjuncts to SRP, 2 were duplicate reports and one each did not include systemic antibiotics, did not report PPD and CAL or was not retrievable. Emails were sent to corresponding authors of the screened papers not reporting response divided by microbial detection. No contact was found for 9 of them, and emails were sent to authors of the remaining 33. Following this, no response was obtained for 27 papers, while 4 authors replied that they could not retrieve the requested data and 2 authors provided individual patient data relevant to the present review were provided resulting in inclusion in the review (Mestnik et al., 2012; Soares et al., 2014). This resulted in 5 papers finally being included. The kappa value for inter-reviewer agreement was 0.54 (95% CI= 0.42-0.67) at title and abstract screening (93.9% agreement) and 0.85 (95% CI= 0.56-1) at full text reading (98.3% agreement).

Study characteristics

Table 1 reports the characteristics of the sample included in the reviewed studies. All 5 included articles were written in English. The countries where the studies were conducted were Brazil (2 studies), United Kingdom, Switzerland and Colombia (one study each). The patient sample ranged from 30 (Mestnik et al., 2012) to 118 patients (Soares et al., 2014). All studies were

RCTs and were published from 2005 to 2017. The target population was generalised aggressive periodontitis (GAgP) in 3 studies (Guerrero et al., 2014; Mestnik et al., 2012; Ardila and Guzman, 2017) and chronic periodontitis (CP) in 2 studies, including moderate CP (Cionca et al., 2010) and moderate to severe CP (Soares et al., 2014). The study follow-ups ranged from 6 and 12 months. The subgingival debridement regime ranged from a full-mouth approach in one (Ardila and Guzman, 2017) or two sessions within 24 hours (Guerrero et al., 2014) or 48 hours (Cionca et al., 2010) to a staged approach over 14 days (Mestnik et al., 2012; Soares et al., 2014). The antibiotic regimes varied too, including Amoxicillin+ Metronidazole for most studies, albeit with differences in dose and duration (Cionca et al., 2010; Guerrero et al., 2014; Mestnik et al., 2012), an additional arm with Metronidazole alone (Soares et al., 2014) and in one case Moxifloxacin (Ardila & Guzman, 2017). A placebo was used for control patients in all included studies. It is worth noting that none of the included studies had differences in clinical response by baseline microbial profile as the main study outcome.

Subgingival plaque sampling was carried out by paper points or curettes, while microbiological methods were also heterogeneous, including cultures (Ardila & Guzman, 2017), polymerase chain reaction (PCR) (Cionca et al., 2010; Guerrero et al., 2014) or checkerboard (Mestnik et al., 2012; Soares et al., 2014). Definition of '*A. actinomycetemcomitans* +ve' was based on level of *A. actinomycetemcomitans* at least 10^5, a threshold suggested as relevant in previous literature (Socransky et al. 1998). Given the heterogeneity in microbial analysis, this threshold was considered in at least 1 site for studies where samples were not pooled (Mestnik et al., 2012; Soares et al., 2014; Ardila & Guzman, 2017) and for detection of *A. actinomycetemcomitans* in papers using PCR from pooled samples (Cionca et al., 2010; Guerrero et al., 2014), where the counts for single sites could not be ascertained, but where in any case most of average counts were $>10^5$. By these definitions, a substantial percentage of patients was *A. actinomycetemcomitans* +ve (respectively 25% (Cionca et al., 2010), 49 % (Guerrero et al., 2014), 50% (Ardila & Guzman, 2017), 33% (Soares et al., 2014) and 80% (Mestnik et al., 2012)).

Synthesis of results

All included studies reported clinical benefits with the use of the adjunctive antibiotics, measured as increased reductions in PPD and CAL in the test compared with control groups (Table 1). Four studies, all using Amoxicillin and Metronidazole as adjuncts, were included in a meta-analysis for reduction in the number of PPD $\geq$ 5 mm at 6 months (see supplemental

material 3), showing increased reduction in the number of PPD ≥ 5 mm for test vs. control group, with WMD = 8.81 sites with PPDs (95% CI= [5.85; 11.76], p<0.001), albeit with high heterogeneity (I^2=82.1%, p_Q=0.001).

