Proposal of a Clinical Endpoint for Periodontal Trials: The Treat-to-Target Approach

Magda Feres,[1] Belen Retamal-Valdes,[1] Marcelo Faveri,[1] Poliana Duarte,[1,2] Jamil Shibli,[1] Geisla Mary Silva Soares,[1,3] Tamires Miranda,[1] Flavia Teles,[4,5] Max Goodson,[5] Hatice Hasturk,[5] Thomas Van Dyke,[5] Benjamin Ehmke,[6] Peter Eickholz,[7] Ulrich Schlagenhauf,[8] Joerg Meyle,[9] Raphael Koch,[10] Thomas Kocher,[11] Thomas Hoffmann,[12] Ti-Sun Kim,[13] Dogan Kaner,[14] Luciene Cristina Figueiredo,[1] Helio Doyle[1]

[1]Department of Periodontology, Dental Research Division, Guarulhos University, Guarulhos, São Paulo, Brazil; [2]Department of Periodontology, School of Advanced Dental Sciences, College of Dentistry, University of Florida, Gainesville, Florida, The United States of America; [3]Department of Stomatology, Federal University of Parana, Parana, Brazil; [4]Center for Innovation & Precision Dentistry, School of Dental Medicine, School of Engineering and Applied Sciences, University of Pennsylvania, Philadelphia, Pennsylvania, The United States of America; [5]The Forsyth Institute, Cambridge, MA, The United States of America; [6]Department of Periodontology, University Hospital Muenster, Muenster, Germany; [7]Department of Periodontology, Johann Wolfgang Goethe-University Frankfurt, Frankfurt, Germany; [8]Department of Periodontology, University Hospital Wuerzburg, Wuerzburg, Germany; [9]Department of Periodontology, University of Giessen, Giessen, Germany; [10]Institute of Biostatistics and Clinical Research, University of Munster, Munster, Germany; [11]Department of Restorative Dentistry, Periodontology, Endodontology, and Preventive and Pediatric Dentistry, University Medicine Greifswald, Greifswald, Germany; [12]Dresden International University, Dresden, Germany; [13]Section of Periodontology, Dept. of Conservative Dentistry, University Hospital Heidelberg, Heidelberg, Germany; [14]Department of Periodontology, Dental School, Faculty of Health, University of Witten/Herdecke, Witten, Germany and Departments of Periodontology and Synoptic Dentistry, Charité Centrum 3, Charité University Medicine Berlin, Berlin, Germany.

ABSTRACT

The selection of proper outcome measures is a critical step in clinical research. Most randomized clinical trials (RCTs) assessing the effects of initial anti-infective periodontal therapies use surrogate outcomes as primary outcome variables, such as mean changes in probing depth (PD) or in clinical attachment. However, these parameters do not reflect disease remission/control at patient level, which has led to subjective interpretations of the data from RCTs and Systematic Reviews. Based on a comprehensive analysis of 724 patients from USA, Germany and Brazil treated for periodontitis, this paper suggests that the clinical endpoint of "≤4 sites with PD≥5mm" is effective in determining disease remission/control after active periodontal treatment and therefore, may represent a pertinent endpoint for applying the treat-to-target concept in RCTs. Furthermore, regression models showed that the presence of >10% and >20% sites with bleeding on probing in the mouth post-treatment increases the risk of a patient leaving the endpoint from 1-2 years (OR=3.5 and 8.7, respectively). Researchers are encouraged to present results on this outcome when reporting their trials, as this will allow for an objective comparison across studies and facilitate systematic reviews, and consequently, the extrapolation of data from research to clinical practice.

Keywords: Endpoint Determination, Periodontitis, Therapeutics, Randomized Controlled Trial, Translational Medical Research

Correspondence to: Magda Feres, Centro de Pós-Graduação e Pesquisa-CEPPE, Universidade Guarulhos, Praça Tereza Cristina, 229 Centro, 07023-070 Guarulhos, SP, Brazil. E-mail: mferes@ung.br

Introduction

One of the most challenging steps when designing a Randomized Clinical Trial (RCT) is the selection of appropriate outcome measures capable of demonstrating the impact of an intervention or exposure. The search for clinically suitable outcomes in research is not a novel concept but has gained new momentum over the past years, along with the growing debate about the clinical applicability of the data generated by RCTs. In this context, two main lines of discussion should be highlighted: (i) the necessity of determining clinical endpoints that reflect real benefits for patients' clinical health (e.g. disease remission/control) or quality of life - and consequently, the need to assess patient-reported outcome measures (PROMs) (Calvert *et al.*, 2013); and (ii) the importance of standardizing the outcomes reported by all RCTs in specific areas of health care (Core Outcome Measures in Effectiveness Trials, 2020).

Most RCTs in periodontology use indirect (i.e. surrogate) measures as primary outcome variables, such as mean changes in probing depth (PD) or in clinical attachment (CA). While these parameters have been largely used for many years and seem to be effective in discriminating the clinical effects of different treatments, they do not necessarily reflect disease remission/control at patient level or indicate tangible benefits for patients. Thus, in recent years, clinical investigators have questioned the relevance of these measures and explored other endpoints for periodontal treatments (Guerrero *et al.*, 2005; Cionca *et al.*, 2009; Feres *et al.*, 2012; Borges *et al.,* 2017). This paper presents a proposal of a clinical endpoint for active periodontal treatment to be used in clinical trials, based on a compilation and analysis of data from studies conducted in the USA, Germany and Brazil. In order to guide the reader through the data presented and discussed, the first part of this paper presents an overview of some concepts that have been directing the debate on this topic.

Choosing primary outcome variables in clinical trials

Choosing a meaningful primary outcome variable and the target effect size is the key to successful clinical studies. These parameters will determine the minimum sample size to assure adequate statistical power for detecting a difference between experimental groups if this difference exists. Regulatory agencies, including the Food and Drug Administration (FDA), have suggested that clinically meaningful (or direct) outcomes should be able to measure how a patient feels (e.g. disease symptoms/quality of life) functions or survives (e.g. survival rates) (FDA, 2012). Nonetheless, is not always possible to use a direct outcome in RCTs, either because the resolution of the disease is not associated with evident symptoms - as in the case of periodontal diseases - or because the endpoint chosen is a rare event that often manifests only long-term – as in the case of mortality/survival rate for certain chronic conditions such as atherosclerosis. In the latter case, very large, long and expensive trials are necessary to test the efficacy of a new treatment.

Whenever it is not feasible to choose a direct outcome, a surrogate outcome such as a biomarker or a clinical sign/measurement can be used as an alternative. Classic examples may be found in the medical field. For instance, RCTs testing a new medication to prevent myocardial infarction or atherosclerotic disease using the clinical events themselves or "death" due to these conditions as the primary outcome could take decades to be completed, since these are low event rates in the general population. Thus, arterial blood pressure is commonly used as a surrogate outcome for myocardial infarction or heart failure, and cholesterol levels for atherosclerotic disease. While surrogate outcomes have been successfully used for clinical practice decision-making, their proper use depends on their ability to predict meaningful benefits to patients (Prentice, 1989; Hujoel and DeRouen, 1995; Fleming and DeMets, 1996; Koch and Paquette, 1997; Hujoel, 2004).

The treat-to-target concept

The treat-to-target concept was introduced in the medical literature in the late 1990s, and its basic principle is to treat a disease until a prespecified clinical or laboratorial target is achieved. This approach has proven efficacy in chronic medical disorders, such as diabetes (target: blood glucose), atherosclerotic disease (target: cholesterol levels) and cerebral vascular accident (target: arterial blood pressure). Although effective, the greatest challenge of this treatment approach is to identify targets that reflect disease remission/control. Patients who stay within the limits of a certain treatment goal are expected to have lower chances of developing future disease progression and complications, worsening of the condition or fatal events. Diabetes is a good example of the effectiveness of the treat-to-target concept. Most RCTs testing new therapies for controlling diabetes evaluate treatments according to their effectiveness in maintaining blood levels of glycated hemoglobin (HbA1c) and fasting plasma glucose within certain thresholds (Nathan *et al.*, 1993). More recently, the treat-to-target concept has been extended to other clinical conditions, such as rheumatoid arthritis (Grigor *et al.*, 2004; Son *et al.*, 2017), psoriasis (Takeshita *et al.*, 2015) and lupus (van Vollenhoven *et al.*, 2014).

