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RANDOMIZED CLINICAL TRIAL

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Clinical benefits of systemic amoxicillin/metronidazole may depend on periodontitis severity and patients' age: An exploratory sub-analysis of the ABPARO trial

Peter Eickholz[1*] | Raphael Koch[2*] | Thomas Kocher[3] | Thomas Hoffmann[4] | Ti-Sun Kim[5] | Joerg Meyle[6] | Doğan Kaner[7,8] | Ulrich Schlagenhauf[9] | Dag Harmsen[10] | Inga Harks[10*] | Benjamin Ehmke[10*]

[1]Department of Periodontology, Johann Wolfgang Goethe-University Frankfurt, Frankfurt, Germany

[2]Institute of Biostatistics and Clinical Research, University of Münster, Münster, Germany

[3]Department of Restorative Dentistry, Periodontology, Endodontology, and Preventive and Pediatric Dentistry, University Medicine Greifswald, Greifswald, Germany

[4]Department of Periodontology, TU Dresden, Dresden, Germany

[5]Section of Periodontology, Department of Conservative Dentistry, University Hospital Heidelberg, Heidelberg, Germany

[6]Department of Periodontology, University of Giessen, Giessen, Germany

[7]Department of Periodontology, Dental School, Faculty of Health, University of Witten/Herdecke, Witten, Germany

[8]Departments of Periodontology and Synoptic Dentistry, Charité-Universitätsmedizin Berlin, Berlin, Germany

[9]Department of Periodontology, University Hospital Würzburg, Würzburg, Germany

[10]Department of Periodontology, University Hospital Münster, Münster, Germany

Correspondence
Benjamin Ehmke, Department of Periodontology, University Hospital Münster, Münster, Germany.
Email: ehmke@uni-muenster.de

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Abstract

Aim: The aim was to identify benefit thresholds for clinical variables. We hypothesize, if variables fall below or exceed these threshold levels, systemic amoxicillin/metronidazole may contribute to reducing progression of periodontitis.

Material & Methods: This is an explorative per-protocol collective analysis (n = 345) conducted on the placebo-controlled, multi-centre ABPARO trial (ClinicalTrials.gov NCT00707369). Patients received debridement with systemic amoxicillin 500 mg/metronidazole 400 mg (3×/day, 7 days, n = 170) or placebo (n = 175) and maintenance therapy every three months. To identify thresholds, each of the following baseline characteristics was classified into two groups (≥threshold value/<threshold value): bleeding on probing, extent of pocket probing depth (PPD) ≥ 5 mm, mean clinical attachment level and age. Treatment effect (% of sites with new attachment loss ≥ 1.3 mm at 27.5 months post-treatment) was calculated.

Results: Adjunctive antimicrobials reduced median new attachment loss in patients < 55 years (5.2%), or with ≥ 35% PPD ≥ 5 mm (4.5%) or with a mean attachment level > 5 mm (5.2%) at baseline compared to the placebo (9.0%, 11.6%, and 12.5%, respectively; p < 0.005).

*Both authors equally contributed to the manuscript.

Conclusions: The clinical benefits of systemic amoxicillin/metronidazole may depend on periodontitis severity and patients' age.

KEYWORDS
amoxicillin/metronidazole, attachment loss, clinical threshold, periodontitis, systemic antimicrobials

1 | INTRODUCTION

In industrialized countries, approximately 50% of the adult population suffer from moderate or severe periodontitis (Eke, Dye, Wei, Thornton-Evans, & Genco, 2012; Holtfreter, Kocher, Hoffmann, Desvarieux, & Micheelis, 2010). Periodontitis is caused by microbial biofilms (Darveau, 2010; Paster et al., 2001; Socransky, Haffajee, Cugini, Smith, & Kent, 1998) and is clinically characterized by periodontal pocket formation and attachment loss. Teeth affected by periodontitis may lose function and need to be extracted, which often requires costly prosthetic rehabilitations. Periodontal therapy usually is aimed to disrupt tooth adhering biofilm and reduce probing depths, followed by lifelong maintenance therapy (AAP, 2000) .

There is a large body of evidence that mechanical debridement in moderate to severe periodontitis patients could be successfully supplemented by systemic antimicrobials, such as amoxicillin and metronidazole (Keestra, Grosjean, Coucke, Quirynen, & Teughels, 2015; Sgolastra, Gatto, Petrucci, & Monaco, 2012). The rationale for the adjunctive antimicrobials is to exert an additional antimicrobial effect during mechanical therapy and further improve the clinical parameters, especially at severely affected sites (Borges et al., 2017; Ehmke, Beikler, Milian, & Flemmig, 2005). Retrospective cohort studies have detected a higher risk of tooth loss as proportions of persistent probing depths increase (Matuliene et al., 2008; McGuire & Nunn, 1996). It is well established that adjunctive amoxicillin and metronidazole are even more effective in reducing deep pockets compared to mechanical debridement alone (Feres et al., 2012; Mombelli et al., 2013). Tomasi and Wennström have recently found that assessing further attachment loss after treatment is far more appropriate than assessing attachment gains (Tomasi & Wennström, 2017). The ABPARO study applied the surrogate parameter, further attachment loss, to evaluate the benefit of systemic antimicrobials adjunctive for subgingival debridement. However, it remains unclear whether disease progression is reduced, *for example* if further attachment loss and furcation involvement changes are limited (Eickholz et al., 2016; Harks et al., 2015). Therefore, regarding a daily routine, it would be very helpful to have clinical thresholds to determine to what extent adjunctive antimicrobials are effective to prevent further disease progression.

The current explorative analysis of a large multi-centre trial aims to identify thresholds for distinct clinical variables for which the application of adjunctive antimicrobials is associated with better clinical outcomes. We hypothesized that younger patients and those whose disease was more severe would receive greater benefits from empiric adjunctive antimicrobials.

