Microbiota around teeth and dental implants in periodontally healthy, partially edentulous patients: is pre-implant microbiological testing relevant?


This study aimed to assess the prevalence of seven periodontal marker pathogens, before implant placement and 1 yr after loading, in periodontally healthy individuals and to assess the long-term effectiveness of pre-implant reduction of pathogens to below threshold levels. In 93 individuals needing single tooth replacement, pooled subgingival microbiological samples from standard sites were cultured and analyzed before implant treatment and 1 yr after loading. Threshold levels commonly used in periodontology to predict periodontal breakdown were applied. Subjects with levels of pathogens above these thresholds received initial periodontal treatment including systemic antibiotics when indicated. At baseline, 49.5% of periodontally healthy subjects harboured one or more marker pathogens above threshold levels. Periodontal treatment reduced the pathogen levels below threshold values in 78.3% of these initially colonized subjects. In all cases Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis were reduced to below threshold. At 1 yr after loading, periodontal pathogens were present above threshold levels in 74.1% of all subjects. It is concluded that in almost half of periodontally healthy individuals the subgingival biofilm harbours periodontal pathogens above threshold values. Long-term effectiveness of pre-implant reduction of the selected marker pathogens appeared limited in our patient population, making pre-implant reduction unpredictable for post-implant levels of these pathogens. Thus, considering the applied microbiological criteria, generalized pre-implant microbiological testing is not contributory in periodontally healthy subjects.

Several studies have indicated a bacterial aetiology for failure of dental implants after osseointegration by demonstrating the presence of high levels of periodontopathic bacteria (e.g. P. gingivalis, Prevotella intermedia, and A. actinomycetemcomitans) in peri-implantitis lesions (1–8). According to the authors of these studies, colonization by periodontopathic bacteria has to be considered as a risk factor for peri-implantitis. Other investigations have indicated that in partially edentulous subjects the microflora of the teeth is a likely source for the microbiota observed around implants by demonstrating the (significant) similarity of bacterial samples obtained from teeth and implants at the same visit to the dentist (7, 9–13). Consequently, several authors have posed that pre-implant elimination of periodontal pathogens might inhibit peri-implant colonization by these pathogens and thus reduce the risk of peri-implantitis (14–17). For a predictable outcome of implant-supported single tooth restorations in partially edentulous subjects, there may be a need to achieve microbiological health before implant placement in order to prevent or reduce the transmission of pathogenic species from teeth to implants and thus to prevent loss of attachment of the peri-implant soft tissues and bone (18–20).

For individuals with a history of susceptibility to periodontal disease, microbiological testing seems rational. However, it is not known whether pre-implant assessment and selective eradication decontamination of potential periodontal pathogens in individuals with no history of periodontal disease is as rational. Furthermore, limited data are available in the literature on the colonization with potential periodontal pathogens of individuals without clinical periodontal disease (19,
21–24), and little is known about the efficacy of reduction of high concentrations (considered to be potentially pathogenic) of periodontal pathogens in colonized healthy individuals, particularly with regard to the long-term results. Therefore, the aim of this study was to assess, in periodontally healthy individuals, the prevalence and proportions of periodontal marker pathogens at teeth before implant placement and 12 months after loading the implants, and at implants 12 months after loading. In addition, the 1-yr effectiveness of pre-implant reduction of periodontal pathogens was assessed.

**Material and methods**

In this study, the prevalence of seven selected putative periodontal pathogens was determined in 93 partially edentulous individuals (49 women and 44 men; mean age 33.3 ± 13.0 yr, range 18–63 yr) who requested an implant-supported crown to replace a single missing tooth in the aesthetic zone of the anterior maxilla (P1–P4). Participants were referred to the Department of Oral and Maxillofacial Surgery and Maxillofacial Prosthetics of the University Medical Center Groningen, the Netherlands, or to the Department of Oral and Maxillofacial Surgery of the Nij Smellinghe Christian Hospital in Drachten, the Netherlands, for implant therapy. Participants had to be free of clinical signs of periodontal disease, determined as the absence of periodontal pockets ≥ 4 mm and the absence of gingival bleeding, oedema, glazing, and redness. All patients were non-smokers and non-diabetic. A titanium endosseous dental implant (ITI-Esthetic Plus; Institut Straumann, Waldenburg, Switzerland) was placed in all patients. Six months after implant placement a crown was placed on the implant.