Primary outcome variables and the treat-to-target concept in periodontal trials

Although there are valid arguments in favor of using tooth loss as a clinically meaningful direct outcome

in RCTs (Hujoel, 2004), there are several difficulties associated with the use of this parameter. First, spontaneous tooth loss (i.e., tooth exfoliation) is a rare event and second, most of the observed losses are due to a clinical decision to extract by a practitioner who deems the tooth hopeless at some point during the periodontal treatment. In fact, the definition of a "hopeless tooth" is in no way standardized between practitioners. Further, tooth loss after active periodontal treatment is even rarer (Graetz *et al.*, 2017) as treatment plans typically include extraction of teeth with very advanced disease as part of initial therapy. The use of PROMs as direct endpoints in periodontal trials has also been discussed (Shanbhags *et al.*, 2012; Baiju *et al.,* 2017; Sharma *et al.*, 2018), but no RCTs to date have used PROMs as primary outcome variables. Thus, most pivotal RCTs published to date testing different periodontal treatments have used surrogate outcomes.

The most commonly used surrogate outcomes in periodontal trials are mean reductions in PD or gain in CA at initially deep sites. Sample size calculations using these parameters normally give rise to very small trials. For example, in a study with a parallel design of two arms using mean PD reduction in sites with PD≥7 mm as the primary outcome and an expected difference of 1 mm between groups, only 14 to 16 patients per group would be necessary to reach a power of 80%. Some authors have used changes in full-mouth variables, which is even worse since full-mouth data are normally diluted by the high number of shallow sites in subjects with periodontitis – even in advanced cases - as these sites do not show considerable changes in PD and CA after treatment. Thus, power calculations based on full-mouth changes in mean PD or CA normally use a 0.5 mm target difference between groups, which has generated many questions and debates about statistical significance versus clinical relevance of the differences observed between therapies. Although there is no evident error in these calculations, hidden pitfalls may exist. For instance, many of these studies may have been underpowered to show a true clinically meaningful benefit from treatments. Unfortunately, underpowered RCTs are not rare occurrences in the dental and medical literature. This may happen for different reasons, such as, for example, underestimating the standard deviation of continuous outcomes, overestimating the effect size, or both (Vickers, 2003). Regrettably, it has been estimated that presentation of sample size calculations in the dental literature is suboptimal, and over 70% of the studies published in leading dental journals have reported inadequate data to allow for replication of sample size assumptions (Koletsi *et al.*, 2014). Another important criticism about using changes in mean PD and CA as primary outcomes in periodontal RCTs is the fact that they do not indicate periodontal health or disease remission/control. Thus, other primary outcome variables have been explored to facilitate the interpretation and comparison of findings of the various RCTs and to make these results more suitable and logical for clinical practitioners (Cionca *et al.*, 2009; Feres *et al.*, 2012; Harks *et al.*, 2015; Borges *et al.*, 2017).

It has been advocated that residual pockets post-treatment may favor persistence/recurrence of periodontal inflammation and infection, and consequently, progression of attachment loss (Claffey and Egelberg, 1995; Renvert and Persson, 2002; Lang and Tonetti, 2003; Matuliene *et al.,* 2008, Graetz et al., 2017). The presence of sites with PD≥ 5 mm was one of the six parameters included in the proposed Periodontal Risk Assessment index by Lang and Tonetti (2003). The presence of ≤ 4, 5-8 and ≥9 of these sites was one of the criteria for defining low, moderate and high risk, respectively, for future disease progression (Lang and Tonetti, 2003). Later on, a robust long-term risk assessment study showed that the presence of 9 or more sites with PD ≥ 5 mm or at least one residual pocket with PD ≥ 6 mm were associated with increased risk of future disease progression in a population of 172 subjects treated for periodontitis and under periodontal maintenance for an average period of 11.3 (range: 3–27 years). These authors also observed that the presence of a pocket ≥ 5 mm (at the tooth level), with or without bleeding on probing, or ≥ 30% of sites with bleeding on probing in the mouth increased the risk of tooth loss (Matuliene *et al.*, 2008). The presence of a pocket ≥ 6 mm in a given tooth after treatment has also been associated with an increased risk of losing the tooth (Salvi et al. 2014, Graetz *et al.*, 2017). Graetz *et al.* (2017) followed 57 patients with generalized aggressive periodontitis under supportive periodontal treatment for an average of 17 years (range: 9–28 years) and observed that the risk of tooth loss was significantly increased with each mm of residual probing depth.

Other authors have advocated that the presence of residual pockets with bleeding on probing (BOP) post-treatment would be the most accurate outcome to evaluate results of treatment (Mombelli *et al.*, 2015). BOP is a very important parameter to measure inflammation burden and infection load at patient level, but it seems less effective as a predictor for disease progression at site level (Matuliene *et al.*, 2008; Farina *et al.*, 2013). In the study of Matuliene *et al.* (2008), although BOP increased the probability of tooth loss at tooth level, the presence of deep sites post-treatment at patient level was associated with future disease progression, independently of the presence/absence of bleeding. It is important to highlight that deep pockets harbor a more dysbiotic subgingival biofilm and higher levels of periodontal pathogens than shallow pockets (Socransky and Haffajee, 2005; Pérez-Chaparro *et al.*, 2018), being

therefore more prone to bleeding than shallower pockets (Farina *et al.*, 2013). Thus, even if bleeding is not detected during a particular appointment in a deep pocket, it may eventually appear. These considerations suggest that deep pockets are risk-associated niches for disease activity, independently of the presence of bleeding.

Altogether, the above-mentioned data indicated that "presence/absence of deep pockets" post-treatment has good potential to be an effective surrogate outcome in periodontal trials, since it has been correlated with disease "recurrence/stability". Defining a maximum threshold for residual pockets after treatment may be an effective treat-to-target approach for RCTs in periodontology: a strategy that has been suggested before (Bartold and Van Dyke, 2017; Bartold *et al.*, 2019), but it remains largely unused mainly due to the lack of established endpoints for periodontal treatment.

Proposal of a patient-centered endpoint for applying the treat-to-target concept in periodontal trials

The presence of at most 4 residual sites with PD ≥ 5 mm post-treatment was reported for the first time by Feres *et al.* (2012) to be a reasonable cutoff for predicting stability with little or no future attachment loss. Afterwards this parameter was reported in other studies evaluating the effects of scaling and root planing (SRP), antibiotics, probiotics and host-modulators in periodontal treatment (Mestnik *et al.*, 2012; Teughels *et al.*, 2013; Faveri *et al.*, 2014; Harks *et al.*, 2015; Laleman *et al.*, 2015; Tekce *et al.*, 2015; Tamashiro *et al.*, 2016; Cosgarea *et al.*, 2017; Araujo *et al.*, 2019; Almeida *et al.*, 2020; Castro dos Santos *et al.*, 2020) and it has also been used as a primary outcome variable (Borges *et al.*, 2017). This outcome was considered a promising endpoint for treatment because it was capable of discriminating the effects of different periodontal treatments and was accurately correlated with microbiological changes brought about by treatments (Feres *et al.*, 2012; 2015; Tamashiro *et al.*, 2016; Borges *et al.*, 2017). In order to test the effectiveness of this outcome in a large population, we consolidated the data sets of the 4 RCTs with time intervals of 1 and 2 years of follow-up, which included patients with periodontitis stages III and IV treated using SRP alone or with adjunctive metronidazole (MTZ), MTZ+ amoxicillin (AMX), local tetracycline fibers and open-flap debridement. Two studies were conducted in Brazil (Feres *et al.*, 2012; Tamashiro *et al.*, 2016), one in the USA (Goodson *et al.*, 2012) and one in Germany - ABPARO study (Harks *et al.*, 2015). From the 385 subjects treated with adjunctive antibiotics, 347 took MTZ+AMX and 38 MTZ. All study protocols were approved by the institutional review committees for human subjects and appropriate informed consents was obtained. All studies included regular maintenance sessions after the active phase of treatment. The demographic and clinical characteristics of the three databases at baseline are presented in Table 1. A total of 724 patients were included in the analysis. Overall, the German study included patients with lower levels of periodontal destruction in comparison with the other centers, and the Forsyth patients had the highest mean full-mouth PD among the 3 centers.

