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REVIEW

Serum antibody levels against *Porphyromonas gingivalis* in patients with and without rheumatoid arthritis – a systematic review and meta-analysis

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Abstract
Objectives Since the peptidyl arginine deiminase of *Porphyromonas gingivalis* is able to citrullinate peptides and proteins, various studies have suggested the species as a possible link between periodontal disease (PD) and rheumatoid arthritis (RA). This systematic review including meta-analysis was aimed to evaluate whether differences in terms of antibody titers against *P. gingivalis* exist between RA patients and systemically healthy individuals with and without PD.
Materials and methods The following focused question was addressed: Are the antibody titers against *P. gingivalis* of RA patients different from systemically healthy individuals with and without PD? A systematic data search was conducted in MEDLINE and EMBASE. The collected data underwent a meta-analysis to detect statistically significant differences in terms of antibody levels between the groups.
Results From 114 articles found by the search 13 articles met the inclusion criteria and provided data suitable for meta-analysis. After analyzing various levels of confinement the meta-analysis revealed a statistically significant higher antibody titer against *P. gingivalis* in patients suffering from RA in comparison with systemically and periodontally healthy controls ($p < 0.01$) and systemically healthy patients with PD ($p < 0.01$).
Conclusion The present findings indicate that RA is often accompanied by the presence of an immune response against *P. gingivalis*.
Clinical relevance The significantly higher antibody response to *P. gingivalis* in comparison to systemically healthy individuals supports the link between PD and RA by *P. gingivalis*. Screening of the regularly taken blood samples of RA patients for *P. gingivalis* antibodies may help to sensitize rheumatologists and RA patients for improving periodontal health.

Keywords *Porphyromonas gingivalis* · Periodontal disease · Rheumatoid arthritis · Serum antibody levels

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Introduction

The possible association between periodontal disease (PD) and rheumatoid arthritis (RA) has been the focus of several investigations, which have reported an increased prevalence of PD in RA patients and an increased prevalence of RA in PD patients [1–3].

Both RA and PD are chronic diseases characterized by a severe inflammation of the respective tissue. They share several inflammatory pathways [4] and both are influenced by similar risk factors such as diabetes mellitus or smoking [5–7]. RA is an immune mediated multigenic arthritis of unknown origin characterized by the presence of disease-specific autoantibodies against citrullinated proteins [8, 9].

It was shown that periodontal treatment in RA patients does not only improve periodontal health as indicated by clinical evaluation, but also lowers disease activity of RA [10, 11]. Opposite, TNFα-blockers used in treatment of RA positively modify the host response of the periodontium [12, 13]. On the other hand, PD may negatively influence the action of TNFα-blockers in RA [14].

In development and progression of periodontitis, *Porphyromonas gingivalis* causes dysbiosis and inflammation by modulating host response and hence it was postulated to be

a keystone-pathogen [15]. Arginine-specific (RgpA and RgpB) and lysine-specific (Kgp) cysteine proteases, referred to as gingipains, are the main virulence factors of *P. gingivalis*, and are absolutely essential for attachment and colonization, nutrient acquisition and evasion of host defenses and to tissue invasion [16].

Recently it was hypothesized that humoral immune response to oral bacteria may stimulate development of RA [17]. *P. gingivalis* is the only human pathogen producing the unique PAD (PPAD) which is also considered as a virulence factor [18, 19]. Because of the PPAD expression, *P. gingivalis* was proposed as an important link between periodontitis and RA [17]. Bacterial endogenous citrullination is abundant in *P. gingivalis* and absent in other oral bacteria; PPAD citrullinates fibrinogen and α-enolase, which are major autoantigens in RA [20]. It has been shown that in patients with RA antibodies against *P. gingivalis* were associated with antibodies against citrullinated proteins/peptides while the antibody levels were higher than in healthy controls [21, 22]. The same studies reported that *P. gingivalis* titers were related to serum levels of C reactive protein (CRP) in RA patients thus emphasizing the possible role of *P. gingivalis* in the pathogenesis of RA.

However, at present no systematic reviews including meta-analysis have analyzed potential differences in terms of antibody titers against *P. gingivalis* between RA patients and systemically healthy individuals with and without PD.

Therefore, the aim of this systematic review and meta-analysis was to evaluate whether differences in terms of antibody titers against *P. gingivalis* exist between RA patients and systemically healthy individuals with and without PD.

