

Periodontal Early Detection and Progression

Microbiome analysis before and during periodontitis

This technical information clarifies the scientific and technical background of NGS-based microbiome analysis and the role it plays in the context of the therapy and prevention of periodontitis.

The basis of the investigation is the presence of subgingival dysbiosis and characteristic microbiome structures which represent the main cause of the formation of periodontal diseases. Today, new molecular biological methods such as the NGS technology enable the in-depth analysis of the patient-specific subgingival flora. This advantage results in suitable therapeutic measures permitting an individual as well as optimised treatment.

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Periodontitis

The consequence of oral microbiome dysbiosis

In the scientific field, there is a general consensus according to which the main cause of periodontal diseases is of a bacterial nature. However, the hypotheses about the etiopathogenesis of the disease have been subject to significant changes over time. Previous hypotheses postulated the increased occurrence of a group of particularly pathogenic bacterial species, the so-called 'key germs', as triggers of an immunological inflammatory response and subsequent destruction of the periodontal holding apparatus^[9].

Nowadays, however, the holistic approach applies, which sees a disturbed balance of the whole subgingival flora as being primarily responsible for the formation of periodontal diseases^[2,6]. The microbiological derailment of the oral microbiome is considered to be the main risk factor for the development as well as the progression of periodontal diseases, whereby the extent of the dysbiosis determines the dimension of the inflammatory response and, consequently, the expected progression of the respective disease^[8].

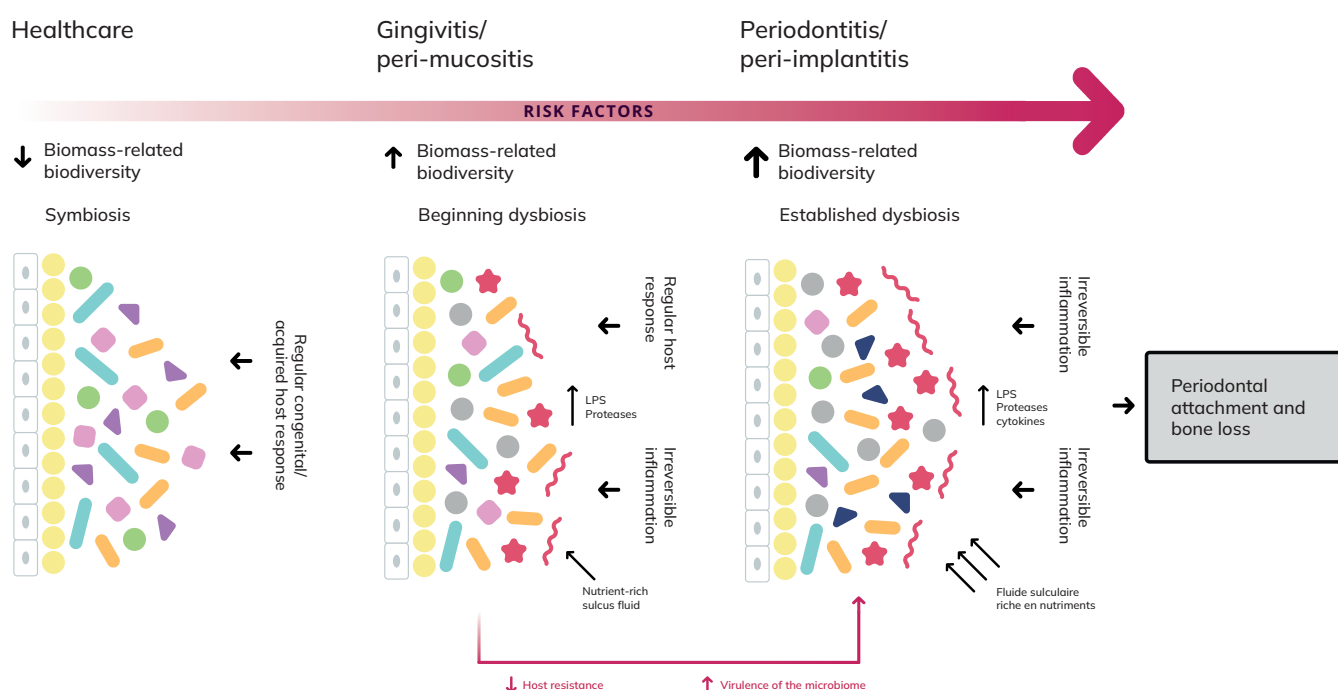


Fig. 1: Changes affecting the subgingival microbiome during the course of periodontal disease. In the healthy periodontium, there is a predominance of symbionts and the host reaction remains under control. The plaque accumulation results in an increased but limited chronic inflammation, which in turn promotes the occurrence of periodontal pathogenic germs. In the further course of the disease, a dysbiosis with dominance of periodontitis-associated bacterial species will be established. The dysbiotic microbiota promotes an increased immune and inflammatory response resulting in a loss of the periodontal supporting apparatus.