Initially, we aimed to check whether the proposed endpoint (≤4 sites with PD ≥ 5 mm) was a frequent or a rare post-treatment event. For this purpose, we tested several thresholds of residual sites (from ≤2 to ≤9). Because there is some debate about whether or not the presence of residual sites with BOP post-treatment would be compatible with disease remission/control, we conducted the analyses accepting in the endpoint: (i) only residual sites without BOP (Figure 1a) or (ii) with and without BOP (Figure 1b). 25% of the population harbored at most 2 residual sites (without BOP) with PD≥5 mm at 1 year post-treatment. This percentage dropped to 15% when at most 3 residual sites (without BOP) were considered, and no subjects had 8 or 9 sites with PD≥5 mm, all of them without BOP (Figure 1a). These findings indicate that achieving a post-treatment endpoint that accepts some remaining pockets provided none of them bleed is an event of very low prevalence, which hampers the feasibility of the endpoint. Data in Figure 1b show that more patients achieved the different clinical thresholds (from 34% to 68%) when residual sites with and without BOP were accepted in the endpoint. The point of intersection between the two curves was detected within the interval ≤4 and ≤5 sites with PD≥5 mm, with 49% and 52% of the subjects, respectively, achieving these clinical statuses (Figure 1b). Thus, these were considered optimal thresholds to define an endpoint for treatment according to the treat-to-target approach. A cut-off point that keeps around 50% of the population within the limits of a surrogate is considered ideal. According to the cross-sectional data of the National Health and Nutritional Examination Survey (NHANES, 1988-2010) in the United States, the overall presence of individuals with diabetes achieving the target of HbA1c <7.0% after treatment was 52.5% (Stark Casagrande *et al.*, 2013). This percentage increases to 62-72% when considering the target HbA1c <8.0%, but the odds of diabetes complications also increase, meaning that more uncontrolled patients fall within the target. Following this line of thought, we hypothesized that accepting more than 5 sites with PD≥5 mm would increase the risk of not accurately distinguishing between periodontal disease remission/control and disease instability. Thus, we then compared the two endpoints ≤4 and ≤5 sites with PD≥5 mm regarding patients' stability from 1 to 2 years, and some advantage was observed for the threshold ≤4 sites. 88% of the subjects achieving this endpoint at 1 year stayed within the

Table 1. Demographic and clinical characteristics of the population evaluated at baseline.

Study	*n*	*Age*	*Systemic condition (# of subjects)*			*Variable (mean ± SD)*				*Treatment*	
			H	*D*	*S*	*PD (mm)*	*CAL (mm)*	*BOP (% sites)*	*PD ≥5 (# sites)*	*SRP*	*SRP + MTZ/MTZ + AMX*
German-ABPARO	403	52.48±10.34^{A}	403	0	135	3.46±0.75^{A}	4.04±0.94^{A}	34.64±18.38^{A}	26.83±20.01A	200	203
Forsyth Institute	187	48.37±10.41^{B}	187	0	75	4.26±0.77^{B}	4.18±1.24	53.57±24.29^{B}	34.07±18.13^{B}	92*	95**
Guarulhos University	134	48.37± 9.56^{B}	82	52	0	3.68±0.73^{C}	4.28±0.95^{B}	57.34±25.00^{B}	30.16±19.13	47	87
Total/ average	724	50.65±10.41	672	52	210	3.71±0.82	4.12±1.03	43.74±23.67	29.31±19.60	339	385

This Table presents the results of 4 previously published clinical trials (Feres *et al.*, 2012; Goodson *et al.*, 2012; Harks *et al.*, 2015; Tamashiro *et al.*, 2016).
Antibiotic protocols: the German-ABPARO (Harks *et al.*, 2015) and Guarulhos University (Feres *et al.*, 2012 and Tamashiro *et al.*, 2016) studies used 500 mg AMX and 400 mg MTZ, thrice a day (TID), for 7 and 14 days, respectively. The Forsyth study used AMX 500 mg , two times a day (BID), and MTZ 250 mg TID for 14 days. From those subjects treated with adjunctive antibiotics, 347 took MTZ+AMX and 38 MTZ.
Mechanical treatments: All subjects received Scaling and Root Planing (SRP). The Forsyth Institute study included the following adjunctive treatments: * 26 subjects received adjunctive local tetracycline fibers (LTC), 21 adjunctive surgeries (SURG) and 22 adjunctive LTC+SURG. ** 28 subjects received adjunctive LTC, 18 adjunctive SURG and 26 adjunctive LTC and SURG.
* Different letters represent statistically significant differences among groups, ANOVA.
PD: Probing Depth; CAL: Clinical Attachment Level; BOP: Bleeding on Probing; SD: Standard Deviation; MTZ: Metronidazole; AMX: Amoxicillin; H: Systemically healthy patients; D: Diabetic patients; S: Smokers

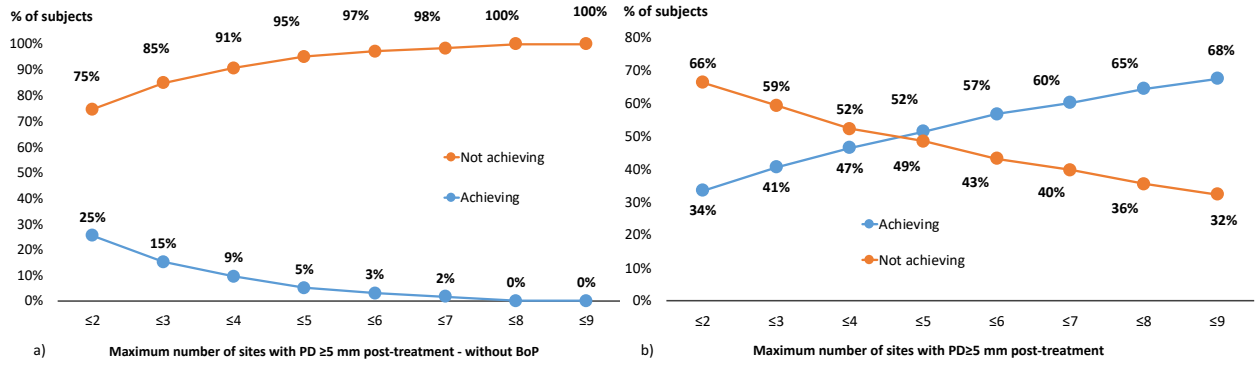

Figure 1. ***Percentage of subjects reaching or not several thresholds of residual sites (from ≤2 to ≤9 sites) post-treatment, a) without bleeding on probing or b) independently of bleeding on probing. The population studied comprises 724 subjects with periodontitis stages 3 and 4 treated in different studies (Feres et al., 2012; Goodson et al., 2012; Harks et al., 2015; Tamashiro et al., 2016).***

endpoint at 2 years, as opposed to 83% of those achieving ≤5 sites. It is worth noting that stability was reduced from 1 to 2 years in the lower thresholds. For instance, 79% and 82% of the subjects presenting, respectively, ≤2 and ≤3 sites at 1 year, stayed at the endpoint at 2 years.