When baseline subgingival microbial data were considered, Ardila and co-workers (using Moxifloxacin as adjunct) reported differences favouring the antibiotic group in *A. actinomycetemcomitans* +ve vs. *A. actinomycetemcomitans* -ve for PPDs ≥7 mm (1.96 mm vs. 1.14 mm respectively) and for PPDs 4-6 mm (0.92 mm vs. 0.51 mm respectively) (Ardila and Guzman, 2017). Similarly, Guerrero and co-workers reported that the percentage of sites changing from ≥ 5 mm to ≤ 4mm PPDs at 6 months was 80.9% for *A. actinomycetemcomitans* +ve individuals treated with adjunctive antibiotics, compared to 71.1% for *A. actinomycetemcomitans* -ve individuals treated with adjunctive antibiotics (Guerrero et al., 2014). In contrast, when no antibiotics were prescribed, the change from ≥ 5 mm to ≤ 4 mm PPDs was found to be 52.6% for *A. actinomycetemcomitans* +ve and 60.9% for *A. actinomycetemcomitans* -ve individuals. Overall, both Guerrero and co-workers and Ardila and co-workers suggested that all patients benefitted from the antibiotic adjunct, although its clinical effect seemed to be more pronounced (but not statistically significant) in *A. actinomycetemcomitans* +ve patients (Guerrero et al., 2014; Ardila & Guzman, 2017). On the contrary, Cionca and co-workers concluded that the adjunctive clinical benefits attributable to the antibiotic were obtained regardless of the baseline presence or absence of the six studied periodontal pathogens (Cionca et al., 2010). The other two included papers did not comment on clinical results for antibiotic and placebo divided by baseline microbial detection, but data were provided to the authors of this review (Mestnik et al., 2012; Soares et al., 2014).

Fixed effects meta-analysis (Figure 2) of the four papers using adjunctive Amoxicillin and Metronidazole revealed no significant differences in the effect of the antibiotic regime on the reduction of PPD ≥ 5 mm at 6 months (adjusted for their baseline number) based on the baseline detection of *A. actinomycetemcomitans*, with moderate heterogeneity (I^2=37.8%, p_Q=0.185).

Meta-analysis for other available clinical data was possible for three papers (Guerrero et al., 2014; Mestnik et al., 2012; Soares et al., 2014) using adjunctive Amoxicillin and Metronidazole. Meta-analyses revealed no statistically significant differences in the effect of the antibiotic regime for *A. actinomycetemcomitans* +ve vs. *A. actinomycetemcomitans* -ve for mean PPD at 6 months in sites which had PPD≥7 mm at baseline (Figure 3), mean CAL at 6

months in sites which had PPD≥7 mm at baseline (Figure 4) and mean CAL at 6 months in sites which had PPD 4-6 mm at baseline (Figure 5). As is common in the meta-analysis of a small number of studies with small sample sizes Cochran's Q test statistic was smaller than the degrees of freedom for these three outcome measures leading to a negative estimate of I^2 which was by convention set to zero (Higgins & Thompson, 2002). Forest plots indicate that there was some heterogeneity which was deemed small enough to justify fixed effect meta-analysis for all outcomes.

All these 6-months outcomes (mean PPD and CAL in sites with PPD≥7 mm at baseline and mean CAL in sites with PPD 4-6 mm at baseline) showed improvements in the test vs. placebo meta-analyses, irrespective of *A. actinomycetemcomitans* status (supplemental material 4, 5 and 6).

Very sparse data were available for the other periodontopathogenic bacteria examined, such as *P. gingivalis* or *T. forsythia*, preventing any conclusion to be drawn.

Risk of bias assessment

Risk of bias analyses (Table 2) showed that most items were considered at low risk of bias. Overall, all studies had low risk of bias. A funnel plot of the main outcome PPD ≥ 5mm (supplemental material 7) provides no evidence of publication bias.

DISCUSSION

This systematic review shows that the adjunctive antimicrobial regime of Amoxicillin and Metronidazole is effective in reducing pocket depths (measured as PPD ≥ 5mm reduction 6 months after treatment), irrespective of *A. actinomycetemcomitans* detection. Similarly, meta-analyses of papers reporting 6-months results of mean PPDs in moderate (4-6 mm PPDs) and deep (≥ 7 mm PPD) pockets and mean CAL in pockets ≥7 mm revealed no significant difference by baseline *A. actinomycetemcomitans* detection. No meta-analysis was possible based on other bacteria or other antibiotics, due to a paucity of data in the 5 included papers. Therefore, although some papers (Guerrero et al., 2014; Ardila & Guzman, 2017) suggested that the adjunctive antibiotics may be more effective in improving clinical outcomes in patients carrying some specific periodontopathogenic bacteria such as *A. actinomycetemcomitans,* this is not supported by the evidence produced by this review.

This paper supports the evidence that some systemic antimicrobials provide additional clinical reductions in PPD and CAL when used as adjuncts to non-surgical periodontal therapy, as clarified by several independent studies and meta-analyses (Jepsen & Jepsen, 2016; Pretzl et al., 2018). However, side effects of systemic antibiotics including risk of allergic/hypersensitivity reactions and onset of antibiotic resistance limit their widespread use. Clinical efficacy and adverse events are thus the two main opposite arguments used by 'pro-antibiotics' and 'anti-antibiotics' periodontists. But what if we could use the principles of personalised medicine (Duarte & Spencer, 2016) to select patients who could benefit the most from the adjunctive antibiotics, therefore moving away from empirical, 'one size fits all' antibiotic use? Some studies employed a targeted microbial approach for the elimination of periodontopathogenic bacteria based on baseline microbial profiles (van Winkelhoff et al. 1989; Goene' et al. 1990). Clinical diagnosis (aggressive vs. chronic periodontitis) was sometimes considered as a rough guide for antibiotic prescription, given the biggest magnitude of adjunctive clinical effect of antibiotics in aggressive periodontitis (AgP) (Haffajee et al., 2003). It has also been suggested that patients with deep pockets, 'active' disease (Herrera et al., 2002) or specific microbiological profiles (van Winkelhoff & Winkel, 2005) might benefit more from this adjunctive therapy. In the new post-AgP/CP era (Tonetti et al., 2018), baseline subgingival microbial profiling could become a useful tool for decision on adjunctive antibiotic use in periodontal non-surgical therapy. A recent study investigating *A. actinomycetemcomitans* isolates in AgP revealed high levels of resistance to systemic antibiotics, suggesting the need for antimicrobial susceptibility analysis prior to systemic use of antibiotics concomitantly to periodontal therapy (Akrivopoulou et al., 2017). However, we found no support for this approach based on meta-analysis of the papers included in this review.