Quote from: Columbo & Tanner, 2019

Healthy or sick

The typical microbiome structures

According to research conducted by Diaz^[2] and Columbo & Tanner^[1], the oral microbiome can be divided into the three following main areas: health-associated species, disease-associated species, and nuclear species. The bacterial species belonging to the individual areas are known; their DNA sequences are stored in the NCBI database.

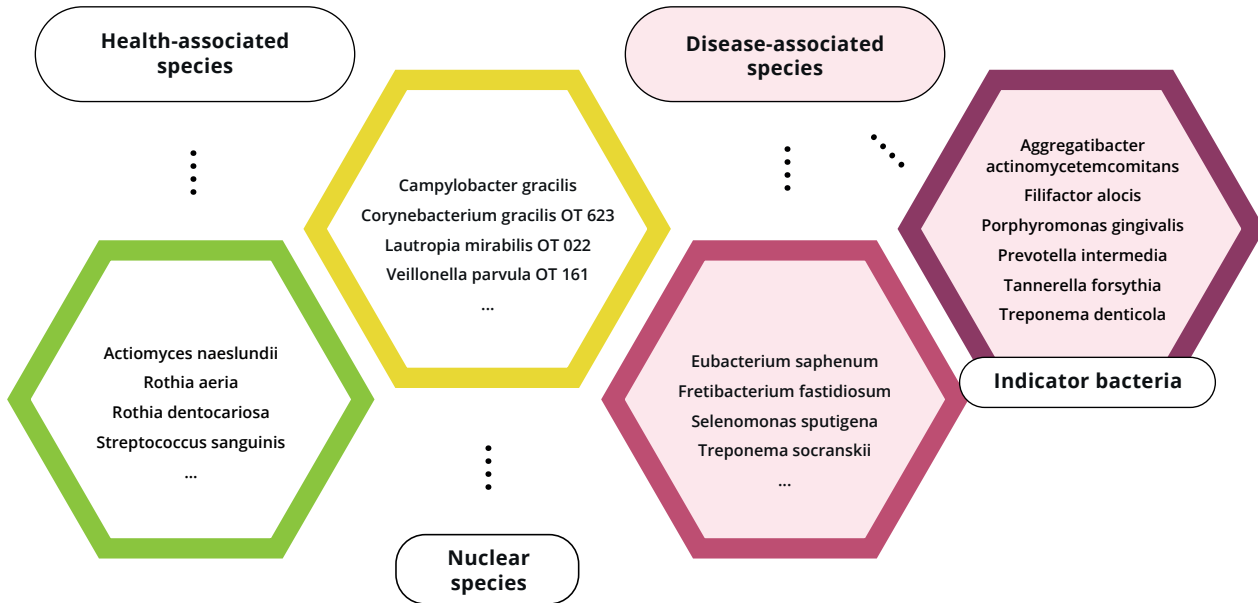
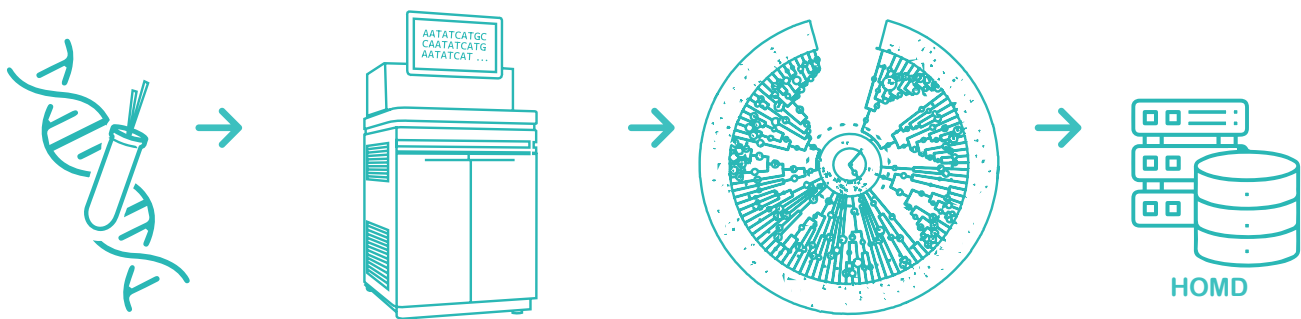


Fig. 2 : Bacterial groups of the subgingival microbiome in periodontally healthy or sick patients.