Next, we aimed to assess whether the proposed endpoint was a good "measuring stick" for post-treatment disease control/remission. Thus, we formulated the following questions: (i) Did those who achieve the endpoint exhibit signs of disease remission/control? (ii) Did those who did not achieve the endpoint exhibit signs of uncontrolled disease? (iii) Are these clinical characteristics (disease remission/no-remission) maintained in the long term (from 1 to 2 years post-treatments)? Table 2 and Figure 2 present the results of these analyses. Subjects who achieved the endpoint "≤4 sites with PD≥5 mm" had fewer residual sites ≥5, ≥6 and ≥ 7 mm at 1 year than those who did not achieve the endpoint. The group that

Table 2. Mean ± SD full-mouth clinical parameters between subjects who achieved or did not achieve the clinical endpoint for treatment at 1 and 2 years post-treatment

Variable	*Time-point*	*Achieved the clinical endpoint for treatment (≤4 sites with PD ≥5 mm)*		*ANCOVA test*
		Yes n=345 (47.7%)	*No n=379 (52.3%)*	
PD (mm)	1-year	2.35 ± 0.44	3.11 ± 0.55	0.000
	2-years	2.35 ± 0.47	3.01 ± 0.65	0.000
CAL (mm)	1-year	3.14 ± 0.86	3.97 ± 0.93	0.000
	2-years	3.22 ± 0.88	3.96 ± 1.01	0.000
% sites with BOP	1-year	12.13 ± 13.51	24.04 ± 16.73	0.000
	2-years	11.98 ± 13.49	22.08 ± 17.66	0.000
Mean number of sites with				
PD ≥5 mm	1-year	1.64 ± 1.47	14.74 ± 10.08	0.000
	2-years	2.29 ± 2.88	13.69 ± 12.13	0.000
PD ≥6 mm	1-year	0.44 ± 0.76	6.55 ± 6.80	0.000
	2-years	0.80 ± 1.55	6.72 ± 8.22	0.000
PD ≥7 mm	1-year	0.12 ± 0.37	2.72 ± 4.04	0.000
	2-years	0.26 ± 0.80	2.89 ± 4.77	0.000

PD: Probing Depth; CAL: Clinical Attachment Level; BOP: Bleeding on Probing ; SD: Standard Deviation

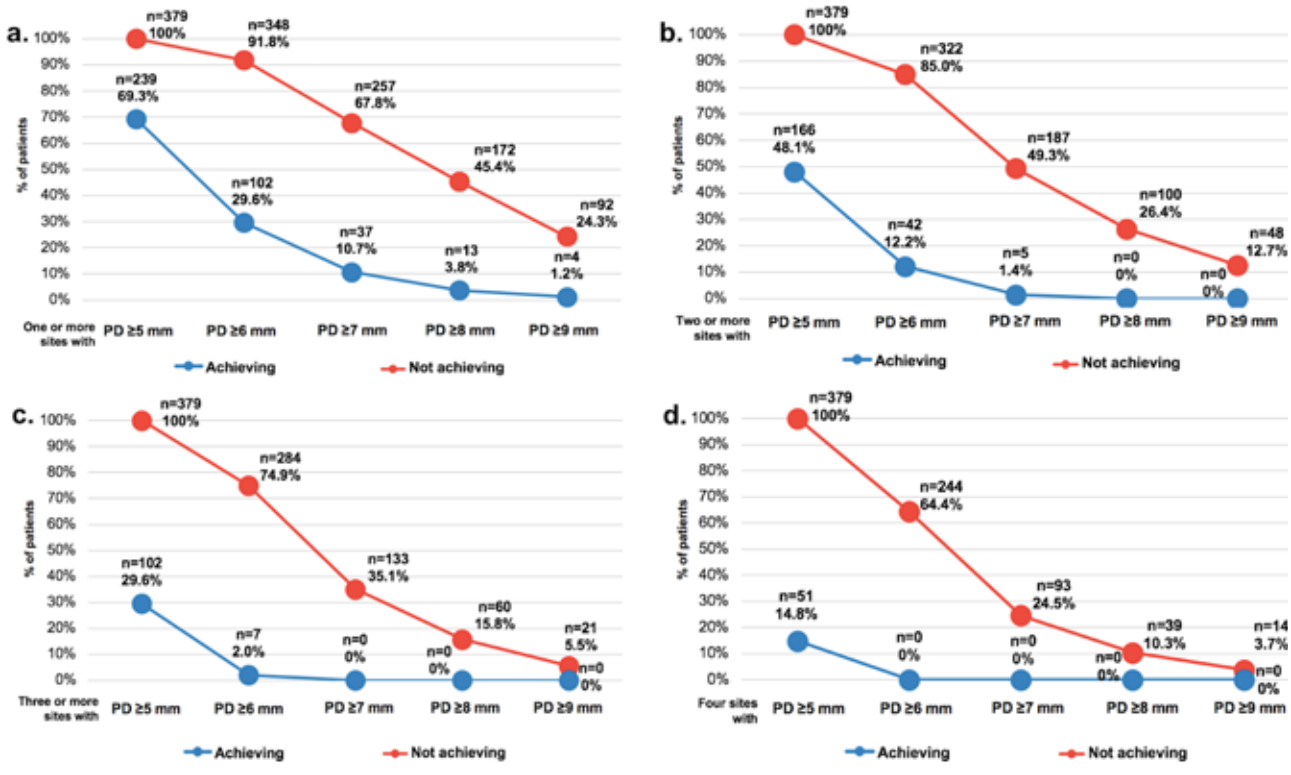

Figure 2. ***Number and percentage of subjects harboring at least 1(a), 2(b), 3(c), 4(d) site/sites with probing depth ≥5mm, ≥6mm, ≥7mm, ≥8mm and ≥9mm among those individuals achieving or not achieving the clinical endpoint for treatment (≤ 4 sites with probing depth ≥ 5 mm) at 1 year post-treatment.***

achieved the endpoint for treatment had an average of 1.64 sites with PD≥5 mm at 1 year, compared with 14.74 sites in the other group. It is interesting to observe is that the group that achieved the endpoint harbored almost no residual sites ≥6 or ≥7mm at 1 year post-treatment (0.44 ±0.76 and 0.12±0.37, respectively), while the group that did not achieve the endpoint still harbored several of these deep pockets at 1 year (Table 2). Figure 2 shows that only 1.4% (n=5 patients) of the subjects achieving the endpoint at 1 year still harbored two or more pockets ≥7 mm, as opposed to 49% of those not achieving the endpoint (n=187 patients). In addition, of the 345 individuals achieving the endpoint at 1 year, only 37 (10.7%), 13 (3.8%) and 4 (1.2%) still harbored ≥1 site with PD ≥7, 8 or 9 mm, respectively. The numbers for the group that did not achieve the endpoint (n=379) were, respectively, 257 (67.8%), 172 (45.4%) and 92 (24.3%).

The traditional outcomes such as mean PD, mean CA and full-mouth BOP were also lower in the group that achieved the endpoint. Most importantly, all these statistically significant differences between the two groups were maintained at 2 years post-treatment (Table 2).

We also examined the microbial profiles of subjects from the Brazilian center. Subgingival biofilm samples were evaluated for 40 bacterial species (Socransky *et al.*, 1998) using Checkerboard DNA–DNA hybridization (Figure 3). The group of individuals that achieved the proposed endpoint maintained a microbial profile compatible with health. Red complex pathogens comprised 4% and 6% of the 40 microorganisms evaluated at 1 and 2 years, respectively, in this group compared with 10% and 13%, respectively, in the group that did not achieve the endpoint. This information is relevant, because previous analyses have suggested attachment instability when the red complex accounted for more than 10% of the 40 bacterial species from the traditional Checkerboard panel (Feres *et al.*, 2015). On the other hand, the proportions of the host-compatible *Actinomyces* species as well as *Veillonella parvula* and *Actinomyces odontolyticus* from the "purple complex" were elevated in subjects who reached the endpoint.

Taken together, these clinical and microbiological data suggest that the proposed clinical endpoint is a good "measuring stick" for determining disease remission/control after the active phase of periodontal treatment.