Materials and methods

The focused question was as follows: Are the antibody titers against *P. gingivalis* of RA patients different from systemically healthy individuals with and without PD?

Sources and search

Two reviewers (P.B. and S.E.) conducted a search in online databases for studies to be included in this review published until December 2015:

- The National Library of Medicine (MEDLINE by PubMed) on December 23rd, 2015 using the strategy: ((antibody OR antibodies) OR (IgG OR immunoglobulin G) OR (IgA OR immunoglobulin A) OR (IgM OR immunoglobulin M)) AND rheumatoid arthritis AND *Porphyromonas gingivalis.*
- Embase on December 23rd, 2015, using the strategy: ((antibody OR antibodies) OR (IgG OR immunoglobulin G) OR (IgA OR immunoglobulin A) OR (IgM OR immunoglobulin M)) AND rheumatoid arthritis AND *Porphyromonas gingivalis*.

There was no language restriction and the reviewers reached a κ-value of 0.99.

Inclusion criteria

In order to be included in this review studies had to meet the following criteria:

- reported antibody titers against *P. gingivalis* in patients with RA
- and reported antibody titers against *P. gingivalis* in periodontally healthy controls
- and / or reported antibody titers against *P. gingivalis* in patients with PD
- titers were measured by using ELISA technique
- each group should consist of at least 10 participants to exclude case reports or pilot studies
- RA definition should meet the 2010 Rheumatoid Arthritis Classification Criteria of the American College of Rheumatology/European League Against Rheumatism Collaborative Initiative [23] or the 1987 criteria of the American Rheumatism Association [24] or an equivalent standardized categorization scheme.

Data abstraction

The titles and abstracts (when available) of all reports identified through the electronic and manual search were screened by two reviewers (P.B. and S.E.). When studies met the inclusion criteria or when the abstract did not show sufficient data for evaluating the inclusion criteria the full article was consulted. The complete texts of all studies of possible relevance were then assessed. All studies meeting the inclusion criteria underwent quality assessment and data recording.

Data analysis

After data extraction it became obvious that there was no established standardized unit to measure the antibody titers against *P. gingivalis* by using ELISA, since every study used an in-house ELISA-technique. Calculation of ELISA results is based on generation of standard curves by using two logarithmic axes but the reference point and the units are most set arbitrarily. Therefore, the values of the studies could not be compared directly to each other. In order to be able to perform a meta-analysis, the published titer values were individually normalized to the mean values of RA, which was considered

Table 1 Articles included in statistical analysis; the column "C″ identifies the confinement level, "1" corresponding to Fig.2 and "2" corresponding Fig.3; RA = rheumatoid arthritis, PD = periodontal disease or chronic periodontitis, AP = aggressive periodontitis, C = control/healthy. * = study excluded due to exaggerated and asymmetric SD; ** = calculated SD

Study	study type	n(RA)	n (PD)	n (AP)	n (C)	Def. RA	Def. PD	Def. AP	Unit for anti-body	Anti-body
Yusof et al. 1995	cross sectional	25	15	25	10	>31y in th.	>35y 1/3 BL	<35y 1/3 BL @ >12 teeth	ELISA unit	IgG
Moen et al. 2003	cross sectional	116	-	-	100	ACR	-	-	ELISA unit	IgG
Ogrendik et al. 2005	cross sectional	30	-	-	20	ACR	-	-	ELISA unit	IgG
Mikuls et al. 2009 *	cross sectional	78	39	-	40	ACR	Pat. in SPT + 2 Pockets >5 mm	-	ELISA unit	IgG
Okada et al. 2011	cross sectional	80	-	-	38	1987 ARA	-	-	ELISA unit	IgG
Arvikar et. Al. 2013	intervention	93	-	-	72	1987 ARA	-	-	ELISA unit	IgG
Laugisch et al. 2015	cross sectional	52	28	-	16	ACR	Amano / PSI	-	ELISA unit	IgG
Quirke et al. 2014	cross sectional	80	44	-	82	1987 ARA	2 Pockets >5 mm	-	ELISA unit	IgG
Konig et al. 2014	cross-sectional	83	-	-	83	1987 ARA	-	-	ELISA unit	IgG
Kharlamova et al. 2015	cross-sectional	1974	-	-	377	1987 ARA	-	-	ELISA unit	IgG
Lee et al. 2015	cross-sectional	248	-	-	85	1987 ARA	-	-	ELISA unit	IgG
Seror et al. 2015	cross-sectional	694	61	-	80	1987 ARA	-	-	ELISA unit	IgG
Shimada et al. 2015	cross-sectional	52	-	-	26	1987 ARA	-	-	ELISA unit	IgG
Scher et al. 2012	cross-sectional	34	-	-	18	1987 ARA	-	-	ELISA unit	IgG

