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Differences in the Oral Microbiome in Patients With Early Rheumatoid Arthritis and Individuals at Risk of Rheumatoid Arthritis Compared to Healthy Individuals

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Objective. It has been suggested that rheumatoid arthritis (RA) may originate at the oral mucosa. The aim of the present study was to assess the oral microbiome and periodontal condition in patients with early RA and individuals at risk of developing RA compared to healthy controls.

Methods. Three groups were recruited (n = 50 participants per group): 1) patients with early RA (meeting the American College of Rheumatology/European Alliance of Associations for Rheumatology 2010 classification criteria), 2) individuals at risk of developing RA (those with arthralgia who were positive for RA-associated autoantibodies), and 3) healthy controls. A periodontal examination was conducted to assess the presence of bleeding on probing (BOP), pocket probing depth (PPD), and periodontal inflamed surface area (PISA). The microbial composition of subgingival dental plaque, saliva, and tongue coating was assessed using 16S ribosomal DNA amplicon sequencing, and findings were compared between groups with permutational multivariate analysis of variance (PERMANOVA).

Results. There were no significant differences in any of the 3 periodontal variables between patients with early RA, at-risk individuals, and healthy controls (P = 0.70 for BOP, P = 0.30 for PPD, and P = 0.57 for PISA, by Kruskal-Wallis test). PERMANOVA analyses comparing microbial composition between the groups showed significant differences in the microbial composition of saliva (F = 2.08, P = 0.0002) and tongue coating (F = 2.04, P = 0.008), but not subgingival dental plaque (F = 0.948, P = 0.51). However, in post hoc tests, no significant differences in microbial composition of the saliva or tongue coating were observed between the early RA group and the at-risk group (F = 1.12, P = 0.28 for saliva; F = 0.834, P = 0.59 for tongue coating). In assessing microbial diversity based on the number of zero-radius operational taxonomic units per sample, *Prevotella* in the saliva and *Veillonella* in the saliva and tongue coating were each found at a higher relative abundance in samples from patients with early RA and at-risk individuals compared to healthy controls.

Conclusion. The results show similarities in the oral microbiome between patients with early RA and at-risk individuals, since in both groups, the oral microbiome was characterized by an increased relative abundance of potentially proinflammatory species when compared to that in healthy controls. These findings suggest a possible association between the oral microbiome and the onset of RA.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease that is frequently accompanied by autoantibodies such as rheumatoid factor (RF) and antibodies against citrullinated proteins (ACPAs) (1). These antibodies are often present several years before the onset of clinically apparent RA (2).

It has been suggested that RA originates at mucosal sites, such as the gut and oral mucosa (3,4). Periodontitis—a chronic inflammation of the gingiva, the tooth-supporting connective

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tissues, and the alveolar bone—displays pathogenic similarities to RA, and several studies have shown an association between periodontal disease and RA (5). In previous studies on the oral microbiome in relation to RA, there was a particular interest in *Porphyromonas gingivalis*, a bacterium associated with periodontal disease (6). Because of its capacity to generate citrullinated proteins, *P gingivalis* could potentially trigger production of ACPAs and thereby initiate an RA-associated immune response (1).

However, findings from previous studies, both in RA patients and in individuals at risk of developing RA, have suggested that further investigations are needed and should focus on the microbiome as a whole, rather than on specific species (7–9). Since the oral microbiome may play a role in the onset of RA, and could thus be a potential target in the prediction or even prevention of RA, information on the oral microbiome in patients with early RA and individuals at risk of developing RA would be relevant. However, data on these specific groups are currently very limited. Our aim was therefore to assess the oral microbiome and the periodontal condition in patients with early RA and individuals at risk of developing RA in comparison to healthy controls.

PATIENTS AND METHODS

Study design and ethics approval. This study is based on baseline data obtained from a larger parent cohort study; a full description of the protocol has been published elsewhere (10) and is outlined in more detail below. Information on the methods used for sample processing is provided in the Supplementary Methods (available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41780/abstract). The protocol was approved by the accredited Medical Ethical Committees of Slotervaart Hospital and Reade (METc Slotervaartziekenhuis and Reade; approval no. U/17.056/P1719), and details on the protocol are included in the Dutch National Trial Register (trial no. NTR6362; https://www.trialregister.nl/trial/6198).