The study was carried out in accordance with the requirements of the national Central Committee on Research involving Human Subjects as active at the start of our trial in 1999. Informed consent was obtained from all patients.

Pooled subgingival plaque samples were taken from pre-determined oral sites to assess the levels and proportions of seven selected major putative periodontal pathogens, namely A. actinomycetemcomitans, P. gingivalis, P. intermedia, Tannerella forsythia, Peptostreptococcus micros, Fusobacterium nucleatum, and Campylobacter rectus. These pathogens were selected because of their association with the onset and progression of periodontal disease, as demonstrated in other studies (25–27).

The pooled samples of the subgingival flora were obtained from the mesiobuccal sites of the first upper molars and the mesiobuccal sites of the first lower molars. Microbiological pooled samples of the implants were obtained at the buccal and palatal approximal sites of the implants. Sampling of the teeth was performed at baseline (i.e. before the implantation procedure), and sampling of both the teeth and the implants was performed 12 months after functional loading of the implant (T12). Before microbiological sampling, supragingival plaque was carefully removed with sterile cotton rolls and cotton pellets after which the sampling site was isolated with cotton rolls and gently air-dried. Sterile paper points (Fine, UDM, West Palm Beach, FL, USA) were inserted in the periodontal or peri-implant sulcus and left in place for 10 s. The paper points were collected in one vial (at baseline: teeth only) or in two separate vials (T12: teeth and implants), containing 1.8 ml of reduced transport fluid (RTF) (28).

The levels and proportions of A. actinomycetemcomitans, P. gingivalis, P. intermedia, T. forsythia, P. micros, F. nucleatum, and C. rectus were assessed. Samples were processed in the laboratory within 18 h of collection. Tenfold serial dilutions of all samples were prepared in RTF. Aliquots of 0.1 ml were inoculated onto 5% horse-blood agar plates (Oxoid no. 2; Oxoid, Basingstoke, UK) containing haemin (5 mg l⁻¹) and menadione (1 mg l⁻¹) for the isolation and growth of obligate anaerobic bacteria, and on tryptic soy-serum-bacitracin-vancomycin (TSBV) plates for the selective isolation and growth of A. actinomycetemcomitans (29). Blood agar plates were incubated anaerobically in 80% N₂, 10% H₂, and 10% CO₂ for up to 14 d. The TSBV plates were incubated in air with 5% CO₂ for 5 d at 37°C (30). Blood agar plates were used to determine the total number of colony-forming units, the presence of dark-pigmented colonies, T. forsythia, F. nucleatum, and P. micros. Representative dark-pigmented colonies were purified and identified using standard techniques (31), including the gram-stain, hemagglutination of 3% sheep erythrocytes, fermentation of glucose, the production of indole from tryptophan, and the production of specific enzymes (32). T. forsythia was identified on the basis of the typical colony morphology, gram-staining, and the production of a trypsin-like enzyme (33).

F. nucleatum and P. micros were identified on the basis of colony morphology, gram-stain, and the production of specific enzymes (determined using the API 32A; Biomerieux, La Balme, Les Grottes, France).

Depending on the microbiological criteria listed below, the participants in the study were divided into three groups: ‘primary-valid’ group; ‘secondary-valid’ group; and ‘colonized’ group.

To qualify for inclusion in the ‘primary-valid’ group, at baseline individuals had to fulfil the following subgingival microbiological conditions, based on the predictive capacity for recurrence of periodontal disease in periodontally compromised patients, as demonstrated by Rams et al. (25), Machtei et al. (26), and Van Winkelhoff et al. (27):

- **A. actinomycetemcomitans** negative;
- **P. gingivalis** negative;
- **P. intermedia** < 2.5%;
- **T. forsythia** < 3.0%;
- **P. micros** < 3.0%;
- **F. nucleatum** < 3.0%; and
- **C. rectus** < 2.0%.