Study	Serum Ig level												p (PD vs. healthy)	p (PD vs. RA)	p (RA vs. Healthy)	C
	Ig(Pg) healthy		Standardized values		Ig (Pg) RA		Standardized values		Ig (Pg) PD		standardized values					
	titers	SE/SD/ range	mean	SD	Titers	SE/SD/ range	mean	SD	titers	SE/SD/ range	Mean	SD				
Yusof et al. 1995	0.590	0.310	0.97	0.51	0.610	0.340	1	0.56	1.390	0.630	2.28	1.03	0.010	<0.001	n.s.	2
Moen et al. 2003	7.700	9.990	0.78	1.01	9.85	12.080	1	1.23	-	-	-	-	-	-	-	2
Ogrendik et al. 2005	0.129	3.100	0.47	0.47	6.54	4.430	1	0.68	-	-	-	-	-	-	-	2
Mikuls et al. 2009 *	200.000	0–204.800	0.25	64.00 **	800.000	0–204.800	1	64.00 **	12,800	0–204.800	16.00	64.00**	-	-	-	1
Okada et al. 2011	-	-	-	-	13.790	0–92.700	1	2.24 **	7.470	0–22.58	0.72	0.55 **	-	-	-	1
Arvikar et. Al. 2013	0.250	0.339	0.54	0.74	0.460	0.588	1	1.28	-	-	-	-	-	-	<0,01	2
Laugisch et al. 2015	0.297	0.200	0.41	0.28	0.722	0.639	1	0.88	0.966	0.642	1.34	0.89**	<0.01	n.s.	n.s.	2
Quirke et al. 2014	87.000	0–1291.74	0.90	3.33 **	97.000	0–1283.86	1	3.31 **	522.000	0–1274.52	5.38	3.29**	<0.01	<0.001	n.s.	1
Konig et al. 2014	0.170	0–0.58	2.74	2.46 **	0.061	0–0.82	1	3.36 **	-	-	-	-	-	-	n.s.	1

Table 1 (continued)

Study	Serum Ig level														C
	Ig(Pg) healthy		Standardized values		Ig (Pg) RA		Standardized values		Ig (Pg) PD		standardized values		p (PD vs. healthy)	p (PD vs. RA)	p (RA vs. Healthy)	
	titers	SE/SD/ range	mean	SD	Titers	SE/SD/ range	mean	SD	titers	SE/SD/ range	Mean	SD				
Kharlamova et al. 2015	100.000	Q 42/21	0.62	0.54 ***	226.000	Q 429/107	1	0.71 ***	-	-	-	-	-	-	<0.001	1
Lee et al. 2015	18.479	1.428	0.54	0.38	34.427	55.2755	1	1.61	-	-	-	-	-	-	<0.003	2
Seror et al. 2015	1.490	0.330	1.01	0.22	1.470	0.420	1	0.29	1.550	0.410	1.05	0.28	-	-	0.660	2
Shimada et al. 2015	0.930	0.070	0.66	0.25	1.410	0.865	1	0.61	-	-	-	-	-	-	<0.03	2
Scher et al. 2012	0.362	0.061	0.01	0.00	0.265	0.048	1	0.91	-	-	-	-	-	-	n.s.	2

as reference. The data (mean and standard deviation) of the other groups were then related as ratio to the RA value.

The first step of the statistical calculation consisted of screening and completing all data for further treatment (Table 1). From the normalized data a confidence interval was calculated and the data of the studies was pooled using a weighted average and weighted standard deviation. This lead to the possibility to deduce a t-test on the pooled data and to generate Forest plots. The data preparation was performed in EXCEL® and for the statistical calculations SAS® 9.4 was chosen [25].