Participants and recruitment. For this study, 3 groups of participants were recruited: 1) patients with early RA, 2) individuals at risk of developing RA, and 3) a healthy control group of subjects without autoimmune conditions. Groups 1 and 2 were recruited at Reade, a rheumatology clinic in Amsterdam, The Netherlands. Group 1 consisted of patients diagnosed as having RA within the previous year and fulfilling the American College of Rheumatology/European Alliance of Associations for Rheumatology 2010 classification criteria for RA (11). For group 2, participants were recruited from among individuals at risk of developing RA in the Reade cohort (12–14). Participants in this at-risk cohort have inflammatory-type arthralgia combined with increased serum levels of RF and/or ACPAs. Participants in group 3 were healthy subjects recruited at the Academic Centre for Dentistry Amsterdam, without selection for oral status, and were matched to the participants in groups 1 and 2 by sex and age (±5 years). All participants were age ≥18 years, had a minimum of 12 natural teeth, and gave written informed consent. All clinical examinations and sampling took place at Reade and were performed by a single trained dentist (JMK).

Outcome variables. *General health.* All participants completed a medical questionnaire prior to the research visit, to identify possible confounders such as comorbid conditions. During the research visit, additional questions were asked about the subject's use of antibiotics during the preceding 3 months. Venous blood samples were collected to determine the serum levels of RF and ACPAs. Blood samples were processed in the hematology laboratory at OLVG Hospital in Amsterdam, using Phadia antibody detection assays (250 EliA IgM and Phadia 250 EliA cyclic citrullinated peptide antibody tests). In accordance with the manufacturer's recommendations, individuals with RF levels >5.0 kU/liter and/or ACPA levels >10.0 kU/liter were considered seropositive; otherwise, participants were considered seronegative.

Oral health. Participants were asked about the amount of time lapsed since brushing their teeth, and about their regular practice of additional oral hygiene measures, e.g., mouth rinse and tongue cleaning. An intraoral examination was performed to determine the total number of teeth present, the number of decayed, missing, and filled teeth, and the presence or absence of a removable (partial) denture.

Collection of samples for microbiome analyses. Participants were instructed not to perform any oral hygiene measures for 24 hours, and not to eat or drink anything except water for 2 hours prior to their research visit. During the visit, a subgingival dental plaque sample, saliva sample, and tongue coating sample were collected. The microbial composition of the samples was assessed using 16S ribosomal DNA amplicon sequencing. A thorough description of the sample collection and processing is available in the Supplementary Methods (http://onlinelibrary.wiley.com/doi/10.1002/art.41780/abstract).

Periodontal examination. The periodontal examination included identification of bleeding on probing (BOP) (absent versus present) and measurement of pocket probing depth (PPD) (measured in millimeters) on 6 sites for each tooth (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual). BOP values are expressed as a percentage of the full-mouth BOP. When calculating this percentage, data from the tooth from which a subgingival plaque sample was collected were not used. The dentist performing the examination of BOP and PPD (JMK) was trained by a periodontist.

Based on the PPD data obtained, a Community Periodontal Index of Treatment Need (CPITN) score (15) was calculated, and this score was used to categorize participants according to periodontitis status. Participants were categorized as either having suspected severe periodontitis (a CPITN score of 4) or not having suspected severe periodontitis (a CPITN score of 0–3). This means

that a PPD of ≥6 mm in at least one tooth would be needed for a patient to be classified as having suspected severe periodontitis.

To quantify the total burden of periodontal inflammation, the total periodontal inflamed surface area (PISA) was calculated. This measurement of inflammatory burden was carried out using the method described by Nesse et al (16).