When discussing this, it is important to remember that none of the studies included in this review had differences in clinical response by baseline microbial profile as the main study outcome. The only study detected in our search which set out to answer this question, although it is not clear how it was powered, had a 3-month follow-up and as such did not qualify for this systematic review (Mombelli et al., 2013). In that study, 82 periodontitis patients (half of whom had *A. actinomycetemcomitans* detected subgingivally) underwent non-surgical periodontal therapy with or without adjunctive antibiotics. The results led to the conclusion that patients benefited from the antibiotics irrespective of sex, age, or smoking status and baseline *A. actinomycetemcomitans* detection. Interestingly, in a post-hoc subgroup analysis molars benefited significantly more from the antibiotics than non-molars (Mombelli et al., 2013).

Low risk of bias was detected in all the papers included in this review. Areas of potential improvement for further reducing risk of bias, based on our assessment, are more specific descriptions of how knowledge of allocated interventions was concealed from patients and researchers (performance bias) and of selective outcome reporting (reporting bias).

An important limitation of these analyses is the lack of sophistication of microbiological analysis. In other words, no specific results relative to different strains of bacteria, in particular *A. actinomycetemcomitans*, were reported, while it is clearly emerging that different strains of the same bacteria may have very different clinical effects (Damgaard et al., 2017). Furthermore, heterogeneity in the microbiological analyses, with different sampling strategies and microbiological technologies (PCR, culture, checkerboard): as well as in the clinical protocols (different antibiotic regimes and different approaches such as quadrant sessions, full-mouth disinfection or chlorhexidine irrigations) employed in the different studies may have had an impact in our findings. The inclusion of smokers in some of the included studies may have also influenced the observed results. This review also suffers from inability to retrieve more unpublished data on clinical outcomes divided by microbiological status from published clinical trials. Aggressive and chronic periodontitis cases were pooled together in the meta-analysis, in line with the new classification of periodontitis (Tonetti et al. 2018). It could be argued that cases should have been separated by disease severity (mild-moderate-severe); however, results seemed consistent both for sites with PPD $\geq$ 5 mm and for sites with more advanced periodontitis (PPD $\geq$ 7 mm).

As part of a drive to reduce unnecessary use of antibiotics and antimicrobial resistance, effective diagnostic microbial testing is now encouraged by governing bodies (WHO Global Action Plan on Antibiotic Resistance 2015, EU Guidelines for the prudent use of antimicrobials in human health 2017). However, entering the era of personalised medicine, this systematic review suggests that there is not enough evidence to support or completely disregard that baseline detection of periodontopathogenic bacteria should be used as criterion for prescribing adjunctive antibiotics, although only limited information on microbial data and specific antibiotics were available for analysis. Other criteria such as disease severity may have stronger ground to be employed as part of the decision on whether to use adjunctive antibiotics or not.

Furthermore, this study stresses the importance of open access databases, which could help in the retrieval of more data for secondary analyses, when original data of interest are not reported in the papers and not easily available.

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FIGURE LEGENDS

Figure 1. Flowchart representing study selection and inclusion

Figure 2. Fixed effects meta-analysis of papers using adjunctive Amoxicillin and Metronidazole on the reduction of PPD ≥ 5mm to PPD<5mm at 6 months (adjusted for their baseline number) based on the baseline detection of *A. actinomycetemcomitans*

Figure 3. Fixed effects meta-analysis of papers using adjunctive Amoxicillin and Metronidazole for mean PPD at 6 months in sites which had PPD≥7mm at baseline based on baseline detection of *A. actinomycetemcomitans*

Figure 4. Fixed effects meta-analysis of papers using adjunctive Amoxicillin and Metronidazole for mean CAL at 6 months in sites which had PPD≥7mm at baseline based on baseline detection of *A. actinomycetemcomitans*

Figure 5. Fixed effects meta-analysis of papers using adjunctive Amoxicillin and Metronidazole for mean CAL at 6 months in sites which had PPD 4-6mm at baseline based on baseline detection of A. actinomycetemcomitans.