Results

Search

The search process is illustrated in Fig. 1. The electronic database search in MEDLINE and EMBASE provided 65 and 49 articles, respectively, published until December 2015. After screening the 114 titles, 73 articles were excluded either because they were listed twice or the title already revealed a complete other focus of the study. Screening the abstracts of the remaining 41 articles resulted in 28 articles that underwent full text screening. Reading of the 28 full texts reduced the number of finally included studies further to 14 (Table 1) [21, 26–38]. The exclusion criteria of the 14 articles excluded after full text review are listed in Table 2 [22, 39–51].

Study characteristics

All studies except those of Arvikar et al. 2013 [30] were cross sectional studies and included between 48 and 2051 patients. All publications presented data for patients with RA and only one study failed to present data for the systemically and periodontally healthy controls [29].

In addition, six studies included separate data for patients with PD, one of them [26] differentiated between aggressive and chronic periodontitis. All studies reported the IgG response while two articles supplemented IgA response. Only one paper assessed also the IgM response. Five studies reported in their data sets only range or quartile values and no standard deviation (Table 1).

Statistical analysis

Since only IgG was the antibody being present in all the articles, this was the only antibody that could be analyzed. As mentioned before, the included articles consisted of data sets of different quality. Therefore, it was decided to analyze the data in several levels based on their inclusion confinement. In a first step, all articles including those where the SD had to be estimated by using the range and/or quartiles were analyzed

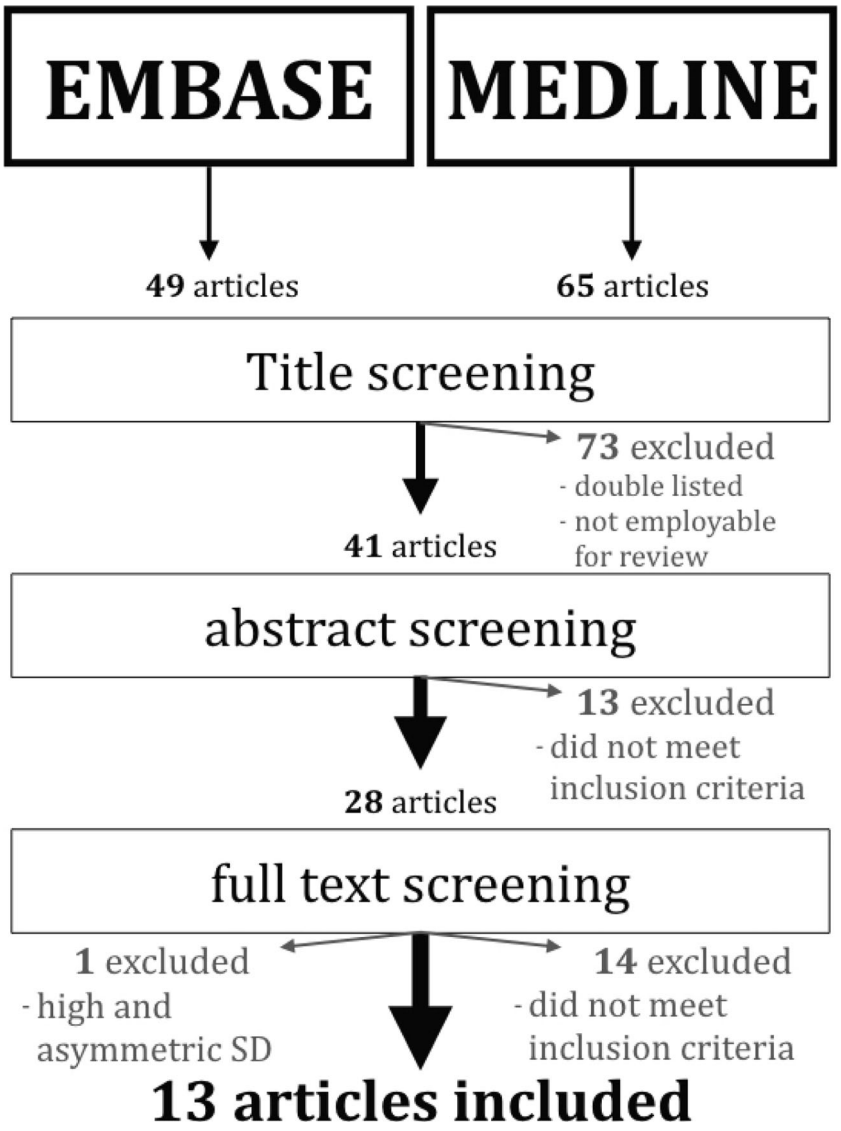

Fig. 1 Search process

(Fig. 2). In a second step, only the articles where the SD could be extracted accurately from the reported data were proceeded to meta-analysis (Fig. 3).