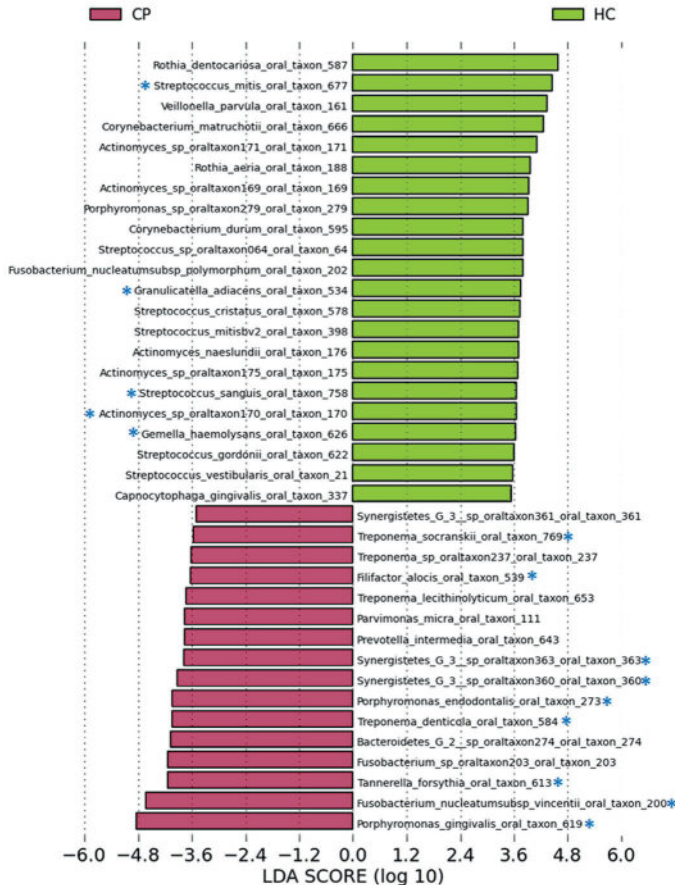
The pros of the NGS technology in the context of the microbiome analysis

In contrast to the previously applied molecular biological methods aimed at the analysis of the subgingival flora, the so-called 'next-generation sequencing' technology allows the simultaneous, automated sequencing of a large number of DNA molecules and their matching to reference DNA sequences. Whereas in the past only the detection of individual indicator germs was feasible, all bacteria present within a patient sample can now be analysed by applying the so-called NGS-based methods. The oral microbiome could be fully decoded in the context of the 'Human Microbiome Project' and the gene sequences of the more than 700 identified bacterial taxa could be stored in a freely accessible database (the so-called 'HOMD').



Proportion of bacterial groups contained in the subgingival biomass

Thanks to the comparison of NGS-based microbiome structures of healthy and periodontally sick patients, it could be shown that their subgingival flora has a fundamentally different composition^[7].



A study by Kirst et al.^[7] showed a different accumulation of bacterial phylotypes depending on the present clinical picture. While the subgingival flora in the healthy periodontium is dominated by actinobacteria and firmicutes, gram-negative anaerobes such as fusobacteria, porphyromonas species, prevotelles and spirochaetes are mainly found in the diseased sulcus.

While the healthy, symbiotic periodontium is characterized by a predominance of aerobic, gram-positive health-associated bacteria, these are increasingly displaced by gram-negative, anaerobic disease-relevant species^[2].

Fig. 3: Different frequency of bacterial phylotypes: The bacterial taxa enriched in healthy sites (in green colour); the bacterial taxa enriched in periodontal sites (in red colour). (according to Kirst et al.^[7]).