Statistical analysis. Descriptive statistics were used to describe the characteristics of the study population. For continuous variables, one-way analysis of variance was performed to compare the mean values for normally distributed variables between the 3 groups; for non–normally distributed variables, a Kruskal-Wallis test was used. Possible differences in categorical variables between groups were tested with a chi-square test. A 2-sided alpha level of 0.05 was used as the threshold for statistical significance.

Microbiome data among the 3 groups were analyzed for both microbial composition and microbial diversity in all 3 periodontal niches: the plaque, saliva, and tongue coating. Details on these analyses are available in the Supplementary Methods (http://onlinelibrary.wiley.com/doi/10.1002/art.41780/abstract).

In addition, since the presence of ACPAs is a more relevant predictor of RA than is the presence of RF (17), we compared 2 subgroups of individuals within the at-risk group according to ACPA status ACPA-positive versus ACPA-negative at-risk individuals. Possible confounding factors and periodontal variables, as well as the microbiome composition in all 3 niches, were compared between these 2 subgroups.

RESULTS

Characteristics of the study population. From November 2017 until July 2019, 150 participants were included, comprising 50 participants per group (Table 1). Patients in the early RA group were included in the study at a mean ± SD 3.1 ± 1.7 months after having received the diagnosis of RA. The majority of patients with early RA had been receiving treatment with methotrexate, mostly in combination with prednisone (Table 1), in accordance with the Dutch Society for Rheumatology national guidelines on drug treatment for RA (18).

The 3 groups were compared with regard to factors that could influence the oral microbiome: smoking status, alcohol consumption, use of drugs, use of painkillers during the preceding 24 hours, use of antibiotics during the preceding 3 months, wearing removable dentures, regular tongue cleaning, regular use of mouth rinse, number of decayed, missing, and filled teeth, time since eating/drinking before the research visit, and time since practice of oral hygiene before the research visit (see Supplementary Table 1, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41780/abstract). No differences in any of these characteristics were observed between the groups, except for the amount of time since practice of oral hygiene, which was carried out significantly more recently by patients in the early RA group compared to subjects in the other 2 groups.

Periodontal health. There was no difference in the percentage of BOP, median PPD, or median PISA between the 3 groups (Table 2). A trend toward a higher prevalence of severe periodontitis was seen in the early RA group and at-risk group compared to the healthy control group, with the prevalence of severe periodontitis incrementally increasing from the healthy control group to the at-risk group, and from the at-risk group to the early RA group. However, no significant between-group differences in the prevalence of severe periodontitis were found (Table 2).

Table 1. Characteristics of the study population by group*

	Early RA (n = 50)	At risk of RA (n = 50)	Healthy controls (n = 50)
Age, mean ± SD years	52.1 ± 13.2	51.4 ± 10.3	51.2 ± 11.0
Sex, female	39 (78)	38 (76)	38 (76)
RF positive	37 (74)	46 (92)	0 (0)
ACPA positive	31 (62)	24 (48)	0 (0)
Both RF and ACPA positive	38 (76)	50 (100)	0 (0)
Pharmacologic treatment for RA			
Methotrexate	44 (88)	–	–
Prednisone	39 (78)	–	–
Other	4 (8)	–	–
No pharmacologic treatment	2 (4)	–	–

* There were no significant differences between the groups in any of the listed characteristics, as determined by one-way analysis of variance or chi-square test. A between-group difference in frequency of rheumatoid factor (RF) and/or anti–citrullinated protein antibody (ACPA) positivity was not tested, because seropositivity was an inclusion criterion for the group of individuals at risk of developing rheumatoid arthritis (RA) and an exclusion criterion for the healthy control group, and therefore the difference was obvious. Additional characteristics are listed in Supplementary Table 1 (available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41780/abstract). Except where indicated otherwise, values are the number (%) of participants.