As one article reported an exaggerating high and asymmetric SD [21], it was decided to exclude this study mainly for the asymmetrical data range. However, it should be mentioned the inclusion or exclusion of this study did not change the statistical significance of the analysis. Finally, 13 studies remained in the two steps of analysis.

At both levels of confinement the statistical analysis revealed a significantly higher antibody titer in the RA group compared to the systemically healthy group. The first step analysis resulted in a t-value of −6.63 ($p < 0.001$). In the second step of analysis when only data with reported SD was analyzed the t-value reached −4.92 ($p < 0.001$). The comparison between the RA patients with systemically healthy PD patients revealed statistically significant lower antibody titers against *P. gingivalis* in RA at both levels of confinement ($t = -13.79/p < 0.001$ and $t = -3.31/p < 0.01$ respectively).

Discussion

The pathogenicity of *P. gingivalis* in PD has been thoroughly investigated during the past decades and it was shown that this species has a variety of virulence factors that support its invasion and facilitate destruction of the tooth's supporting tissues as well as the modulation of the host response [52–54]. Successful periodontal treatment has been demonstrated to result in a significant reduction of sites colonized by *P. gingivalis* [55].

Infection with *P. gingivalis* implies a host response leading to generation of specific antibodies evidenced by the correlation between bacterial presence and the corresponding IgA

Table 2 Excluded papers after fulltext screening with exclusion criteria

#	Author	Year	Exclusion criterion
1	**Hitchon et al.**	2010	RA relatives served as control
2	**Mikuls et al.**	2012	Only pre-RA patients or first degree relatives included
3	**Jeong et al.**	2012	No SD, range or SE reported – not suitable for meta analysis
4	**de Smit et al.**	2012	Only patients with RA included; no healthy or PD reference group
5	**Lappin et al.**	2013	No RA group in study
6	**Okada et al.**	2013	Interventional study on only RA patients, no control/reference group
7	**Kobayashi et al.**	2014	Only patients with both PD and RA were included
8	**Mikuls et al.**	2014	Only RA and osteoarthritis patient included, no reference patients
9	**de Smit et al.**	2014	Only pre-RA patients included, no PD or healthy reference patients
10	**Kimura et al.**	2015	Only RA patients included, no PD or healthy reference patients
11	**Janssen et al.**	2015	No RA Patients included; only PD, bronchiectasis and cystic fibrosis patients
12	**Bello-Gualtero et al.**	2015	No specific sera titers reported, only percentage of IgG-positive sera per group are reported
13	**Reichert et al.**	2015	No RA patients included
14	**Fisher et al.**	2015	Only pre-RA patients included

and IgG levels [56]. *P. gingivalis* positive patients with chronic periodontitis clearly showed an enhanced antibody response compared to patients where the species could not be detected [57]. Thus, assessing IgG titers against that *P. gingivalis* appears to be helpful in diagnosing periodontal disease, since the increased levels correlated well with the severity of the disease [58]. One study evaluating the antibody levels against 19 different bacterial species has shown that those against *P. gingivalis* were most strongly associated with periodontitis [59]. In another study, the changes in clinical variables were strongly correlated with IgG antibody serum levels against *P. gingivalis* [60].

In the present review, literature databases evaluating the antibody levels against *P. gingivalis* related to RA were searched. The most common antibody class used for host response detection and in all studies included in this review is IgG as the predominant class present in serum [61]. However, the increase of IgG serum levels can be directed to several different antigens of *P. gingivalis* [62]. All studies used in-house ELISA techniques, as no standardized commercial ELISA kit focusing on *P. gingivalis* is available. Since reporting of the results differed too, only standardization/normalization to RA as the reference allowed comparison between the studies. In most studies, whole bacterial cells from