If microorganisms were present above these thresholds, the following interventions were applied after initial periodontal treatment:

- **A. actinomycetemcomitans** plus P. gingivalis: amoxicillin 500 mg + metronidazole 500 mg, three times per day for 7 d;
- **A. actinomycetemcomitans** without P. gingivalis: amoxicillin 375 mg + metronidazole 250 mg, three times per day for 7 d;
- **P. gingivalis** without **A. actinomycetemcomitans**: metronidazole 500 mg, three times per day for 7 d;
- **T. forsythia** without **A. actinomycetemcomitans** or P. gingivalis: metronidazole 500 mg, three times per day for 7 d; or
- **P. intermedia, P. micros, F. nucleatum,** and/or **C. rectus** (and not fulfilling any situation mentioned above): repeated initial periodontal treatment and rinsing with chlorhexidine (0.12%), twice daily for 4 wk.
After periodontal treatment (i.e., before placement of the implant), microbiological analysis was repeated: when the above-mentioned subgingival microbiological conditions were met at the second sampling, the patient was classified as ‘secondary-valid’. When the levels of the pathogens were still above thresholds, the individual was classified as ‘colonized’.

Differences in the number of individuals colonized with detectable levels, or above-threshold levels, of microorganisms were statistically analyzed using Chi-square tests with Yates’ continuity correction, using a level of significance of 0.05.

Results

The prevalence of indicator microorganisms at baseline is shown in Table 1. Before any implantation treatment and before any periodontal intervention, *P. micros* and *F. nucleatum* were the predominant microorganisms present in our population of periodontal healthy individuals. The prevalence of indicator microorganisms exceeding threshold levels is shown in Table 2. Again, *P. micros* and *F. nucleatum* were the predominant microorganisms, exceeding threshold levels in about one-third of the subjects participating in this study.

At baseline only about half of the population (n = 47) had levels of putative periodontal pathogens below threshold and was classified as ‘primary-valid’ (Table 2).

The other half of the subjects included in this study (n = 46) were colonized with one or more of the seven selected major putative periodontal pathogens above threshold level. Of these colonized individuals, seven (7.5%) were colonized with *A. actinomycetemcomitans* and three (3.2%) were colonized with *P. gingivalis*, including one individual colonized with both *A. actinomycetemcomitans* and *P. gingivalis*.

After periodontal therapy according to the study protocol, the proportions of pathogens were reduced to a level below threshold in 36 out of 46 colonized individuals at baseline (Fig. 1). These individuals were classified as ‘secondary-valid’. Colonization with pathogens persisted in the remaining 10 individuals, who were classified as ‘colonized’. *P. micros* was the most common persistent species (eight individuals) followed by *F. nucleatum* (four individuals), *T. forsythia* (two individuals), and *C. rectus* (one individual). In all cases, *A. actinomycetemcomitans*, *P. gingivalis*, and *P. intermedia* were reduced to below detection levels.

At T12, 91 individuals were available for follow-up (48 women, 43 men). Two individuals were lost as a result of implant loss: one individual in the primary-valid group and one individual in the secondary-valid group. The implant loss occurred owing to failure in osseointegration within the first 4 months after implantation and before functional loading. This failure could not be related to the microbial status of the patients.

| Table 1 |
|-----------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
|                | AA                 | PG                 | PI                 | TF                 | PM                 | FN                 | CR                 |
| Baseline: teeth only (n = 93) | 7 (7.5%) | 3 (3.2%) | 16 (17.2%) | 33 (35.5%) | 73 (78.5%) | 88 (94.6%) | 20 (21.5%) |
| T12: teeth + implant (n = 91) | 2 (2.2%) | 6 (6.6%) | 18 (19.8%) | 45* (49.5%) | 80 (87.9%) | 89 (97.8%) | 11 (12.1%) |


*P = 0.077 (TF: ‘Baseline’ vs. ‘T12-teeth + implant’).