Clinical Relevance

Scientific rationale for study: Systemic amoxicillin and metronidazole are established adjuncts for periodontitis therapy; however, it is unclear which patients will benefit from this. Identifying benefit thresholds from clinical parameters may also help to identify patients in whom, if their values fall short of or exceed these thresholds, the progression of periodontitis may be reduced.
Principal findings: The identified thresholds are based on the baseline proportion of deep pockets, number of deep pockets, mean attachment level at baseline and age. If thresholds were met, the patient benefited from adjunctive antimicrobials with less additional attachment loss.
Practical implications: Information concerning the patient's age and probing pocket depth are easily obtained at the start of periodontal therapy. Clinicians treating patients similar to the population presented in this sub-analysis may consider the reported beneficial thresholds as an additional decision-making aid, either for or against the use of systemic amoxicillin/metronidazole.

2 | PATIENTS AND METHODS

2.1 | Study design

This is an exploratory analysis of the per-protocol collective from the prospective, randomized, stratified, double-blind, multi-centre ABPARO trial (Clinical Trials.gov NCT00707369) over a period of 27.5 months (Eickholz et al., 2016; Harks et al., 2015). The trial examined the effect of systemic amoxicillin (500 mg) and metronidazole (400 mg; 3×/day for 7 days) adjunctive on mechanical subgingival debridement using clinical parameters in patients suffering from moderate to severe periodontitis. Antimicrobials were prescribed empirically, *that is* without prior analysis of intra-oral bacteria. As formerly described (Harks et al., 2014, 2015), patients who followed the protocol and took the two prescribed drugs as scheduled

represent the per-protocol collective. Thus, in the following, only a brief description is provided:

Patients between 18 and 75 years with untreated moderate to severe chronic and aggressive periodontitis were included in this trial. Key clinical inclusion criteria were as follows: at least 10 natural teeth in situ and pocket probing depths (PPD) of ≥ 6 mm in a minimum of four teeth. Key exclusion criteria were as follows: confirmed or assumed allergies or former hypersensitive skin reactions to amoxicillin and/or metronidazole, systemic medications affecting periodontal health and pregnancy. The institutional review boards (IRB) of the participating centres approved the protocol and all patients provided written informed consent. Moreover, an independent data and safety monitoring board reviewed the safety data throughout the trial. Participants were divided into four strata according to the severity of their periodontitis [localized: <38%; generalized: ≥38% of teeth with PPD ≥ 6 mm], as well as their smoking habits [non-/light smoker: <7 ppm CO in exhaled air; moderate to heavy smoker: ≥7 ppm (Bedfont-Smokerlyzer®, Bedfont, UK)]. For stratification purposes, the clinics' patient population was analysed according to the severity of periodontitis, and the median for sites with PPD ≥ 6 mm was determined to be 38% (Harks et al., 2014).

Quad-block patient randomization lists were computer-generated for each stratum per centre (1:1 allocation ratio). This statistician was not involved in further trial affairs. Randomization lists for participating centres were stored exclusively at the study centre.

2.2 | Examinations and endpoints

All measurements were conducted by blinded and calibrated examiners not involved in therapy (Harks et al., 2014, 2015). Full-mouth periodontal measurements were performed at six sites for each tooth: primarily relative attachment level (RAL) measurements, corresponding to the distance from occlusal surface to the bottom of the periodontal pocket (Florida Disk probe, Gainesville, FL, USA). The differences between baseline and 27.5-month RAL readings reveal the changes to the clinical attachment level (gain or loss of tooth-supporting tissue). The primary endpoint was the proportion of sites per patient with new clinical attachment loss (PSAL) ≥ 1.3 mm between the initial visit and the post-27.5-month visit. Among others, the following secondary endpoints were assessed exploratorily: PPD, attachment level (sum of gingival recession and PPD) and gingival bleeding on probing (BOP, Lang, Adler, Joss, & Nyman, 1990). These parameters were used to define a clinical threshold value for the prescription of adjunctive systemic antimicrobials (see statistical analysis).

2.3 | Periodontal therapy

Each patient received 12 examinations and/or therapy visits over the 27.5-month study period. After the baseline examination, patients received supra- and subgingival debridement in up to two sessions on two consecutive days. All mechanical therapy was performed with hand instruments and/or machine-driven scalers. Upon completion of the mechanical debridement, the antimicrobial group of patients received two empiric antimicrobials [amoxicillin $3H_2O$ 574 mg (Amoxicillin-ratiopharm 500 mg®, Ratiopharm, Germany); metronidazole 400 mg (Flagyl® 400, Sanofi-Aventis, Germany)], and the placebo group of patients received two placebo pills, each to be taken three times per day for seven days. The medication was repacked in neutral capsules so that it would appear identical. Patients were re-evaluated at least two months after mechanical debridement. Thereafter, all patients received maintenance therapy, including full-mouth supra- and subgingival debridement and oral hygiene instruction at three-month intervals. Sites with PPD ≥ 4 mm also received subgingival re-debridement.

2.4 | Statistical analysis

Standard univariate statistical analyses were applied. Categorical variables are depicted as absolute and relative frequencies. Fisher's exact tests were used to quantify the evidence between categorical variables. Continuous variables are presented as the mean ± the standard deviation or median (25% quantile (Q25)/ 75% quantile (Q75)). Groups were compared using Mann–Whitney U tests for continuous variables and Fisher's exact tests for categorical variables. Spearman correlation coefficients were calculated between continuous variables.