We then used logistic regression analysis to look at parameters that could possibly have influenced patients' response relative to achieving the endpoint at 1 and 2 years. The dependent variable for these analyses was "achieving or not achieving the endpoint". Extensive logistic regression models were fitted to the data according to different parameters and thresholds (Supplementary section). To evaluate any possible factors interfering with a patient achieving or not achieving the endpoint at 1 year, the following categorical variables were selected:

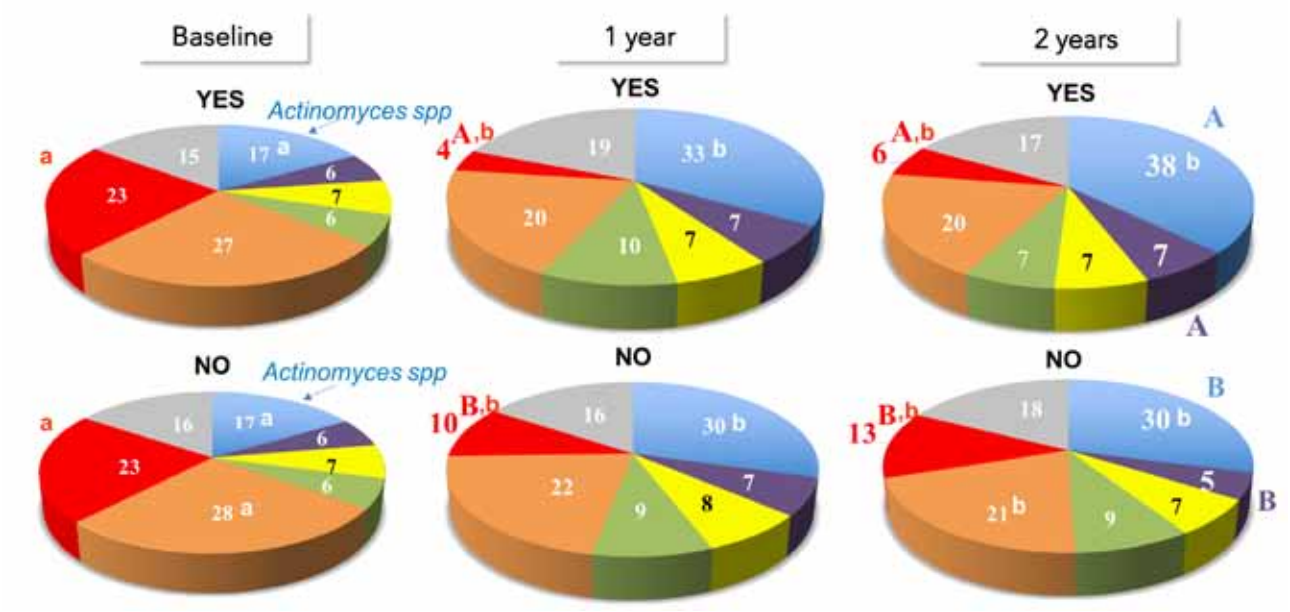

Figure 3. ***Subgingival microbial profiles of subjects who achieved (YES) or did not achieve (NO) the clinical endpoint for treatment "≤ 4 sites with probing depth ≥ 5 mm" at 1 year post-treatment. The population studied comprises 134 subjects with periodontitis stages III and IV treated in two studies (Feres et al., 2012; Tamashiro et al., 2016). Nine subgingival biofilm samples were taken from each subject at each time point and were analyzed separately to determine their content of the 40 species described by Socransky et al. (1998). The grey color represents species that did not fall into any complex. The significance of differences within each group over the course of the study was assessed using Friedman and Dunn's multiple comparison tests (different small letters indicate significant differences, p<0.05). The significance of differences between groups at each time point was determined using the Mann–Whitney U-test (different capital letters indicate significant differences, p < 0.05).***

full-mouth mean PD, number of sites with PD ≥5 mm, BOP, plaque, age, gender, diabetes, smoking and study center. The only factors that significantly interfered with the chances of a patient achieving the endpoint at 1 year were: full-mouth mean PD, number of sites with PD≥5 mm at baseline and smoking. Thus, we stratified the data according to different thresholds of sites with PD≥5 mm at baseline. Non-smokers presenting 15-30 sites with PD≥5 mm at baseline had higher chances of achieving the endpoint than smokers, with an OR=1.9 (Supplementary section).

To evaluate any possible parameters interfering with a patient staying/achieving or entering/leaving the endpoint from 1 to 2 years, the logistic regression models were repeated using the same variables previously described but with different cutoffs for the quantitative variables. The cutoffs were based on their quartiles at 1 year as well as on biological premises and the power of the data (i.e. sample sizes in each clinical scenario described in Supplementary Tables 3a and 3b). The results are described in Table 3. Of those who did not achieve the endpoint at 1 year, the presence of at most 8 residual sites with PD≥ 5 mm (from 5-8 sites) and low levels of full-mouth BOP would increase the chances of the subject achieving the endpoint at 2 years, with an OR=6 for those presenting ≤10% of sites with BOP, and OR=3.4 when 11-20% of the sites exhibited BOP. Of those who achieved the endpoint (≤4 sites with PD ≥5 mm) at 1 year, the presence of more than 10% of sites with BOP increased the chances of leaving the endpoint at 2 years, with an OR=3.5 if 11-20% of the sites in the mouth exhibited BOP, and OR=8.7 if more than 20% of the sites still presented BOP after treatment (Table 3).

The logistic regression analysis did not show a trend towards clinical improvements from 1 to 2 years for patients presenting more than 8 sites with PD≥5 mm. This was in agreement with the findings of the risk assessment study by Matuliene *et al.* (2008) showing that the presence of ≥9 sites with PD≥5 mm post-treatment contributed significantly to the increased risk of periodontitis progression (Matuliene *et al.,* 2008).

Summary of findings from the analyses presented

- The proposed clinical endpoint for treatment (≤4 sites with PD ≥5 mm) was effective in distinguishing between patients showing signs of post-treatment periodontal disease remission/control from those showing signs of uncontrolled disease, at 1 and 2 years post-treatment.
- The presence of >10% (especially >20%) of sites with BOP at 1 year post-treatment increased the risk of a patient leaving the clinical endpoint from 1-2 years.
- The presence of at most 8 sites with PD≥5 mm (from 5 to 8) and ≤ 20% (especially ≤10%) of sites with BOP at 1 year post-treatment increased the chances of a patient achieving the clinical endpoint from 1-2 years.

Discussion

Over the years, the interpretation of clinical trials in periodontology and the extrapolation of the findings from these studies to clinical practice have been hampered by the heterogeneity and subjectivity of the outcome measures used in different studies. Thus, despite the existence of a plethora of RCTs and Systematic Reviews

Table 3. Results of the Logistic Regression Analysis evaluating the impact of different parameters* on the odds ratio of a patient entering or leaving the endpoint from 1 to 2 years post-treatment. The only parameters showing a statistically significant association with these clinical fluctuations were number of residual sites with PD ≥5 mm and BOP.

Endpoint	*Number of sites with PD ≥5 mm at 1 year*	*BOP*	*OR (95% CI)*	
Did not achieve the endpoint at 1 year	5 – 8 sites	BOP-2 years (≤ 10%)	6.0 (2.3 – 16.1)	OR for entering the endpoint at 2 years
		BOP-2 years (11 - 20%)	3.4 (1.2 – 9.6)	
Achieved the endpoint at 1 year	≤ 4 sites	BOP-2 years (11 - 20%)	3.5 (1.4 – 8.4)	OR for leaving the endpoint at 2 years
		BOP-2 years (≥ 21%)	8.7 (3.7 – 20.1)	

PD: Probing Depth; BOP: Bleeding on Probing; OR: Odds Ratio
* The independent variables used in the regressions were: # sites with PD≥5 mm (5|—|8; ≥9), BOP (≤10%; 11%|—|20%; ≥21%), plaque (<20%; ≥20%), Age (≤ 40; 41|—|50; ≥51), Gender (female, male), Smoking (yes, no), Diabetes (yes, no), Center (Forsyth institute, Guarulhos University, German-ABPARO).