Table 2. Periodontal assessments in the study groups*

	Early RA (n = 50)	At risk of RA (n = 50)	Healthy controls (n = 50)
BOP, median (IQR) %	19.3 (9.9–35.4)	15.4 (7.4–32.5)	17.5 (8.5–27.5)
PPD (total 6 sites per tooth)			
Plaque sample tooth, median (IQR) mm	2.5 (2.3–3.0)	2.6 (2.3–3.0)	2.5 (2.2–2.8)
All samples, median (IQR) mm	2.2 (2.0–2.6)	2.2 (2.0–2.5)	2.1 (1.9–2.5)
No. of pockets ≥6 mm, median (IQR)	0 (0–0.25)	0 (0–0)	0 (0–0)
PISA, median (IQR) mm^2	258.3 (108.4–398.3)	191.3 (72.5–431.8)	181.4 (91.2–369.4)
CPITN score 4, no. (%)	12 (24)	10 (20)	7 (14)

* There were no significant differences between the groups in any of the 3 periodontal variables assessed (P = 0.70 for bleeding on probing [BOP], P = 0.30 for pocket probing depth [PPD], and P = 0.57 for periodontal inflamed surface area [PISA], by Kruskal-Wallis test) or in the Community Periodontal Index of Treatment Needs (CPITN) PPD score (P = 0.21, by chi-square test with linear association). RA = rheumatoid arthritis; IQR = interquartile range.

ACPA-positive versus ACPA-negative at-risk individuals. A separate analysis was performed to compare at-risk individuals who were ACPA positive (n = 24) to those who were ACPA negative (n = 26) with regard to all of the above-mentioned possible confounding factors and periodontal variables. No significant differences were found between these 2 subgroups of at-risk individuals stratified by ACPA status (data not shown).

Microbiologic signatures. After processing, the periodontal samples obtained from all subjects were found to have a total of 948 zero-radius operational taxonomic units (zOTUs). After subsampling at 3,500 reads per sample, 942 zOTUs remained, with a mean of 130 zOTUs per sample. Eight samples (2 plaque, 4 saliva, and 2 tongue coating samples) were excluded from further analyses because the number of reads was too low. Because patients in the early RA group had performed oral hygiene practices significantly more recently before the research visit as compared to participants in the other 2 groups, and brushing could influence the oral microbiome, this variable was taken into account when analyzing group differences by permutational multivariate analysis of variance (PERMANOVA).

Microbial diversity of the 3 periodontal niches (the plaque, saliva, and tongue coating) in all samples was measured using the Shannon Diversity Index, as well as by determining the number of zOTUs per sample and the Bray-Curtis distance as an index of microbial diversity. The results in each niche are reported below and in Supplementary Table 2 (available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41780/abstract).

Plaque samples. There was no difference among the groups in the microbial composition of the plaque samples, as determined in analyses based on the number of hours since practice of oral hygiene (F = 1.58, P = 0.07 by two-way PERMANOVA) and in analyses based on the groups being compared (F = 0.948, P = 0.51 by two-way PERMANOVA). Results of principal components analysis (PCA) did not reveal any clustering of plaque microbial composition by group (Figure 1A).

Saliva samples. The microbial composition of saliva differed significantly among the groups, irrespective of the number of hours since practice of oral hygiene (F = 2.27, P = 0.004 by two-way PERMANOVA) and irrespective of the groups being compared (F = 2.08, P = 0.0002 by two-way PERMANOVA). PCA showed that the microbial composition of saliva samples from the healthy control group clustered together (Figure 1B). This was confirmed by results of the post hoc, pairwise two-way PERMANOVA analyses based on the number of hours since practice of oral hygiene and those based on the groups being compared, with evidence of a significant difference in the saliva microbial composition between the healthy control group and the early RA group (F = 2.66, P < 0.001) and between the healthy control group and the at-risk group (F = 2.56, P = 0.001), but not between the early RA group and the at-risk group (F = 1.12, P = 0.28).