The primary endpoint in the ABPARO trial was the proportion of sites per patient with new clinical attachment loss ≥ 1.3 mm between the baseline and 27.5 months (PSAL ≥ 1.3 mm). In the present sub-analysis, this endpoint is also used to determine clinical threshold values of the baseline values of BOP (%), extent PPD (% of sites with PPD ≥ 5 mm, number of sites with PPD ≥ 5 mm), mean attachment level (mm) and age (years). Therefore, these continuous variables were classified into two groups each (≥ threshold value/<-threshold value). The effect of the adjunctive antibiotic or placebo therapy on PSAL ≥ 1.3 mm, between the baseline and 27.5 months, was calculated for these groups. Based on the comparison of ranks in each threshold group (≥threshold value/<threshold value), the empirical probability was calculated for the case that PSAL ≥ 1.3 mm in the antimicrobial group is smaller than in the placebo group. By using the *p*-values of the Mann–Whitney U tests to compare the antimicrobial group and placebo group in each threshold subgroup, and based on a clinical meaningful median difference between the treatment groups and sufficient sample size in each group, arbitrary clinical cut-off values were determined.

The combination of thresholds for age and % PPD ≥ 5 mm (% PPD ≥ 5 mm < 35 and age < 55% PPD ≥ 5 mm < 35 and age ≥ 55% PPD ≥ 5 mm ≥ 35 and age < 55% PPD ≥ 5 mm ≥ 35 and age ≥ 55) was examined in an univariate analysis to determine if the treatment effect on clinical attachment loss differed among the categories. A multivariable analysis was performed to adjust for possible imbalances between the subgroups. Confounders were first identified using univariate methods (Supporting Information Tables S1, S2). Subsequently, a multivariable linear regression model was fitted,

including the cut-off variables and potential confounders. A full description of the model is given in Supporting Information Table S3. The results are reported using least-square mean estimates, with corresponding 95% confidence limits and *p*-values obtained from Wald tests.

Statistical analyses were performed using SAS software, version 9.4 of the SAS System for Windows (SAS Institute, Cary, NC, USA). Inferential statistics, like *p*-values and confidence intervals, were intended to be exploratory, not confirmatory. Therefore, neither global nor local significance levels were determined, and no adjustment for multiplicity was applied. Consequently, explorative *p*-values ≤ 0.05 were denominated as statistically noticeable instead of significant.

3 | RESULTS

3.1 | Patients

Of the 506 randomized patients, 345 patients who followed the study protocol during the 27.5-month period and took all tablets within 6–8 days, according to their medication diaries, were included in the per-protocol collective (345 patients, placebo: *n* = 175, antimicrobials: *n* = 170). For baseline, demographic and clinical characteristics see Tables 1 and 2, respectively. Based on the clinical and demographic characteristics, the patients were rather a sample of chronic periodontitis.

Before the thresholds were determined, Spearman correlations (r) between the continuous measurements and the proportion of sites exhibiting new attachment loss after 27.5 months were calculated. The aim was to examine whether a multivariable prediction model using continuous predictors was reasonable. In the entire population as well as in the placebo and antimicrobial group, the correlations between new attachment loss and the variables percentage of PPD ≥ 5 mm, age at baseline, mean attachment level and proportion of initial BOP were low (−0.36 < *r* < 0.25). The correlation between percentage of PPD ≥ 5 mm and initial mean attachment level was high (*r* > 0.77), based on the calculation of attachment level (PPD plus recession). Furthermore, the correlation between the number of sites per patient of PPD ≥ 5 mm and the percentage of PPD ≥ 5 mm was *r* = 0.96.

TABLE 1 Patient demographics at baseline by treatment groups[a]

	Placebo group	Antimicrobial group
n	175	170
Age—years.	52.3 ± 10.8	53.5 ± 10.1
Female sex—no. (%)	87 (50)	85 (50)
Active smokers—no. (%)	44 (25)	49 (29)
Former smokers—no. (%)	64 (44)	63 (44)
non-smoker—CO (ppm)[b]	0.7 ± 1.1	0.7 ± 1.1
smoker—CO (ppm)[b]	13.7 ± 8.7	13.5 ± 10.7

Notes. [a]Categorical variables are shown as absolute and relative frequencies. Continuous variables are shown as mean ± standard deviation. No statistically noticeable differences were noted between the groups at baseline (Fisher's exact tests, Mann–Whitney U tests). [b]CO (ppm) : carbon monoxide (part per million).

3.2 | Clinical threshold values

Through the use of descriptive analysis, clinical threshold values were identified for the percentage of PPD ≥ 5 mm, age at baseline and mean baseline attachment level (Figure 1a–c). For initial BOP, no clinical threshold could be identified (Figure 1d).

3.2.1 | Initial percentage of sites showing PPD ≥ 5 mm

A clinical threshold value was identified for the initial percentage of %PPD ≥ 5 mm. If the percentage was ≥ 35%, then 4.5% of sites exhibited further attachment loss in the antimicrobial group (Q25/Q75: 2.3%/6.1%; *n* = 28). In the placebo group, the rate of attachment loss was 11.6%, which is noticeably higher (*p* < 0.001; Q25/Q75: 5.8%/16.7%; *n* = 30). In this subgroup, the rate of new attachment loss in the antimicrobial group was halved compared to the placebo group (Figure 2a). In contrast, if the initial percentage of PPD ≥ 5 mm was < 35%, the antimicrobial group patients (*n* = 142) exhibited a median of 5.7% (Q25/Q75: 3.3%/10.1%) of sites with further attachment loss after 27.5 months, whereas in the placebo group (*n* = 145), this was the case in 6.8% (Q25/Q75: 4.3%/13%) of sites. The percentage of attachment loss was noticeably different between the placebo and antimicrobial groups (*p* = 0.022, Figure 2a).