Two individuals were lost during follow-up.

| Table 2 |
|-----------------|------------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
|                | Free of pathogens | Colonized > threshold | AA | PG | PI | TF | PM | FN | CR |
| Baseline (n = 93) | 47 (50.5%) | 46 (49.5%) | 7 (7.5%) | 3 (3.2%) | 1 (1.1%) | 13 (14.0%) | 32* (34.4%) | 28† (30.1%) | 4 (4.3%) |
| T12 all individuals (n = 91) | 23 (25.3%) | 68 (74.7%) | 2 (2.2%) | 6 (6.6%) | 3 (3.3%) | 10 (11.0%) | 56 (61.5%) | 40 (44.0%) | 4 (4.4%) |
| T12 valid group (n = 81) | 21 (25.9%) | 60† (74.1%) | 2 (2.5%) | 3 (3.7%) | 3 (3.7%) | 9 (11.1%) | 49 (60.5%) | 36 (44.4%) | 4 (4.9%) |

Two individuals were lost during follow-up.

The valid group is the group of individuals who met the inclusion criteria of the study with or without previous intervention (i.e. the primary valid + secondary valid groups).


*P = 0.002 (PM: ‘Baseline’ vs. ‘T12-All individuals’).

†P = 0.048 (FN: ‘Baseline’ vs. ‘T12-All individuals’).

‡P = 0.0007 (Colonized > threshold: ‘Baseline’ vs. ‘T12-All individuals’).

§P = 0.0016 (Colonized > threshold: ‘Baseline’ vs. ‘T12-Valid group’).
A considerably larger number of individuals at T12 had become colonized with periodontal pathogens above threshold compared with the number of individuals at baseline [46 individuals at baseline (49.5% of the study population) vs. 68 individuals at T12 (74.7% of the study population); \( P = 0.0007 \)]. This occurred irrespective of whether subjects belonged to the primary-valid group (0 individuals at baseline vs. 32 individuals at T12) or to the secondary-valid group [0 individuals at (second) baseline after periodontal treatment vs. 28 individuals at T12] (Tables 2 and 4; Fig. 1). Combining the primary-valid and secondary-valid groups as being the microbiologically healthy group at the start of the study (‘valid-group‘), only about one-quarter (25.9%) of the assessed individuals remained free from pathogens (i.e. pathogens below threshold levels) at T12. When comparing these data with the data obtained in the colonized group 1 yr after loading the implants, about the same percentage of individuals (20%) appeared to be free from pathogens at T12 (Fig. 1).

At baseline, nine individuals were colonized with A. actinomyctemcomitans (7×) and/or P. gingivalis (3×) (Tables 1 and 2). In all subjects the levels of A. actinomyctemcomitans and P. gingivalis were reduced to below-detection levels after periodontal intervention, according to protocol. At T12, six of these individuals remained free from A. actinomyctemcomitans (5×) and/or P. gingivalis (2×), while two individuals previously colonized with A. actinomyctemcomitans and one individual previously colonized with P. gingivalis showed detectable levels of A. actinomyctemcomitans and P. gingivalis again. In addition, five new individuals were observed to be colonized with P. gingivalis at T12; these individuals were free from detectable levels of P. gingivalis at baseline. No new colonizations with A. actinomyctemcomitans were found at T12.

Sampling of the peri-implant sulcus revealed colonization of the implants 12 months after loading (Table 3). All selected putative periodontal pathogens were found to be able to colonize the peri-implant region. Similarly, all selected putative periodontal pathogens were found to be able to colonize the peri-implant region and exceeded threshold levels (Table 4). The highest prevalence was for P. micros and F. nucleatum. Implants were as frequently colonized with periodontal pathogens above...
Discussion