Diaz et al.^[2] demonstrated by means of 16S-rRNA sequencing that the total number of germs in the sulcus increases by 3 log stages in the course of the disease progression. This fact is primarily due to the above-average growth of periodontitis-associated bacterial species, which account for almost the half of the subgingival flora of patients suffering from periodontitis. The ratio of health and disease-specific taxa changes significantly in as the periodontitis develops and is an indicator of the extent of subgingival dysbiosis.

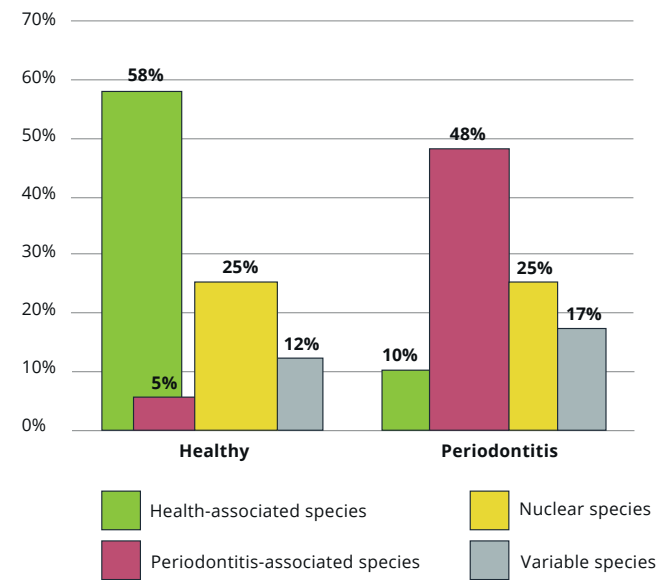


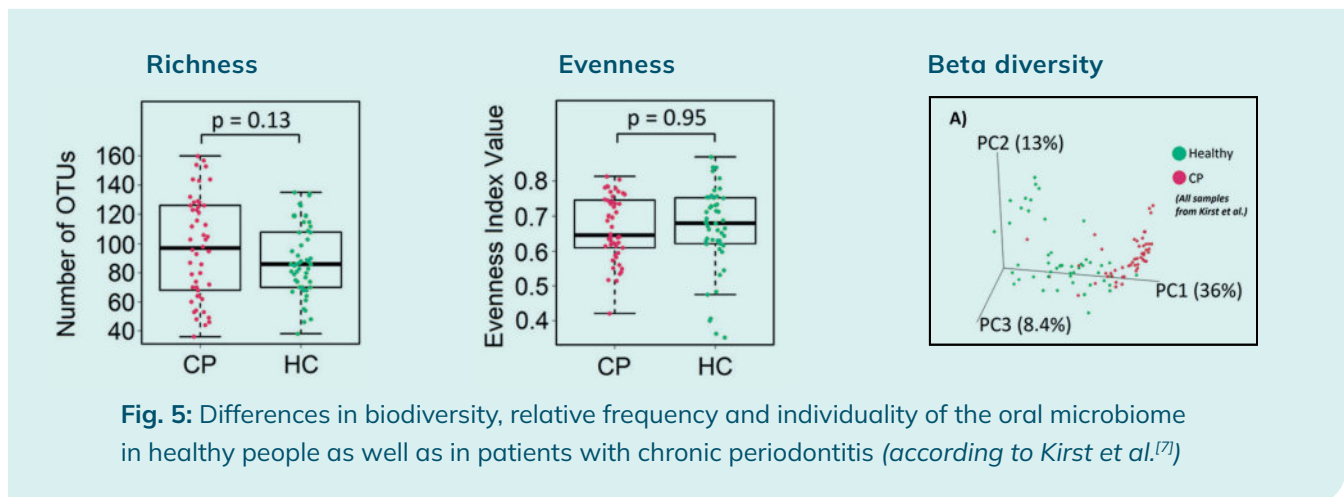
Fig. 4: Changes relating to the composition of the oral microbiome in the healthy vs. diseased periodontium as a proportion of the individual groups in the subgingival biomass. (as modified according to Diaz et al.^[2])

Effects of periodontal therapy on the oral microbiome

The studies conducted by Kirst et al.^[7] and Hagenfeld^[3] show that the parameters richness and evenness for the assessment of a microbiome differ significantly between periodontally healthy and sick patients. On the other hand, they can be significantly improved through an efficient periodontitis therapy which aims at a re-establishment of the symbiotic conditions.