3.2.2 | Initial number of sites showing PPD ≥ 5 mm

If a patient had more than or equal to 48 sites with PPD ≥ 5 mm, the median rate of attachment loss (PSAL ≥ 1.3 mm) over the observation period was 10.4% in the placebo group and 4.2% in the antimicrobial group (*p* = 0.001; Q25/Q75: 4.5%/16.7%; *n* = 31 and Q25/Q75: 4.5%/16.7%; *n* = 27). If the number of deep sites was less than 48, the difference in PSAL ≥ 1.3 mm decreased between the placebo group (7.1%; Q25/Q75: 4.6%/13.3%, *n* = 144) and the antimicrobial group (5.4%; Q25/Q75: 3.3%/10.1%, *n* = 143) (*p* = 0.008, Table 3, Supporting Information Figure S1).

3.2.3 | Age at baseline

Patients were classified based on their age at baseline into the following two subgroups: age < 55 years (placebo: *n* = 103, antimicrobials: *n* = 94) and ≥ 55 years (placebo: *n* = 71, antimicrobials: *n* = 76). If the patients' age at baseline was < 55 years, PSAL ≥ 1.3 mm after 27.5 months was noticeably lower in the antimicrobial group (5.2%; Q25/Q75: 2.8%/10%) compared to the placebo group (9%; Q25/Q75: 4.6%/15.3%) (*p* < 0.001, Figure 2b). On the other hand, in patients aged ≥ 55 years, no statistically noticeable difference in further attachment loss were detected between the patients in either

TABLE 2 Patient periodontal characteristics at baseline and 27.5 months follow-up[a]

	Placebo group (*n* = 175)			Antimicrobial group *n* = (170)		
	Baseline	27.5 months	Difference 27.5 months - Baseline	Baseline	27.5 months	Difference 27.5 months - Baseline
Total no. of teeth	25.0 ± 4.3 26 (23,28)	24.1 ± 4.6 25 (22, 27)	−0.9 ± 1.7 0 (−1, 0)	24.5 ± 4.0 25 (22, 27)	23.8 ± 4.2 24 (21, 27)	−0.7 ± 1.4 0 (−1, 0)
Mean probing depth—mm	3.5 ± 0.7 3.3 (3.0, 4.0)	2.6 ± 0.7 2.5 (2.1, 3.1)	−0.9 ± 0.7 −0.8 (−1.3, −0.4)	3.5 ± 0.7 3.3 (3.0, 3.9)	2.3 ± 0.5** 2.3 (2.0, 2.6)	−1.1 ± 0.7** −1.0 (−1.6, −0.7)
Proportion of probing depths						
% ≤ 3.4—mm	59.9 ± 17.6 63.5 (47.2, 73.2)	79.9 ± 14.8 83.3 (71.1, 90.0)	20.0 ± 14.8 18.1 (9.2, 29.9)	59.6 ± 16.0 62.3 (47.8, 71.8)	85.9 ± 12.6** 89.6 (80.8, 95.5)	26.3 ± 16.4** 25.3 (13.1, 37.5)
% 3.5–6.4—mm	32.2 ± 12.5 30.4 (22.4, 41.7)	17.4 ± 12.1 15.3 (8.6, 24.6)	−14.8 ± 11.5 −14.2 (−20.9, −7.1)	33.0 ± 11.4 32.0 (23.6, 41.2)	13.2 ± 11.7** 9.6 (4.4, 17.6)	−19.8 ± 13.4** −20.7 (−28.9, −10.2)
% ≥ 6.5—mm	7.9 ± 8.2 4.8 (2.2, 11.3)	2.7 ± 4.2 1.2 (0.0, 3.5)	−5.2 ± 7.5 −2.8 (−7.9, −0.7)	7.4 ± 8.4 4.2 (1.6, 10.5)	0.9 ± 1.6** 0.0 (0.0, 1.1)	−6.5 ± 8.2 −3.6 (−8.7, −1.2)
Mean attachment level—mm	4.1 ± 0.9 3.9 (3.5, 4.7)	3.6 ± 1.0 3.5 (2.9, 4.2)	−0.4 ± 0.7 −0.4 (−0.9, 0.0)	4.0 ± 0.9 4.0 (3.4, 4.6)	3.0 ± 0.9** 3.3 (2.7, 4.0)	−0.6 ± 0.6** −0.6 (−1.0, −0.2)
Sites with gingival bleeding—%	34.3 ± 16.8 32.6 (21.4, 47.1)	18.9 ± 14.2 15.9 (7.4, 27.4)	−15.4 ± 18.3 −12.3 (−28.4, −3.0)	36.4 ± 19.6 34.1 (24.4, 48.5)	12.8 ± 12.0** 9.3 (3.9, 17.7)	−23.6 ± 21.4** −22.4 (−36.7, −9.0)
Sites with detectable plaque—%	35.8 ± 23.8 30.0 (15.2, 54.5)	36.7 ± 23.3 33.0 (18.8, 51.0)	0.8 ± 28.2 −3.0 (−17.6, 22.4)	38.2 ± 24.2 35.8 (18.5, 53.1)	38.9 ± 24.7 34.3 (19.8, 58.3)	0.7 ± 27.6 0.7 (−17.8, 17.2)

Notes. [a]Continuous variables are shown as mean ± standard deviation and median (25% quantile, 75% quantile). All variables are means or proportions per patient. No statistically noticeable differences were noted between the groups at baseline. **Statistically noticeable differences between placebo and antimicrobial groups; $p < 0.01$; Mann–Whitney U test.