Various authors have reported on the composition of the microflora in periodontal disease (34–39), but only a few reports are available on the presence of putative periodontal pathogens in periodontally healthy individuals (19, 21–24, 40). We observed that at baseline no more than half of the participating subjects who were considered as being clinically periodontally healthy also had a healthy oral microflora, according to the microbiological standards reported in the literature (25–27). The other half of our study population carried putative periodontal pathogens at above-threshold levels, but without clinical signs of active periodontitis. These observations question the value of pre-implant reduction of periodontal pathogens when aiming for the long-term prevention of recurrence of periodontal pathogens around implants. It also demonstrates that the mere presence of periodontal pathogens, even *A. actinomycetemcomitans* and *P. gingivalis*, is compatible with health, although absolute or relative numbers may be related to disease progression (41). To focus on the threshold levels and numbers of potentially periodontal pathogens alone is attractive, but questionable (41). The numbers of pathogens play an obvious role in health and disease. However, at the same time it must be considered that the number of bacteria alone cannot be predictive for future diseases such as peri-implantitis, because confounding factors, for instance host factors and the condition of the pathogens are, as well as the time span during which they express their virulence, contribute to the number of pathogens and disease severity. Recently, crosstalks have been observed in, for example, viral and mycobacterial infections, which may explain the development of pathologies in a restricted part of the population after colonization (42, 43). This is also observed at gene-expression levels of gingival tissues, which may be related to the subgingival bacterial composition and levels of specified bacterial species (44). Although this cannot elucidate whether a certain bacterial composition will influence the gene expression of the host or whether a genetic profile will facilitate the colonization of microorganisms, it becomes more obvious that interplay is present between the phenotype of the host and microorganisms at the site of interest.

In this study, it was possible to reduce the numbers of periodontal pathogens to below-threshold levels in the majority of the colonized individuals (78.3%). However, this reduction was followed by the re-appearance of these periodontal pathogens in the majority of the colonized individuals (78.3%). Only reduction of *A. actinomycetemcomitans* and *P. gingivalis* to below-detection levels was shown to be effective in the long term, but even then not in all cases. Whether, in these cases, the renewed finding of *A. actinomycetemcomitans* and *P. gingivalis* is caused by a true recolonization with new microorganisms or by regrowth of reduced numbers of *A. actinomycetemcomitans* and *P. gingivalis* could not be confirmed. In a study

### Table 3

<table>
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<th>n = 81</th>
<th>AA</th>
<th>PG</th>
<th>PI</th>
<th>TF</th>
<th>PM</th>
<th>FN</th>
<th>CR</th>
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<td>0</td>
<td>6 (7.4%)</td>
<td>10 (12.4%)</td>
<td>47 (58.0%)</td>
<td>70 (86.4%)</td>
<td>10 (12.4%)</td>
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<td><strong>T12</strong> – at teeth only</td>
<td>1 (1.2%)</td>
<td>1 (1.2%)</td>
<td>3 (3.7%)</td>
<td>11 (13.6%)</td>
<td>10* (12.4%)</td>
<td>6* (7.4%)</td>
<td>4 (4.9%)</td>
</tr>
<tr>
<td><strong>T12</strong> – at implant only</td>
<td>0</td>
<td>2 (2.5%)</td>
<td>10 (12.4%)</td>
<td>15 (18.5%)</td>
<td>10* (12.4%)</td>
<td>3* (3.7%)</td>
<td>3 (3.7%)</td>
</tr>
<tr>
<td><strong>T12</strong> – at teeth + implant</td>
<td>1 (1.2%)</td>
<td>0</td>
<td>4 (4.9%)</td>
<td>12 (14.8%)</td>
<td>50 (61.7%)</td>
<td>70 (86.4%)</td>
<td>3 (3.7%)</td>
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*P < 0.0001 (PM: 'T12-at teeth only' or 'T12-at implant only' vs. 'Baseline' or 'T12-at teeth + implant').