Tongue coating samples. The microbial composition of the tongue coating samples was also significantly different among the groups, irrespective of the number of hours since practice of oral hygiene (F = 1.97, P = 0.033 by two-way PERMANOVA) and irrespective of the groups being compared (F = 2.04, P = 0.008 by two-way PERMANOVA). Similar to the findings in the saliva, the microbial composition of the tongue coating samples from the healthy control group clustered together (Figure 1C). This was confirmed by post hoc, pairwise two-way PERMANOVA analyses based on the number of hours since practice of oral hygiene and those based on the groups being compared with a significant difference in tongue coating microbial composition observed between the healthy control group and the early RA group (F = 2.75, P = 0.005) and between the healthy control group and the at-risk group (F = 2.59, P = 0.01), but not between the early RA group and the at-risk group (F = 0.834, P = 0.59).

ACPA-positive versus ACPA-negative at-risk individuals. For all 3 niches, a separate one-way PERMANOVA analysis was performed to compare ACPA-positive and ACPA-negative individuals at risk of developing RA. No significant differences in microbial composition in any of the 3 niches were found between these 2 subgroups (data not shown).

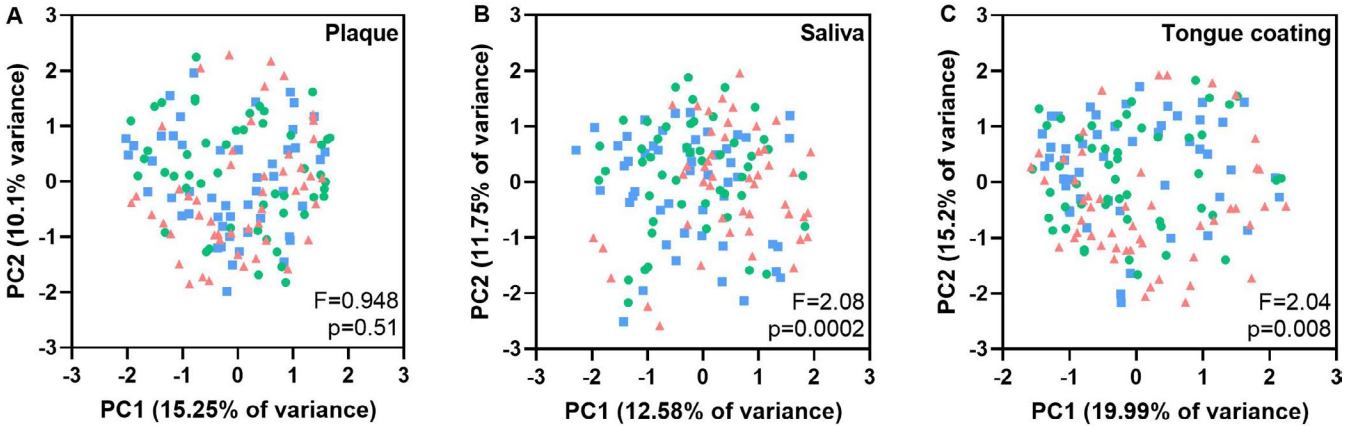

Figure 1. Principal components analysis plots, including the first and second principal components (PC1 and PC2, respectively), displaying microbiologic composition signatures of the subgingival dental plaque (**A**), saliva (**B**), and tongue coating (**C**) in patients with early rheumatoid arthritis (RA) (blue), individuals at risk of developing RA (green), and healthy controls (orange). The F value was calculated using two-way permutational multivariate analysis of variance, based on the number of hours since practice of oral hygiene and based on the groups being compared.

Discriminative zOTUs. *Saliva samples.* Twenty-five zOTUs in the saliva significantly discriminated among the groups, of which 7 had a relative abundance of ≥0.01 in at least 1 group (see Supplementary Table 3, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41780/abstract). Post hoc Mann-Whitney U tests showed that the significant results were predominantly attributable to the difference between the early RA and at-risk groups and the healthy control group, rather than to the difference between the early RA group and the at-risk group (Supplementary Table 3 [http://onlinelibrary.wiley.com/doi/10.1002/art.41780/abstract]). *Prevotella salivae* (zOTU 25), *Veillonella* (zOTU 4), and *Prevotella* (zOTU 10) were more abundant in the early RA group and at-risk group compared to the healthy control group (Figures 2A–C), while *Neisseria flavescens/subflava* (zOTU 7), *Porphyromonas pasteri/sp._oral_taxon_278* (zOTU 15), and *Veillonella parvula* (zOTU 12) were more abundant in the healthy control group compared to the other 2 groups. Only *Fusobacterium periodonticum* (zOTU 13) was more abundant in the at-risk group and the healthy control group compared to the early RA group, while no difference was found between the at-risk group and the healthy control group.