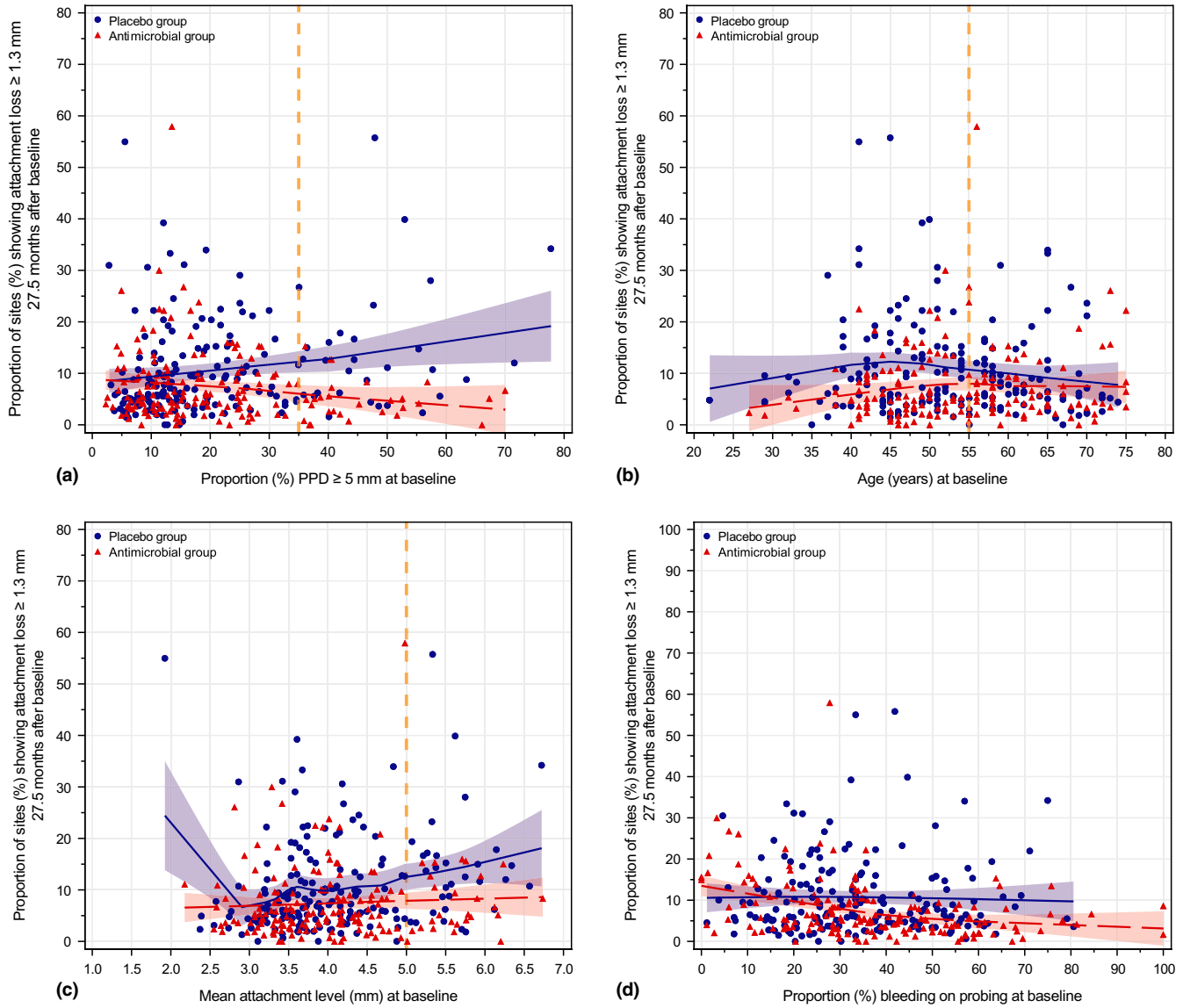

FIGURE 1 Scatterplots of clinical and demographic baseline parameters in relation to the proportion of new attachment loss (PSAL) ≥ 1.3 mm after 27.5 months for the antimicrobial group (▲) and placebo group (●). Lines represent fitted, locally weighted regression (LOESS) curves using linear interpolation and optimal smoothing parameters based on the AICC criterion. A descriptive clinical threshold was identified for the proportion of sites that exhibit pocket probing depths ≥ 5 mm (a), for age (b) and mean attachment level at baseline (c) (dashed orange line, respectively). No threshold could be identified for initial bleeding on probing (d)

the antimicrobial (5.8%; Q25/Q75: 3.5%/9.4%) or placebo group (6.5%; Q25/Q75: 3.9%/11.8%), in regard to sites that exhibited new attachment loss after 27.5 months (p = 0.194, Figure 2b).

3.2.4 | Mean attachment level at baseline

Regarding initial mean clinical attachment level, a threshold value was identified when the mean value was < 5 mm or ≥ 5 mm. If the mean clinical attachment level was ≥ 5 mm, the PSAL ≥ 1.3 mm after 27.5 months in the antimicrobial group (n = 26) was 5.2% (Q25/Q75: 3.3%/12.7%) and moreover was noticeably higher in the placebo group (n = 31), with 12.5% (Q25/Q75: 6.3%/17.2%) (p = 0.005, Figure 2c). At a mean initial attachment level of < 5 mm, the patients in the antimicrobial group (n = 144) exhibited a loss of attachment at 5.3% of sites (Q25/Q75: 3.2%/9.6%), which is comparable to the amount of attachment loss in the placebo group (n = 144) of 6.7% (Q25/Q75: 4.4%/12.6%), respectively (p = 0.005).