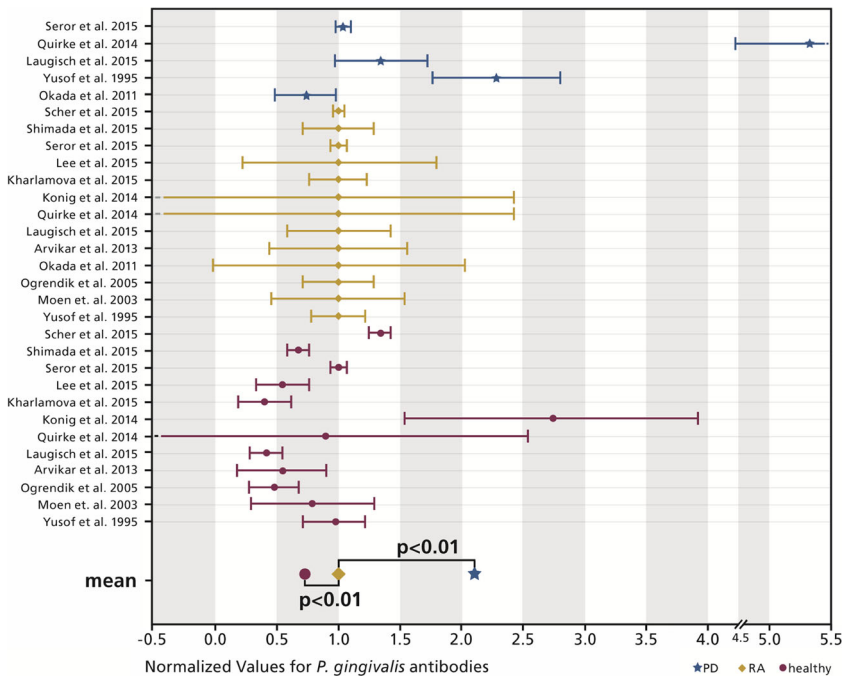

Fig. 2 Forest plot of normalized values for *Porphyromonas gingivalis* antibodies in study groups (patients with rheumatoid arthritis (RA) as well as in systemically healthy individuals without (healthy) and with periodontal disease (PD)) of the articles including data with approximately calculated SD in patients

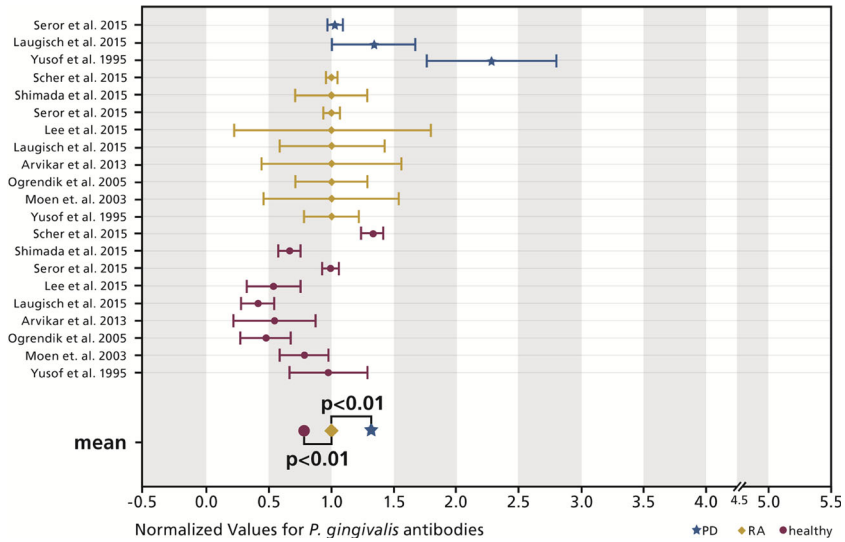

Fig. 3 Forest plot of normalized values for *Porphyromonas gingivalis* antibodies in study groups (patients with rheumatoid arthritis (RA) as well as in systemically healthy individuals without (healthy) and with periodontal disease (PD)) articles including only data that was extractable directly from the article

laboratory strains were used and prepared as antigens [21, 26–30], only one study used clinical isolates [41]. In some other studies bacterial constituents like PPAD [32, 63], RgpB [31, 32] or cell wall components [45] were used and prepared. The RgpB antigen used in two studies was identical [31, 32], but interestingly, the study by Quirke et al. [32] differentiated more between RA and systemically healthy subjects despite that there was no difference in the presence of *P. gingivalis* in subgingival biofilm [31]. Only two studies in this review included the detection of both IgA [27] and IgM [41]. In these studies, the results were either similar or less differentiating when comparing RA patients with systemically healthy individuals.