(SRs) comparing different periodontal therapies, it is still difficult to determine the best periodontal treatment for different clinical conditions. Difficulties associated with the establishment of outcomes that reflect periodontal health or disease remission/control bring even more complexity to this equation. The 2017 World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions (Caton *et al.*, 2018) for the first time provided a clear definition of periodontal health in an intact and in a reduced periodontium. The parameters established in the workshop for defining post-treatment health (periodontal stability) were "<10% of sites BOP and no sites with PD≥4 mm and BOP" (Chapple *et al.*, 2018). While very low prevalence of BOP and absence of bleeding residual pockets are undoubtedly effective parameters for defining periodontal health after treatment (Lang *et al.*, 1990; Joss et al, 1994; Claffey and Egelberg, 1995; Matuliene *et al.*, 2008; Matuliene *et al.*, 2010; Salvi *et al.*, 2014; Graetz et al., 2017), the applicability of this composite endpoint in RCTs may not be always feasible. When applied to the 724 patients with periodontitis stages III and IV evaluated in the present study, less than 3% of the patients reached both outcomes ("<10% of sites BOP and no sites with PD≥4 mm and BOP").

In one of the expert papers of Workgroup 1 of the 2017 World Workshop, Lang & Bartold (Lang and Bartold, 2018) stressed the need for defining clinical endpoints for treatment that are capable of reflecting disease remission/control, and differentiating between cases of these patients and those who still present high levels of inflammation and would be considered unstable. The clinical endpoint suggested in this paper is in line with this perception and is the first attempt to validate a surrogate outcome in periodontology that reflects disease remission/control after the active anti-infective phase of periodontal treatment (Figure 4). In addition, because the proposed outcome is suggested as an endpoint for treatment at patient level and not a mean change in full-mouth parameters, it is in line with the treat-to-target concept that is already widely used in medicine, but still incipient in dentistry. The analyses provided in this paper indicate that the proposed endpoint has good potential to be an objective measure for RCTs, but caution should be taken while attempting to extrapolate these results to clinical practice. It is important to highlight that this endpoint is open-ended, and it is clearly not the same to have 4 residual sites with 5 mm or with 10 mm after the active phase of

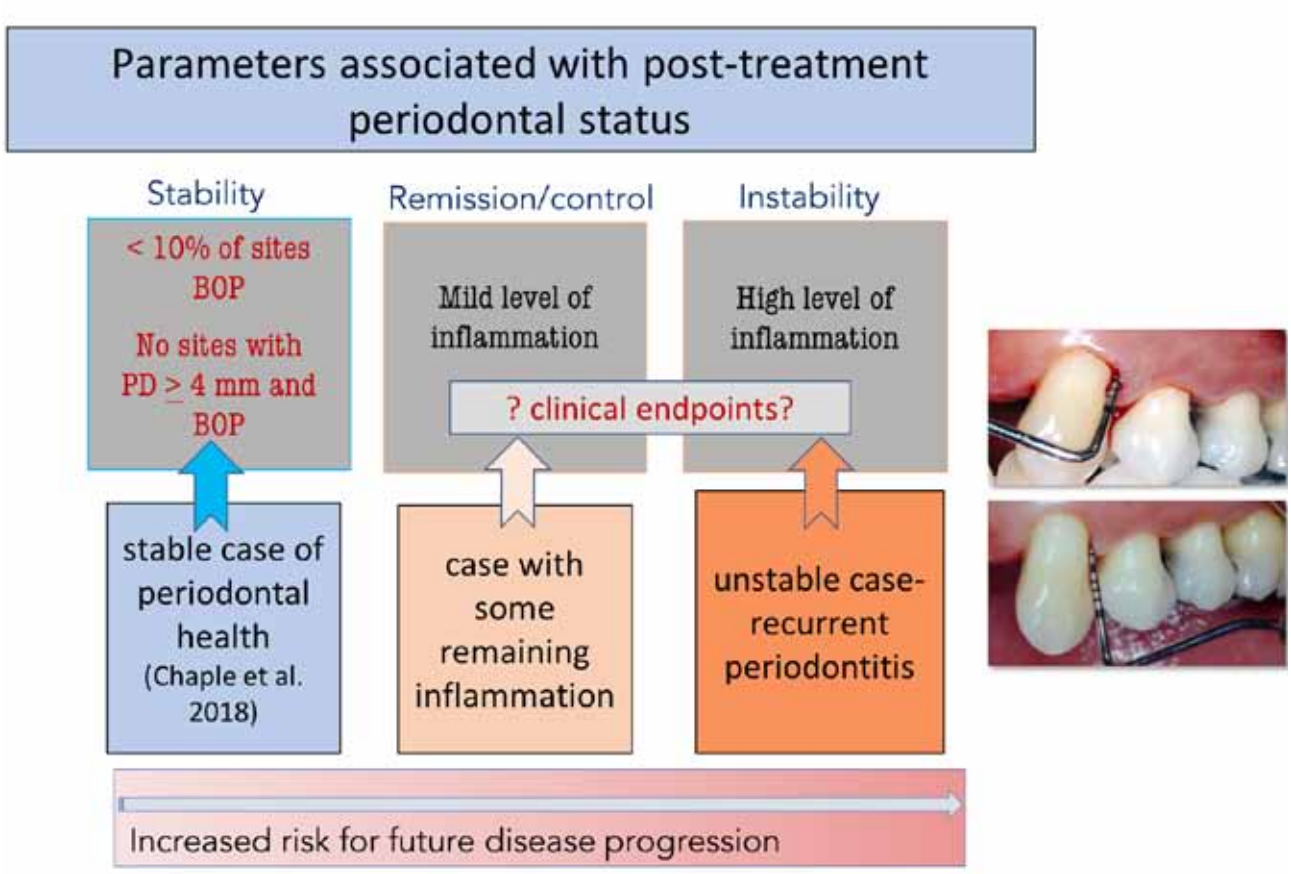

Figure 4. ***Illustration representing the rationale for determining clear parameters that reflect post-treatment disease remission or control. Criteria for periodontal health/stability after treatment have been recently proposed (Chapple et al., 2018). However, achieving total resolution of inflammation is not always possible, leading to the need of defining other criteria/endpoints that reflect disease remission/control with periodontal stability (Lang and Bartold, 2018) and differentiates these cases from those with an unstable clinical situation.***

periodontal treatment. While the chances of a patient that achieves the endpoint still has very deep sites (i.e., PD ≥7 or ≥8 mm) are quite low (Figure 2), this may happen, and very deep pockets would require further treatment. It is also important to bear in mind that deep pockets at site/tooth level increase the risk of tooth loss in the long term (Matuliene *et al.*, 2008, Salvi *et al.*, 2014, Graetz *et al.*, 2017). Therefore, to establish the need for additional treatment in clinical practice, each patient and each tooth should be evaluated individually according to a number of criteria, such as the depth of the pocket, the presence of furcation involvement, the feasibility of reconstructive/regenerative treatments, and the presence of risk factors, such as smoking or diabetes. The clinician should bear in mind that although the definition of disease remission/control is not fully established, the fewer bleeding residual sites post-treatment, the greater the chances of keeping periodontal stability longitudinally (Chapple *et al.*, 2018; Lang and Bartold, 2018; Dietrich *et al.*, 2019).