3.2.5 | Combination of PPD ≥ 5 mm and age at baseline

If both of the above-identified thresholds for baseline probing depth and age are combined, it becomes even more evident that younger patients exhibit larger numbers of deep pockets, the more the individual can benefit more from adjunctive antimicrobials in terms of less new attachment loss (Figure 3). In other words, if patients

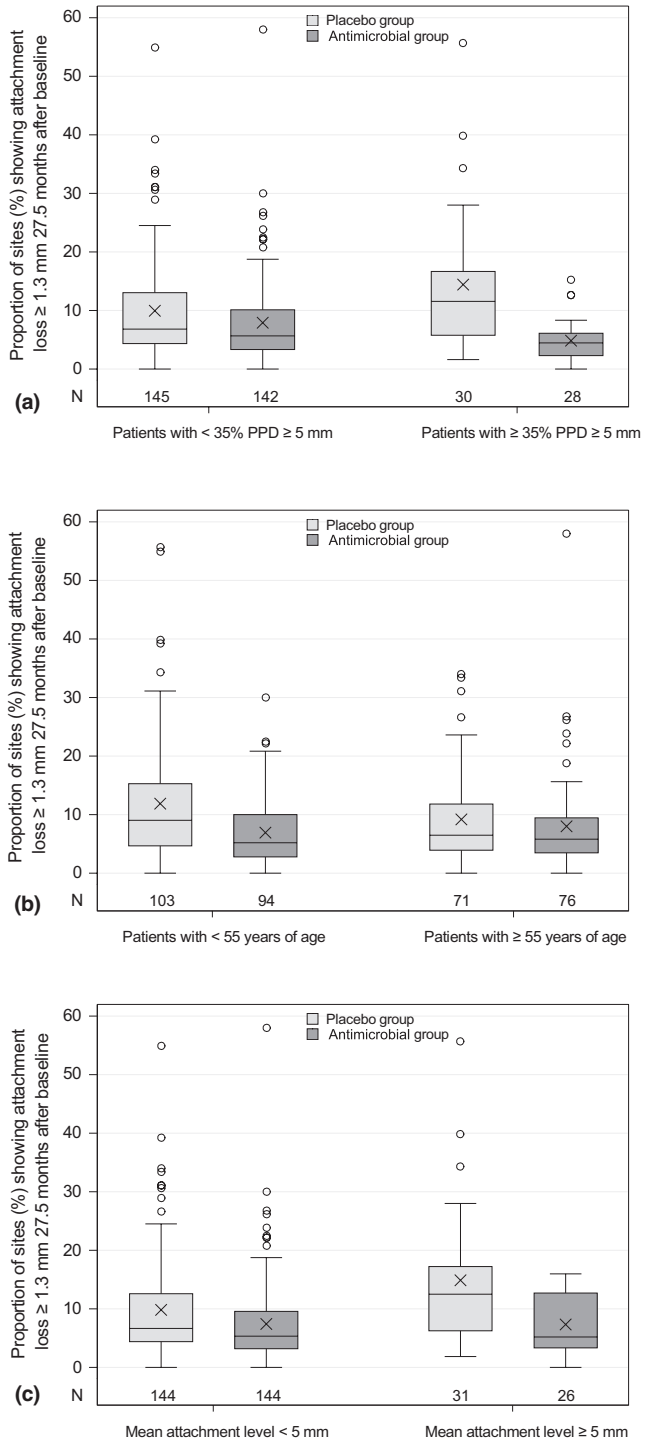

FIGURE 2 Boxplots of the proportion of new attachment loss ≥ 1.3 mm in the antimicrobial group (■) and placebo group (□) in: (a) patients falling below (<35%) or exceeding (≥ 35%) the baseline threshold (%) of pocket probing depth ≥ 5 mm, (b) patients falling below (≤55 years) or exceeding (>55 years) the baseline age threshold and (c) patients falling below (<5 mm) or exceeding (≥ 5 mm) the baseline attachment level threshold. X refers to the mean

are < 55 years of age and exhibit ≥ 35% PPD ≥ 5 mm, those in the placebo group exhibited three times more site-attachment loss compared to patients in the antimicrobial group (12% vs. 4%, respectively; $p = 0.003$). In contrast, if patients are ≥ 55 years of age and exhibit < 35% PPD ≥ 5 mm, those in both the placebo and antimicrobial groups exhibited comparable results concerning new attachment loss (6.2% vs. 6.0%, respectively; $p = 0.730$). For more details see Table 3.

3.2.6 | Multivariable analysis

The conducted multivariable analysis confirms the above findings (Supporting Information Table S3).

4 | DISCUSSION

The present sub-analysis identified benefit thresholds for the following parameters: age, initial percentage and numbers of sites with PPD ≥ 5 mm and mean initial attachment level > 5 mm. At these levels, the application of adjunctive systemic amoxicillin and metronidazole is associated with better clinical outcomes. However, it was not possible to define a threshold value for initial BOP.

For the present analysis, clinical and demographic parameters had already been routinely obtained before or during the periodontal examinations and could be calculated from those (*e.g.* clinical attachment level). Therefore, the suggested threshold-related strategy to determine whether or not to prescribe adjunctive antimicrobials can easily be adapted to one's daily routine without additional effort. However, the threshold values should be seen as a helpful orientation, and by no means as a strict rule regarding the application of antimicrobials, because the mean variation of our data indicates that individual patients above or below or above the threshold may benefit or not. This is also documented in the probabilities presented in Table 3.

The main effect of adjunctive systemic antimicrobials is the improved reduction of deep pocket sites compared to mechanical therapy alone (Mombelli, Almaghlouth, Cionca, Courvoisier, & Giannopoulou, 2015; Silva et al., 2011). Small proportions of deep pockets after therapy may reduce the need for surgery and ease maintenance therapy, because higher numbers of teeth with residual deep pockets would plausibly require a more laborious maintenance therapy. In the present study, the results regarding the percentage of sites with new attachment loss were statistically noticeable different ($p < 0.05$) between the antimicrobial and placebo groups, in both patients exceeding the 35% threshold for PPD ≥ 5 mm and in patients with PPD ≥ 5 mm below the 35% threshold (Figure 2a). However, the absolute difference between antimicrobials and placebo patients at and above the 35% threshold was approximately 7% points, and a difference of this magnitude could be rated as clinically relevant (Harks et al., 2015). In contrast, the absolute difference between the antimicrobial and placebo patients below the 35% threshold was approximately 1%, and such a small difference cannot be considered clinically relevant, despite its statistical noticeability (Figure 2a). Because of the high correlation between the number of