Tongue coating samples. Nineteen zOTUs in the tongue coating samples significantly discriminated among the groups, of which 4 had a relative abundance of ≥0.01 in at least 1 group (see Supplementary Table 4, available on the

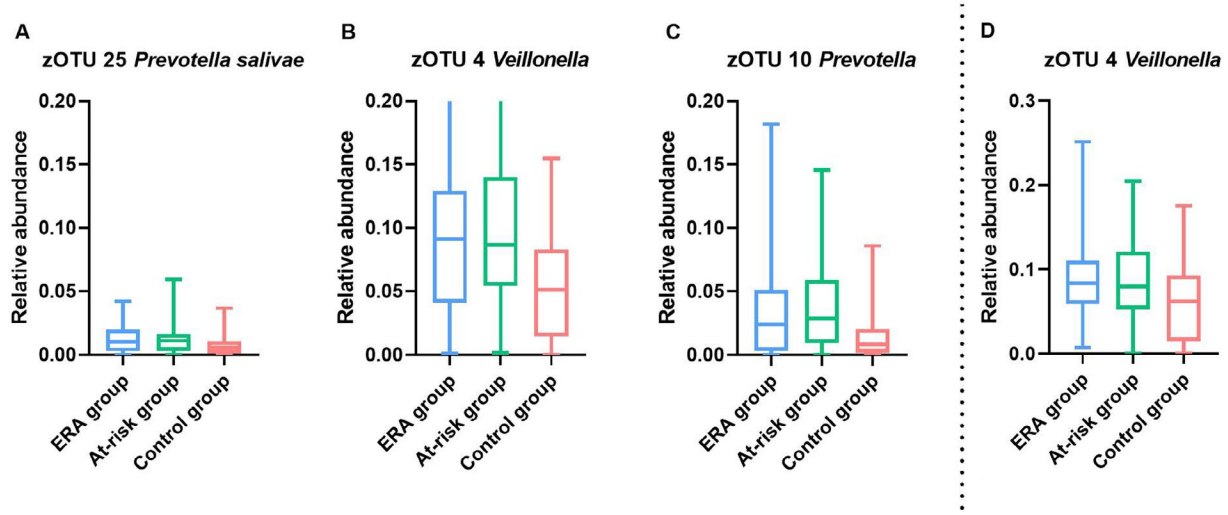

Figure 2. Relative abundance of zero-radius operational taxonomic units (zOTUs) in the saliva (*Prevotella salivae*, *Veillonella*, and *Prevotella* species) (**A–C**) and tongue coating (*Veillonella* species) (**D**) in patients with early rheumatoid arthritis (ERA), individuals at risk of developing RA, and healthy controls. The relative abundance of microbial species was assessed as the number of zOTUs, which helped discriminate among the groups according to linear discriminant analysis incorporating effect size. Results are shown as box plots. Each box represents the 25th to 75th percentiles. Lines inside the boxes represent the median. Lines outside the boxes represent the minimum and maximum.

Arthritis & Rheumatology website at http://onlinelibrary.wiley.com/doi/10.1002/art.41780/abstract). Again, the significant results were predominantly attributable to the difference between the early RA and at-risk groups and the healthy control group, rather than to the difference between the early RA group and the at-risk group (Supplementary Table 4). While *Veillonella* (zOTU 4) was more abundant in the early RA group and the at-risk group (Figure 2D), *Neisseria flavescens/subflava* (zOTU 7) and *Streptococcus dentisani/infantis/mitis/oralis/sp._oral_taxon_058* (zOTU 1) were more abundant in the healthy control group compared to the other 2 groups. *Fusobacterium periodonticum* (zOTU 13) was more abundant in the healthy control group compared to the early RA group, while no differences were found in the at-risk group compared to both other groups.