Future robust risk assessment studies should be conducted in order to establish if the endpoint "≤4 sites with PD ≥5 mm" can reflect how a patient feels, functions or survives, in other words, how it correlates with periodontal stability, tooth survival or health-related quality of life, in the long term. Nonetheless, it is important to bear in mind that these associations have never been established for the most traditional outcome measures based on mean changes in PD or CAL. On the other hand, although the proposed endpoint has not yet been directly tested in risk assessment studies of long periods of follow-up, the presence of multiple sites with PD ≥5 mm post-treatment has already been associated with future disease progression in a comprehensive risk assessment study (Matuliene *et al.* 2008). Moreover, Sharma et al. (2019) evaluated 14,568 dentate patients and demonstrated almost linear associations between a composite outcome (including the presence of deep pockets, bone loss and BOP) and PROMs of "discomfort", "restricted eating" and "unhappiness with appearance". The authors also observed that patients with alveolar bone loss, but no deep periodontal pockets were less likely to report dental pain or restrictions in the diet compared to patients with deep periodontal pockets. Taken together, these data suggest that the present endpoint, based on residual sites with PD≥5mm, has good potential to reflect direct benefits to the patient, increasing its value as a parameter of choice for the treat-to-target approach.

Conclusion

The endpoint for active periodontal treatment proposed in this paper (≤4 sites with PD ≥5 mm) seems to be an effective measure for applying the treat-to-target concept in periodontal trials. Clinical researchers are encouraged to present results on this outcome as this would allow for an objective comparison among studies and make it easier to conduct SRs. A global effort to standardize the outcomes reported by all RCTs in specific areas of health care is in course (Core Outcome Measures in Effectiveness Trials, 2020) and the periodontal field should follow such initiatives. Furthermore, the information generated by the regression models suggesting that the presence of >10% and, specially, >20% of sites with BOP in the mouth may worsen the periodontal clinical status between 1 and 2 years post-treatment is also relevant. These findings indicate that full-mouth BOP should be carefully monitored during supportive periodontal maintenance. Researchers could also consider using "≤10% or ≤20% of bleeding sites" post-treatment as clinical outcomes for BOP in RCTs. In the future, the value of a composite endpoint including "≤4 sites with PD ≥5 mm" and these different thresholds of BOP could be also explored.

Finally, researchers should investigate direct outcomes, including PROMs, such as those suggested by Sharma *et al.* (2019), as well as other surrogates, such as biological parameters (e.g. cytokines or metabolites). These measures may be used as alternatives or in combination with the endpoint proposed in this paper in an attempt to establish effective composite outcomes. Large risk assessment studies are necessary to determine if these and other new endpoints being proposed for periodontal treatment would reflect attachment stability, tooth survival and good quality of life in the long term. The combination of large data sets may be the easiest and fastest way to conduct such analyses.

Acknowledgments and conflict of interest

The authors would like to thank Sigmund Socransky, Anne Haffajee and Ricardo Teles for their willingness to share the data from some of the studies included in the present analyses. The authors would also like to express their profound appreciation and gratitude for the overall contributions of these leaders to the field of clinical periodontology. Their passion, generosity and dedication, along with their insightful discussions, continue to shape the field and don't cease to inspire us. The authors also thank Nidia Castro for her comments and suggestions. The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

Sources of funding statement

This study was supported by the following research grants: São Paulo State Research Foundation (FAPESP, São Paulo, SP, Brazil): grants 2007/55291-9, 2011/14872-4 and 2013/01072-5; National Institute of Dental and Craniofacial Research: grants DE12861

and RR025771, and GCRC funding from RR00533, RR025020 and RR01032; German Research Foundation (Deutsche Forschungsgemeinschaft, DFG): grant EH 365 1-1, and the Open Access Publication Fund of the University of Munster. The authors also acknowledge the Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES), the National Council for Scientific and Technological Development (CNPq, Brazil) and Latin American Oral Health Association (LAOHA) for supporting some researchers involved in this study.

REFERENCES

Almeida ML, Duarte PM, Figueira EA *et al.* Effects of a full-mouth disinfection protocol on the treatment of type-2 diabetic and non-diabetic subjects with mild-to-moderate periodontitis: one-year clinical outcomes. *Clinical Oral Investigations* 2020; **24**(1):333-341.

Araujo CF, Andere NMRB, Castro Dos Santos NC *et al.* Two different antibiotic protocols as adjuncts to one-stage full-mouth ultrasonic debridement to treat generalized aggressive periodontitis: A pilot randomized controlled clinical trial. *Journal of Periodontology* 2019; **90**(12):1431-1440.

Bartold PM, Kantarci A, Feres M, Iacono V and Meyle J. Meeting Report 1st International Academy of Periodontology Research Conference (IAPRC). *Journal of the International Academy of Periodontology* 2019**; 20**(1):1-3.

Bartold PM and Van Dyke TE. Host modulation: controlling the inflammation to control the infection. *Periodontology 2000* 2017; **75**(1):317-329.

Baiju RM, Peter E, Varghese NO and Anju P. Patient Reported Outcome Assessment of Periodontal Therapy: A Systematic Review. *Journal of Clinical and Diagnostic Research* 2017; **11**(8):ZC14-ZC19.

Borges I, Faveri M, Figueiredo LC *et al.* Different antibiotic protocols in the treatment of severe chronic periodontitis: A 1-year randomized trial. *Journal of Clinical Periodontology* 2017; **44**(8):822-832.

Calvert M, Blazeby J, Altman DG *et al.* Reporting of patient-reported outcomes in randomized trials: the CONSORT PRO extension. *Journal of the American Medical Association* 2013; **309**(8):814-22.

Castro Dos Santos NC, Andere NM, Araujo CF *et al.* Omega-3 PUFA and aspirin as adjuncts to periodontal debridement in patients with periodontitis and type 2 diabetes mellitus. Randomized clinical trial. *Journal of Periodontology* 2020. doi: 10.1002/JPER.19-0613.

Caton JG, Armitage G, Berglundh T *et al.* A new classification scheme for periodontal and peri-implant diseases and conditions - Introduction and key changes from the 1999 classification. *Journal of Periodontology* 2018; **89** (Suppl 1):S1-S8.

Chapple ILC, Mealey BL, Van Dyke TE *et al.* Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *Journal of Clinical Periodontology* 2018; **45** (Suppl 20):S68-S77.

Cionca N, Giannopoulou C, Ugolotti G and Mombelli A. Amoxicillin and metronidazole as an adjunct to full-mouth scaling and root planing of chronic periodontitis. *Journal of Periodontology* 2009; **80**(3):364-371.

Claffey N and Egelberg J. Clinical indicators of probing attachment loss following initial periodontal treatment in advanced periodontitis patients. *Journal of Periodontology* 1995; **22**(9):690-696.

Cosgarea R, Heumann C, Juncar R *et al.* One year results of a randomized controlled clinical study evaluating the effects of non-surgical periodontal therapy of chronic periodontitis in conjunction with three or seven days systemic administration of amoxicillin/metronidazole. *PLoS One* 2017; **12**(6):e0179592.

Core Outcome Measures in Effectiveness Trials [Internet]. 2020; Available from: http://www.comet-initiative.org/

Dietrich T, Ower P, Tank M *et al.* Periodontal diagnosis in the context of the 2017 classification system of periodontal diseases and conditions - implementation in clinical practice. *British Dental Journal* 2019; **226**(1):16-22.

Farina R, Tomasi C and Trombelli L. The bleeding site: a multi-level analysis of associated factors. *Journal of Clinical Periodontology* 2013; **40**(8):735-742.

Faveri M, Rebello A, de Oliveira Dias R *et al.* Clinical and microbiologic effects of adjunctive metronidazole plus amoxicillin in the treatment of generalized chronic periodontitis: smokers versus non-smokers. *Journal of Periodontology* 2014; **85**(4):581-91.

FDA. Clinical Trial Endpoints [Internet]. 2012; Available from: https://www.fda.gov/media/84987/download

Feres M, Figueiredo LC, Soares GMS and Faveri M. Systemic antibiotics in the treatment of periodontitis. *Periodontology 2000* 2015; **67**:131-186.

Feres M, Soares GM, Mendes JA *et al.* Metronidazole alone or with amoxicillin as adjuncts to non-surgical treatment of chronic periodontitis: a 1-year double-blinded, placebo-controlled, randomized clinical trial. *Journal of Clinical Periodontology* 2012; **39**(12):1149-1158.