- Alpha diversity** = state of the microbiome
- *Richness*: biodiversity, number of different bacterial species
 - *Evenness*: relative frequency and distribution

- Beta diversity** = individuality, difference between the number of organism types
- » Calculation of a microbiome index for interpretation of results



Thus, the biodiversity (richness) of the microbiome increases in the course of periodontitis because the PA-associated bacterial species increasingly colonize the sulcus. In parallel, the evenness decreases as microbiomes of periodontally sick patients are increasingly dominated

by single species. As a consequence, the beta divergence also decreases with the disease progression because the dominance of the PA-associated species makes the microbiomes of diseased patients increasingly similar.

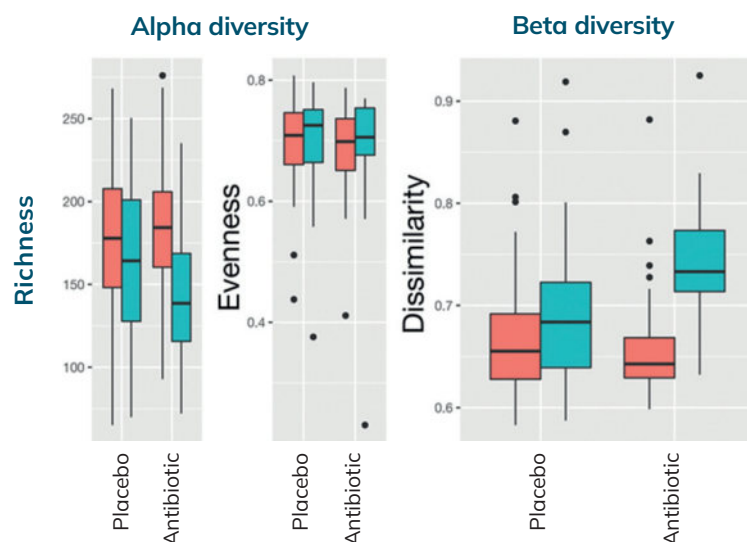


Fig. 6: Effects of periodontal therapy on the diversity indices of the oral microbiome before the treatment (in red colour) and 2 months after the treatment (in turquoise colour). The supportive administration of a systemic antibiotic results in a significant improvement in microbiome structure^[3].

What's the innovating aspect of PadoBiom®?

Generation of the crucial advantage

PadoBiom® analyses and evaluates the periodontal microbiome and its equilibrium by means of next-generation sequencing. In contrast to the examination of individual pathogenic bacteria, this method enables the comprehensive and **early assessment** of developing periodontitis.

The dysbiosis index, the **identification of risk patients**, the evaluation of key parameters and the examination of antibiotic resistance genes lead to outcome recommendations that optimise the treatment timing and treatment planning of every dental practice.



Detection of the dysbiosis at an early stage and initiation of the prophylaxis measures



Diagnostics as a quality feature and for patient retention



Interruption of periodontitis by a timely transition into the therapy phase



Identification of at-risk patients with progression and assured provision of (antibiotic) adjuvant

Based on statistical comparative analyses, **PadoBiom®** can also identify patients who, due to their subgingival flora, may expect a high progression of the disease and who can therefore profit from particularly intensive therapeutic measures and close monitoring.

This makes it possible for the first time to decide at an early stage which patients should be advanced from the regular check-up phase to prophylaxis or even to the therapy.

The result recommendation is made as a classification in one of 3 practise-oriented categories with measures from the known dental treatment spectrum.

This increases the success of the treatment and also prevents the need for therapy in the event of early diagnosis.



The new method for evaluating the gingival sulcus

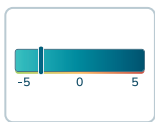
How does PadoBiom[®] work?

Knowledge for practitioners

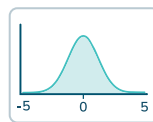
For practise-oriented result recommendations

With the patient's clinical picture as a starting point, the analysis of the oral microbiome by using various indices, such as the microbial dysbiosis index, progression and other key parameters, enables individualised therapeutic recommendations matching the patient's actual treatment need. Such recommendations can be implemented through practice-oriented therapeutic measures that can be easily integrated into existing treatment plans by dental practice.

Dysbiosis index and progression *Early detection prior to periodontitis and identification of risk patients*



Assessment of symbiosis / dysbiosis
The ratio of health- and disease-related bacteria results in the dysbiosis index of the oral microbiome.



Identification of progression
The deviating microbial load in the statistical comparison enables the targeted therapy of risk patients.