In other systemic diseases the findings were similar or opposite. In coronary vascular diseases, IgG levels against *P. gingivalis* were also associated with hypertension and atherosclerosis [64]. On the contrary, in the Third National Health and Nutrition Examination Survey, higher concentrations of IgG antibodies to *P. gingivalis* were significantly associated with lower prevalence of asthma [65]. The authors explained that finding with a potential protective role of microbes [65]. However, it should be also kept in mind that in patients with allergic diseases, the host response to *P. gingivalis* infection seems to be lower. Another example for an obviously diminished host response is related to the finding that women with high counts and low serum levels of IgG against *P. gingivalis* appear to have an increased risk for preterm delivery [66].

RA patients show a high responsiveness to *P. gingivalis*. Infrequently data about the PD disease status in RA patients were reported but prevalence was closed to 100 % if reported. One study [28] excluded RA patients with gingivitis or periodontitis; however no information is given, how periodontal health was diagnosed. In two papers [31, 63] RA patients with periodontitis were compared with systemically and periodontally healthy individuals. Also, periodontal disease status in systemically healthy population is in part unclear. In the study by Quirke et al. [32], RA patients were compared with clearly defined PD patients, but the "healthy" people were not screened for periodontal disease. In the present meta-analysis, only four of the ten included studies compared data of RA patients with those of periodontitis patients without RA. In RA patients, IgG levels were higher in one study [41], while they were lower in two others [31, 45]. In our recent study [31] the lower RgpB antibody levels in RA than in PD patients might be explained by recruitment of severe periodontitis patients at a specialized center for periodontal therapy. Another study confirmed that result by using RgpB as antigen [32]. Results were differently reported for using PPAD as an antigen. Higher Anti-PPAD [32] as well as lower AB titers against PPAD [63] in RA in comparison with systemically and periodontally healthy individuals were seen. Only rarely clearly defined RA patients without PD were analyzed in these studies. In the study by de Smit et al. [41] AB level corresponded with periodontitis disease severity. In our recent study two of the four RA patients without PD had high IgG levels against RgpB, however it should be mentioned that these Anti-RgpB levels were associated with presence of *P. gingivalis* in subgingival biofilm [31].

Except for *P. gingivalis*, antibody levels against other bacterial species being associated with periodontal disease have been occasionally measured. Antibody levels in serum against *Aggregatibacter actinomycetemcomitans* were lower [29] or equal [28], against *Prevotella intermedia* most equal [27, 29, 45] and once higher [28] and against *Tannerella forsythia* lower [27] in one report and higher [28] in the other report in RA patients in comparison with systemically healthy individuals.

RA patients are in continuous therapy control where blood samples are regularly taken. Screening for antibodies against *P. gingivalis* may give a hint to the rheumatologist for an

existing periodontal disease and for referring the patient to a periodontist. As mentioned above, there is a multitude of studies reporting, at least in part, promising results on the influence of periodontal therapy in patients with PD suffering from RA [11, 67]. In those studies improvement of periodontal health was correlated with decreased disease activity of RA as well as with decreased IgG levels to *P. gingivalis* [42, 43]. The reported disabilities in handling and perception of patients with RA [68] may also explain the poor oral hygiene found in this population [69]. All these findings support the need for providing a thorough periodontal treatment combined with frequently performed oral hygiene recall visits which may improve the quality of life together with the decrease in inflammatory load in RA patients.

In summary, the present data indicate that patients with RA show a significantly higher antibody response to *P. gingivalis* in comparison to systemically healthy individuals. These findings may support the link between PD and RA by *P. gingivalis*. Screening of the regularly taken blood samples of RA patients by a standardized ELISA kit for *P. gingivalis* antibodies may help to sensitize rheumatologists and RA patients for improving periodontal health.

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Compliance with ethical standards

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Conflict of interest Author Philip Bender declares that he has no conflict of interest. Author Walter B. Bürgin declares that he has no conflict of interest. Author Anton Sculean declares that he has no conflict of interest. Author Sigrun Eick declares that she has no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent For this type of study, formal consent is not required.

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