Fleming TR and DeMets DL. Surrogate end points in clinical trials: are we being misled? *Annals of Internal Medicine Ann Intern Med* 1996; **125**(7):605-613.

Goodson JM, Haffajee AD, Socransky SS *et al.* Control of periodontal infections: a randomized controlled trial I. The primary outcome attachment gain and pocket depth reduction at treated sites. *Journal of Clinical Periodontology* 2012; **39**(6):526-536.

Graetz C, Sälzer S, Plaumann A *et al.* Tooth loss in generalized aggressive periodontitis: Prognostic factors after 17 years of supportive periodontal treatment. *Journal of Clinical Periodontology* 2017; **44**(6):612-619.

Grigor C, Capell H, Stirling A *et al.* Effect of a treatment strategy of tight control for rheumatoid arthritis (the TICORA study): a single-blind randomised controlled trial. *Lancet* 2004; **364**(9430):263-269.

Guerrero A, Griffiths GS, Nibali L *et al.* Adjunctive benefits of systemic amoxicillin and metronidazole in non-surgical treatment of generalized aggressive periodontitis: a randomized placebo-controlled clinical trial. *Journal of Clinical Periodontology* 2005; **32**(10):1096-1107.

Harks I, Koch R, Eickholz P *et al.* Is progression of periodontitis relevantly influenced by systemic antibiotics? A clinical randomized trial. *Journal of Clinical Periodontology* 2015; **42**(9):832-842.

Hujoel PP and DeRouen TA. A survey of endpoint characteristics in periodontal clinical trials published 1988-1992, and implications for future studies. *Journal of Clinical Periodontology* 1995; **22**(5):397-407.

Hujoel PP. Endpoints in periodontal trials: the need for an evidence-based research approach. *Periodontology 2000* 2004; **36**:196-204.

Joss A, Adler R, Lang NP. Bleeding on probing. A parameter for monitoring periodontal conditions in clinical practice. *Journal of Clinical Periodontology* 1994; **21**:402–408.

Koch GG and Paquette DW. Design principles and statistical considerations in periodontal clinical trials. *Annals of Periodontology / the American Academy of Periodontology* 1997; **2**(1):42-63.

Koletsi D, Fleming PS, Seehra J, Bagos PG and Pandis N. Are sample sizes clear and justified in RCTs published in dental journals? *PLoS One* 2014; **9**(1):e85949.

Laleman I, Yilmaz E, Ozcelik O *et al.* The effect of a streptococci containing probiotic in periodontal therapy: a randomized controlled trial. *Journal of Clinical Periodontology* 2015; **42**(11):1032-1041.

Lang NP and Bartold PM. Periodontal health. *Journal of Clinical Periodontology* 2018; **45** (Suppl 20):S9-S16.

Lang NP, Adler R, Joss A, Nyman S. Absence of bleeding on probing. An indicator of periodontal stability. *Journal of Clinical Periodontology* 1990; **17**:714–721.

Lang NP and Tonetti MS. Periodontal risk assessment (PRA) for patients in supportive periodontal therapy (SPT). *Oral Health & Preventive Dentistry* 2003; **1**(1):7-16.

Matuliene G, Pjetursson BE, Salvi GE *et al.* Influence of residual pockets on progression of periodontitis and tooth loss: results after 11 years of maintenance. *Journal of Clinical Periodontology* 2008; **35**(8):685-695.

Matuliene G, Studer R, Lang NP *et al.* Significance of Periodontal Risk Assessment in the recurrence of periodontitis and tooth loss. *Journal of Clinical Periodontology* 2010; **37**(2):191-199.

Mestnik MJ, Feres M, Figueiredo LC *et al.* The effects of adjunctive metronidazole plus amoxicillin in the treatment of generalized aggressive periodontitis: a 1-year double-blinded, placebo-controlled, randomized clinical trial. *Journal of Clinical Periodontology* 2012; **39**(10):955-961.

Mombelli A, Almaghlouth A, Cionca N, Courvoisier DS and Giannopoulou C. Differential benefits of amoxicillin-metronidazole in different phases of periodontal therapy in a randomized controlled crossover clinical trial. *Journal of Periodontology* 2015; **86**(3):367-375.

Nathan DM, Genuth S, Lachin J *et al.* The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *The New England Journal of Medicine* 1993; **329**(14):977-986.

Pérez-Chaparro PJ, McCulloch JA, Mamizuka EM *et al.* Do different probing depths exhibit striking differences in microbial profiles? *Journal of Clinical Periodontology* 2018; **45**(1):26-37.

Prentice RL. Surrogate endpoints in clinical trials: definition and operational criteria. *Statistics in Medicine* 1989; **8**(4):431-440.

Renvert S and Persson GR. A systematic review on the use of residual probing depth, bleeding on probing and furcation status following initial periodontal therapy to predict further attachment and tooth loss. *Journal of Clinical Periodontology* 2002; **29** (Suppl 3):82-89; discussion 90-1.

Salvi GE, Mischler DC, Schmidlin K *et al.* Risk factors associated with the longevity of multi-rooted teeth. Long-term outcomes after active and supportive periodontal therapy. *Journal of Clinical Periodontology* 2014; **41**(7):701-7.

Shanbhag S, Dahiya M and Croucher R. The impact of periodontal therapy on oral health-related quality of life in adults: a systematic review. *Journal of Clinical Periodontology* 2012; **39**(8):725-35.

Sharma P, Yonel Z, Busby M, Chapple IL and Dietrich T. Association between periodontal health status and patient-reported outcomes in patients managed in a non-specialist, general dental practice. *Journal of Clinical Periodontology* 2018; **45**(12):1440-1447.

Socransky SS, Haffajee AD, Cugini MA, Smith C and Kent RL. Microbial complexes in subgingival plaque. *Journal of Clinical Periodontology* 1998; **25**(2):134-144.

Socransky SS and Haffajee AD. Periodontal microbial ecology. *Periodontology 2000* 2005; **38**:135-187.

Son KM, Lee SY, Seo YI, Choi JE and Kim HA. Contribution of subjective Disease Activity Score 28 (DAS28) components to the response to treatment of rheumatoid arthritis. *Clinical Rheumatology* 2017; **36**(6):1221-1227.

Stark Casagrande S, Fradkin JE, Saydah SH, Rust KF and Cowie CC. The prevalence of meeting A1C, blood pressure, and LDL goals among people with diabetes, 1988-2010. *Diabetes Care* 2013; **36**(8):2271-2279.

Takeshita J AA, Mease PJ and Gelfand JM. Treat-to-Target and Improving Outcomes in Psoriasis: A Report from the GRAPPA 2014 Annual Meeting. *The Journal of Rheumatology* 2015; **42**:4.

Tamashiro NS, Duarte PM, Miranda TS *et al*. Amoxicillin Plus Metronidazole Therapy for Patients with Periodontitis and Type 2 Diabetes: A 2-year Randomized Controlled Trial. *Journal of Dental Research* 2016; **95**(7):829-836.

Tekce M, Ince G, Gursoy H *et al*. Clinical and microbiological effects of probiotic lozenges in the treatment of chronic periodontitis: a 1-year follow-up study. *Journal of Clinical Periodontology* 2015; **42**(4):363-72.

Teughels W, Durukan A, Ozcelik O, Pauwels M, Quirynen M and Haytac MC. Clinical and microbiological effects of *Lactobacillus reuteri* probiotics in the treatment of chronic periodontitis: a randomized placebo-controlled study. *Journal of Clinical Periodontology* 2013; **40**(11):1025-1035.

van Vollenhoven RF, Mosca M, Bertsias G *et al*. Treat-to-target in systemic lupus erythematosus: recommendations from an international task force. *Annals of the Rheumatic Diseases* 2014; **73**(6):958-967.

Vickers AJ. Underpowering in randomized trials reporting a sample size calculation. *Journal of Clinical Epidemiology* 2003; **56**(8):717-720.