**Table 4**

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<th>n = 81</th>
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<th>PI</th>
<th>TF</th>
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<th>FN</th>
<th>CR</th>
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<tr>
<td><strong>T12 &gt; threshold</strong> – at teeth only</td>
<td>1 (1.2%)</td>
<td>1 (1.2%)</td>
<td>1 (1.2%)</td>
<td>3 (3.7%)</td>
<td>12 (14.8%)</td>
<td>12 (14.8%)</td>
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<tr>
<td><strong>T12 &gt; threshold</strong> – at implant only</td>
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<td>2 (2.5%)</td>
<td>2 (2.5%)</td>
<td>5 (6.2%)</td>
<td>18 (22.2%)</td>
<td>13 (16.1%)</td>
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</tr>
<tr>
<td><strong>T12 &gt; threshold</strong> – at teeth + implant</td>
<td>1 (1.2%)</td>
<td>0</td>
<td>0</td>
<td>1 (1.2%)</td>
<td>19 (23.5%)</td>
<td>13 (16.1%)</td>
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on the effectiveness of subgingival debridement it was found that a high proportion of treated root surfaces (from 5 to 80%) still harboured plaque and/or calculus; the remaining bacteria were considered as the primary source for subgingival re-colonization (45). In addition, cross-colonization between partners and family members might influence the composition of the oral microflora in individuals (46, 47). In future similar studies, genotyping might be useful to decide upon regrowth or re-colonization, as may sampling of partners to eliminate possible bias caused by cross-colonization.

This study used well-defined microbiological criteria to decide upon colonization and non-colonization. The criteria used were based on the threshold levels of the selected putative periodontal pathogens reported in the literature. These threshold levels were considered useful as they have been shown to be predictive in patients with refractory periodontal disease for recurrence of the disease (25–27). The results of our study suggest, however, that the threshold levels reported in the literature are not applicable for the microbiological testing of periodontally healthy individuals aiming for the long-term prevention of recurrence of the periodontal marker pathogens above threshold levels. It is obvious from our results that periodontal intervention (initial periodontal treatment and antibiotics) is unstable and unpredictable in healthy subjects. Thus, there is a great need for longitudinal studies on the microbiology in periodontally healthy subjects and on its association with peri-implant disease, in order to rate the burden of periodontal marker pathogens in the health of peri-implant tissues. Furthermore, there is a great need to develop more applicable microbiological criteria and indications for periodontal intervention, as well as effective methods to reduce the burden of periodontal marker pathogens. Thus, on the basis of the results of our study, pre-implant testing on periodontal marker pathogens is not relevant in clinically periodontally healthy subjects. However, we cannot exclude the possibility that in future a better understanding of the role of microorganisms can lead to valuable testing. This will depend, amongst others, on a better understanding of the association between thresholds of known periodontopathogens and the prediction of periodontal and/or peri-implant disease, of the influence of newly recognized clusters that are more pathogenic than single periodontopathogens alone, and of the discovery of different (yet unculturable) species.

Colonization of the marginal region of the implant with periodontopathic microorganisms has been shown to occur within 7–14 d, whereas subgingival colonization has been shown to occur within 1 month (4, 48, 49). Six months after loading of the implants, the majority of the implant sites are colonized with detectable levels of many periodontal bacterial species (14). After 3 yr, the periodontitis marker bacteria were found as frequently around implants as around natural teeth in the same individual (48). The observation in our study suggests that as early as 12 months after loading (i.e. 18–20 months after implantation), the implants are as frequently colonized with the marker pathogens at above-threshold levels as the teeth (Tables 3 and 4).

From this study, it is concluded that almost half of a periodontally healthy population carries putative periodontal pathogens in concentrations exceeding threshold levels, as described by Rams et al. (25), Machtei et al. (26), and van Winkelhoff et al. (27). In about three-quarters of the individuals with pathogen levels above threshold, it was possible to reduce the pathogen levels to below threshold, and in all cases A. actinomycetemcomitans and P. gingivalis were eliminated to below detection levels. This pre-implant gain in microbiological health appeared to be a short-term achievement, however. Re-evaluation of the periodontal flora, 1 yr after loading the implant, showed re-colonization with periodontal pathogens above threshold levels in three-quarters of the individuals in whom pathogen levels were below threshold at (second) baseline after periodontal treatment. Therefore, the value of generalized pre-implant microbiological testing for the purpose of prevention of post-implant prevalence of these periodontal marker pathogens above threshold levels (and thus in the possible prevention of peri-implantitis) in clinically periodontally healthy subjects seems limited.

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References


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