P gingivalis. The genus *P gingivalis* was not identified as a discriminative zOTU. One of the zOTUs–*P gingivalis* zOTU 116–did classify as *P gingivalis*, but had an overall low abundance in all 3 niches and did not differ among the groups; in all 3 groups, the median relative abundance of *P gingivalis* zOTU 116 in all 3 niches was 0 (in the plaque, $P = 0.37$; in the saliva, $P = 0.47$; in the tongue coating, $P = 0.12$, by Kruskal-Wallis test).

DISCUSSION

The microbial composition of the stimulated saliva and tongue coating, but not that of the subgingival dental plaque, in patients with early RA and individuals at risk of RA significantly differed when compared to an age- and sex-matched healthy control group. *Prevotella* and *Veillonella*—both being gram-negative anaerobes—were found at a higher relative abundance in the saliva, and *Veillonella* was also found at a higher relative abundance in the tongue coating, both in the patients with early RA and in the at-risk individuals compared to healthy controls. However, in saliva, there was also another zOTU that classified as a different species, belonging to the genus *Veillonella*, which was present at a higher relative abundance in the healthy control group compared to the other 2 groups.

The increased relative abundance of the genus *Prevotella* in patients with early RA is consistent with the findings from a study by Scher et al in patients with new-onset RA (7), and with the findings from a study by Correa et al in patients with established RA (19). Furthermore, studies on the gut microbiome have also shown an increased relative abundance of *Prevotella* species in patients with early RA (3) and at-risk individuals (20). Some *Prevotella* strains are capable of promoting chronic inflammation, by stimulating local cytokine production and induction of mucosal inflammation, which in turn can lead to systemic dissemination of inflammatory mediators (21). Furthermore, results of one study suggested that a possible translocation of oral *Prevotella* species or their DNA to joint tissues occurs in patients with RA (22). The increased relative abundance of this potentially proinflammatory genus in patients with early RA and at-risk individuals suggests a link between the oral microbiome and RA (8), and microbiome dysbiosis may contribute to the induction of arthritis. Additionally, dysbiosis in both the oral and gut microbiome is partially resolved after the start of pharmacologic treatment for RA (23), further supporting an association between the microbiome and RA development. However, the currently available literature is insufficient to establish a causal link and to fully elucidate the biologic mechanisms involved (22).

Furthermore, the potential presence of a reversed pathway needs to be considered, whereby active inflammation or the characteristics of inflammation in susceptible subjects may provide an environment in which *Prevotella* species would attain ecologic advantages and emerge at larger numbers. When RA has been treated with specific medications (23), the ecology tends to revert to a situation less favorable for *Prevotella*. Thus, the possible influence of treatments for systemic RA on the oral microbiome is overall a factor to consider when interpreting the findings in patients with early RA (24).

Due to the early stage of the disease, most patients with early RA in the present study had been receiving the same treatment, and thus heterogeneity within this group was not an issue. However, it does mean that there was a notable difference in comparison to the at-risk group and the control group, potentially leading to a bias in the results. By including the patients with early RA during the first few months after the start of the treatment, we attempted to limit this bias. Interestingly, the results showed an increased relative abundance of *Prevotella* in the early RA group, with similar findings in the at-risk group, possibly indicating that the influence of pharmacologic treatment was limited. However, it should be mentioned that future research should preferably include patients with early RA who are not receiving any immunomodulatory therapy.

This study is the first to report on the microbial composition of several oral niches in individuals at risk of RA. In a study by Mankia et al, ACPA-positive at-risk individuals were assessed for the presence of *P gingivalis* and *Aggregatibacter actinomycetemcomintans* (in subgingival plaque only), with the results showing an increased relative abundance of *P gingivalis* in at-risk individuals compared to healthy controls (25). However, the current study showed an overall low abundance of *P gingivalis*, and did not identify *P gingivalis* as a relevant bacterium for discrimination between the groups. Also, we did not observe any differences between the ACPA-positive and ACPA-negative at-risk groups. Interestingly, our results do show an increased relative abundance of the *Veillonella* and *Prevotella* species in the at-risk group, similar to the findings in the early RA group. This is consistent with the findings from a study by Tong et al, in which both patients with RA and at-risk individuals showed an increased abundance of *Prevotella* in saliva (26).