TABLE 3 Proportion of sites showing new attachment loss ≥ 1.3 mm between baseline and 27.5 months for the threshold groups [a]

	Proportion of new attachment loss ≥ 1.3 mm between baseline and 27.5 months			
	Placebo group	**Antimicrobial group**	***p*-value**	**P(A < P)**
Percentage of pocket probing depths ≥ 5 mm at baseline				
<35%	*n* = 145 6.8 (4.3, 13.0)	*n* = 142 5.7 (3.3, 10.1)	0.022	57.6%
≥ 35%	*n* = 30 11.6 (5.8, 16.7)	*n* = 28 4.5 (2.3, 6.1)	<0.001	81.4%
Number of sites per patient with pocket probing depths ≥ 5 mm at baseline				
<48	*n* = 144 7.1 (4.5, 13.3)	*n* = 143 5.4 (3.3, 10.1)	0.008	58.8%
≥ 48	*n* = 3110.4 (4.5, 16.7)	*n* = 27 4.2 (2.1, 7.2)	0.001	76.2%.
Age				
<55 years	*n* = 103 9.0 (4.7, 15.3)	*n* = 94 5.2 (2.8, 10.0)	<0.001	66.1%
≥55 years	*n* = 71 6.5 (3.9, 11.8)	*n* = 76 5.8 (3.5, 9.4)	0.194	56.0%
Mean attachment level at baseline				
<5 mm	*n* = 144 6.7 (4.4, 12.6)	*n* = 144 5.3 (3.2, 9.6)	0.005	59.4%
≥5 mm	*n* = 31 12.5 (6.3, 17.2)	*n* = 26 5.2 (3.3, 12.7)	0.005	72.3%
Percentage of pocket probing depths ≥ 5 mm and age at baseline				
% PPD ≥ 5 mm < 35% & age ≥ 55 years	*n* = 60 6.2 (3.3, 10.1)	*n* = 65 6.0 (3.6, 9.5)	0.730	51.5%
% PPD ≥ 5 mm < 35% & age < 55 years	*n* = 84 8.1 (4.6, 14.7)	*n* = 77 5.3 (3.1, 10.5)	0.007	62.1%
% PPD ≥ 5 mm ≥ 35% & age ≥ 55 years	*n* = 11 10.8 (5.6, 14.7)	*n* = 11 4.9 (1.9, 7.2)	0.037	78.5%
% PPD ≥ 5 mm ≥ 35% & age < 55 years	*n* = 19 12.0 (5.8, 23.2)	*n* = 17 4.0 (2.5, 5.4)	0.003	82.0%

Note. [a]Results are shown as frequencies (n), median (25% quantile, 75% quantile), and rank-based empirical probabilities P (A < P). The proportion of sites exhibiting new attachment loss ≥ 1.3 mm is smaller in the antimicrobial group (A) than in the placebo (P) group. *p*-values used to compare the placebo and antimicrobial groups were obtained from Mann–Whitney U tests.

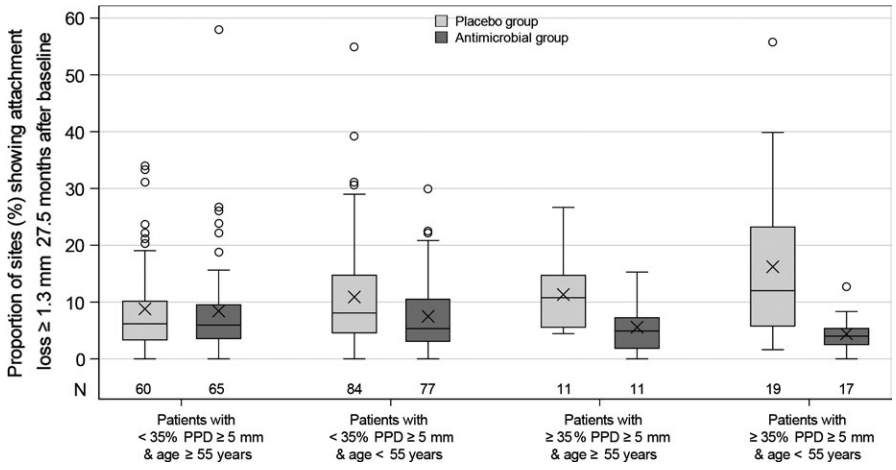

FIGURE 3 Boxplots of the proportion of new attachment loss ≥ 1.3 mm in the antimicrobial group (■) and placebo group (□) in patients falling below or exceeding the combined baseline thresholds identified for pocket probing depth ≥ 5 mm (%) and the age threshold. X refers to the mean

sites and the percentage of pocket probing depths ≥ 5 mm, our main focus was on the proportion variable. The high correlation is mainly due to the fact that all patients have approximately the same number of teeth (95% of the patients initially had 16–31 teeth). The cut-off value of 48 sites almost corresponds to the 35% threshold for the proportion of PPD ≥ 5 mm. Therefore, in our study, the results for both parameters can be transferred approximately to each other.

Considering the patients' age, a risk-related therapeutic approach might be more appropriate, as this could lead to a more individual treatment strategy (Wennström, Papapanou, & Gröndahl, 1990). Similar clinical signs at different ages may express different susceptibilities to the disease, and thus may result in a diverging appraisal of the clinical relevance of therapeutic approaches. The results of the present analysis indicate that younger patients (< 55 years) may benefit more from adjunctive amoxicillin and metronidazole than older patients (≥ 55 years). This finding is evidenced by lower rates of new attachment loss when younger patients were treated adjunctively with the antimicrobials (Figure 2b). For patients younger than 55 years of age, the rate of further attachment loss in the antimicrobial group (5.2%) was lower compared to the placebo group (9%). However, this difference is larger than for the whole patient sample without considering age (antimicrobial group: 5.3%; placebo group: 7.5%; see Harks et al., 2015). Thus, the age threshold may be justified. A similar risk-related strategy was suggested by Lang and Tonetti. They have proposed using a periodontal risk assessment tool for patients in supportive therapy (Lang & Tonetti, 2003). According to this tool, a quotient should be calculated based on the loss of periodontal support in relation to the patients' age to estimate the supportive periodontal therapy intervals. The results of this calculation indicate that considering similar amounts of loss of periodontal supporting tissue, older patients are at less of a risk and should, therefore, receive supportive therapy less frequently than younger patients. The importance of the ratio between periodontal bone loss and age has also been introduced into the new Classification of Periodontal and Peri-implant Diseases and Conditions for grading (Tonetti, Greenwell, & Kornman, 2018). Indeed, the larger the ratio of bone loss by age, the faster the disease progression and the worse the prognosis (Tonetti et al., 2018).

For the mean initial attachment level, a threshold value could only be identified in patients with high initial attachment loss (<5 mm vs. ≥ 5 mm). The magnitude of attachment loss at a distinct moment in a patient's life may be related to the individual's susceptibility to periodontitis or to lifelong lasting exposure to risk factors. The first may fit for younger patients, whereas the second may explain disease severity in older patients. Possibly due to these different and/or mixed reasons behind similar amounts of attachment loss, our patient sample was not sufficient to identify a clinical threshold appropriate to make a decision regarding adjunctive antimicrobials. However, when there is a mean baseline attachment level between 5 mm and 5.5 mm, it can be observed that new attachment loss after 27.5 months increases more in the placebo group than in the antimicrobial group (Figures 1c and 2c). Unfortunately, in clinical practice, attachment loss is not regularly recorded. This was acknowledged by the new classification by introducing not only attachment loss, but also bone loss, as a measure to determine the severity of periodontitis (Tonetti et al., 2018). Prior to therapy, the amount of PPD ≥ 5 mm and mean attachment loss are strongly related. However, PPD is more frequently recorded in clinical practice than is attachment level. Thus, the amount of PPD ≥ 5 mm is a more pragmatic and practical measure to determine adjunctive antimicrobial use.

Bleeding on probing is one extensively evaluated clinical parameter (Joss, Adler, & Lang, 1994; Lang, Schätzle, & Löe, 2009; Lang et al., 1990). Absence of BOP during maintenance is associated with periodontal stability (Joss et al., 1994). However, the level of this parameter at baseline failed to indicate the predictive value of either the antimicrobial group or the placebo group, in regard to the proportion of new attachment loss after 27.5 months. Due to even distribution of initial bleeding and later attachment loss events, no clinical thresholds could be identified (Figure 1d). The identification of patients benefitting from adjunctive systemic antimicrobials is a different issue than the identification of patients prone to further attachment loss during supportive periodontal therapy (SPT) (i.e. after accomplishment of active periodontal treatment). Repeated BOP at certain sites in SPT patients indicates subgingival infection and inflammation, which may induce further attachment loss. Supra- and subgingival cleaning is likely to address this. However, frequent BOP has a low positive predictive value for attachment loss. Whereas, infrequent BOP has a good negative predictive value (Joss et al., 1994). Moreover, BOP alone cannot distinguish between gingivitis and periodontitis. Thus, BOP is not a helpful parameter to determine adjunctive systemic antimicrobial use. Therefore, prescribing adjunctive systemic antimicrobial due to high initial bleeding on probing scores is not recommended.

This study contains several limitations. Due to the interaction setting between the threshold classification variables and the appropriate threshold value determination, which is based on the treatment group effect on PSAL ≥ 1.3 mm within each threshold group, statistical standard methods for cut-off determination like ROC analyses could not be applied. Because the correlations between the continuous threshold variables and the PSAL ≥ 1.3 mm were low (Figure 1), no prognostic multivariable model could be developed to precisely predict new attachment loss. Furthermore, validation and sensitivity analyses of the determined threshold were not possible due to the limited sample size. Therefore, a new prospective independent data sample is needed to confirm the proposed threshold values. The authors were aware that, if conducted on their own, per-protocol analyses could lead to bias. However, in Harks et al. (2015), both analyses were presented, and both analyses were going in the same direction. However, in the present analysis, the authors attempted to identify subgroups that benefit from antimicrobial therapy. These subgroups are based on new thresholds for various clinical variables associated with better clinical outcomes. To evaluate the clinical response of the therapy, all patients must take the medication as prescribed in the protocol. Therefore, analysing the per-protocol collective appears to be suitable, because this sample completed the study without any major protocol violations.

Against the background and danger of increasing microbiological resistance, systemic antibiotics for treating non-life threatening diseases should be prescribed with caution. Increased appearance of bacterial resistance is strongly related to the frequency of antibiotic drug consumption. Therefore, the described thresholds for clinical parameters may help to identify groups of patients which profit more than others, *that is* with less new attachment loss, from adjunctive systemic antimicrobial therapy.

In conclusion, the clinical benefits of systemic amoxicillin/metronidazole may depend on periodontitis severity and patients' age. Clinicians treating patients similar to the population presented in this sub-analysis may consider the reported beneficial thresholds as an additional decision-making aid in regard to the use of systemic amoxicillin/metronidazole. In terms of generalizability, it would be interesting to investigate whether these newly identified beneficial thresholds are also suitable for other populations.

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CONFLICT OF INTEREST

The authors have explicitly stated that no conflicts of interest exist in connection with this article.

ORCID

Peter Eickholz https://orcid.org/0000-0002-1655-8055
Joerg Meyle https://orcid.org/0000-0003-0495-6926
Benjamin Ehmke https://orcid.org/0000-0002-2418-6765

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SUPPORTING INFORMATION

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