Furthermore, for most of the discriminative zOTUs, the relative abundance did not significantly differ between the early RA group and the at-risk group. This corresponds to our observations of overall similarities in the oral microbiome between patients with early RA and at-risk individuals, despite the microbial variety within

these groups, which should always be considered when interpreting Bray-Curtis distances between groups. Taken together, these findings indicate a possible role for oral microbial dysbiosis in the induction of arthritis, similar to previous findings on the gut microbiome (3). It also corresponds to the findings from a study by Cheng et al, in which at-risk individuals, patients with early RA, and controls showed differences in bacterial diversity and composition, and a role for oral microbiome dysbiosis in RA onset was suggested (9). Collectively, the current findings and previously published data suggest that bacterial colonization on any mucosal tissue might trigger aberrant inflammatory reactions; this is not, per se, restricted to bacteria from periodontal pockets. However, the possibility of a reversed pathway, as mentioned earlier, cannot be excluded, due to the cross-sectional nature of the data.

Regarding measures of periodontal health and possible confounding factors, this study did not show any between-group differences. This is a major strength of the current study, since clinical differences between groups are often an issue that complicates the interpretation of the results in microbiome studies (27). In contrast to our study findings, the study by Mankia and colleagues showed that ACPA-positive at-risk individuals had a higher prevalence of periodontal disease compared to the control group (25). These contradictory findings might be explained by differences in the case definition of periodontitis. Furthermore, the percentage of subjects with severe periodontitis in the healthy control group in the current study is representative of the overall prevalence of severe periodontitis observed in Western populations (28), while in the study by Mankia and colleagues, the prevalence of periodontitis was relatively high, but severe cases were not specified (25). Nonetheless, although not significant, the current results do show a trend toward a higher prevalence of severe periodontitis in patients with early RA and at-risk individuals compared to healthy controls.

A somewhat surprising result of this study is the absence of a difference among the groups in the microbial composition of the subgingival dental plaque. Whereas a role for periodontal disease and associated bacteria in the onset of RA was previously hypothesized (25), which would thus lead to a profound difference in the plaque microbiome between the groups, the results of the present study do not support this hypothesis. A possible explanation for this is that a significant difference in periodontal disease was not observed among the 3 groups, and thus there was no difference in the relative abundance of associated bacteria. Furthermore, the composition of dental plaque can be influenced by several factors, e.g., local immunologic reactions, diet, and oral hygiene, whereas the tongue coating was shown to be the most stable oral niche in terms of microbiome composition (29). Although we attempted to limit the influence of systemic treatment for RA, as described earlier, it may also partly explain the similarities in the plaque microbiome between the early RA group and the healthy control group, because the use of prednisone was shown to be associated with a healthier subgingival microbiome in patients with RA (24). This effect may be more pronounced in the subgingival plaque compared to the saliva and tongue coating, due to the direct contact of the subgingival niche to the circulatory system. However, further research is necessary to support this hypothesis.

To complement the currently available cross-sectional data, future studies should also include a longitudinal aspect, preferably with large cohorts and consistent data collection, to aid in the application of advanced methods, including the use of artificial intelligence, for prediction of an oral–systemic RA link (27). The results of the present study indicate that there are similarities in the oral microbiome between patients with early RA and at-risk individuals, as both groups had an increased relative abundance of potentially proinflammatory species when compared to healthy controls. These findings thus point toward a possible role of the oral microbiome in the onset of RA.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Ms Kroese had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Kroese, Crielaard, Lobbezoo, Loos, van Schaardenburg, Volgenant.

Acquisition of data. Kroese, van Boheemen.

Analysis and interpretation of data. Kroese, Brandt, Buijs, Loos, Zaura, Volgenant.

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