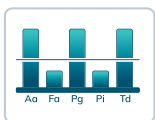
Key parameters *For an extended assessment*



Evaluation of richness
The lower the microbial species diversity, the healthier the oral microbiome.



Evaluation of evenness
The frequency of individual bacteria enables an evaluation of balance.



Evaluation of pathogenicity
The identification of indicator bacteria for classifying pathogenicity in the oral microbiome.



Determination of Aa serotypes
Detection of *Aggregatibacter actinomycetemcomitans* a - f and the JP2 clone in the subgingival flora for individual antibiotic therapy.

Antibiotic resistance genes *Bacterially induced therapy failure*



Detection of resistance genes
Existing antibiotic resistance genes from five dentally-relevant antibiotic classes as information for optimised antibiotic therapy.

Illustrations are symbolic

Identification of “special care” patients

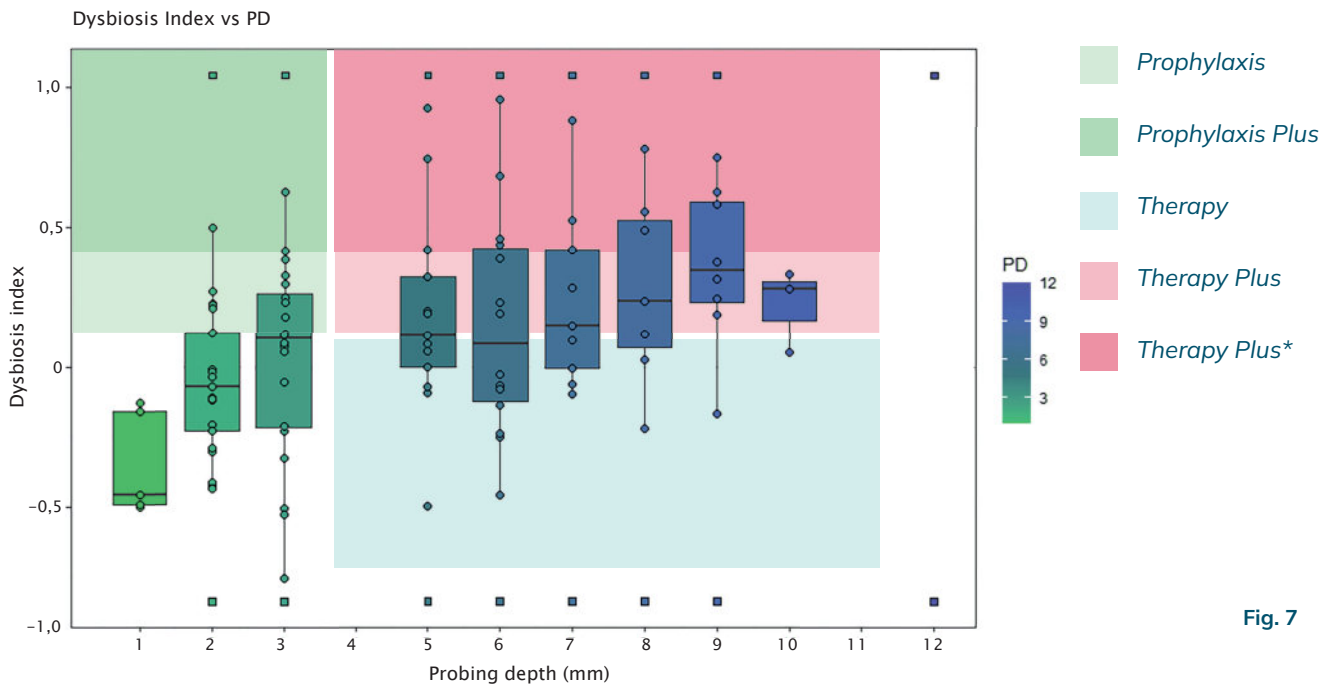


Fig. 7

Early intervention ensures that healthy, high-risk and gingivitis patients who are being continually monitored in the regular check-up phase will not suffer a progression of the disease and will not have to transition to periodontal therapy (Fig. 7: in green colour).

PA patients with existing dysbiosis require an intensified therapy for the reestablishment of the symbiotic conditions. Patients with above-average dysbiotic microbiomes, in particular, are at increased risk for disease progression, and can therefore benefit from supporting the instrumental therapy with adjuvants as well as more close-knit dental care (Fig. 7: in red colour).

Verification of grading

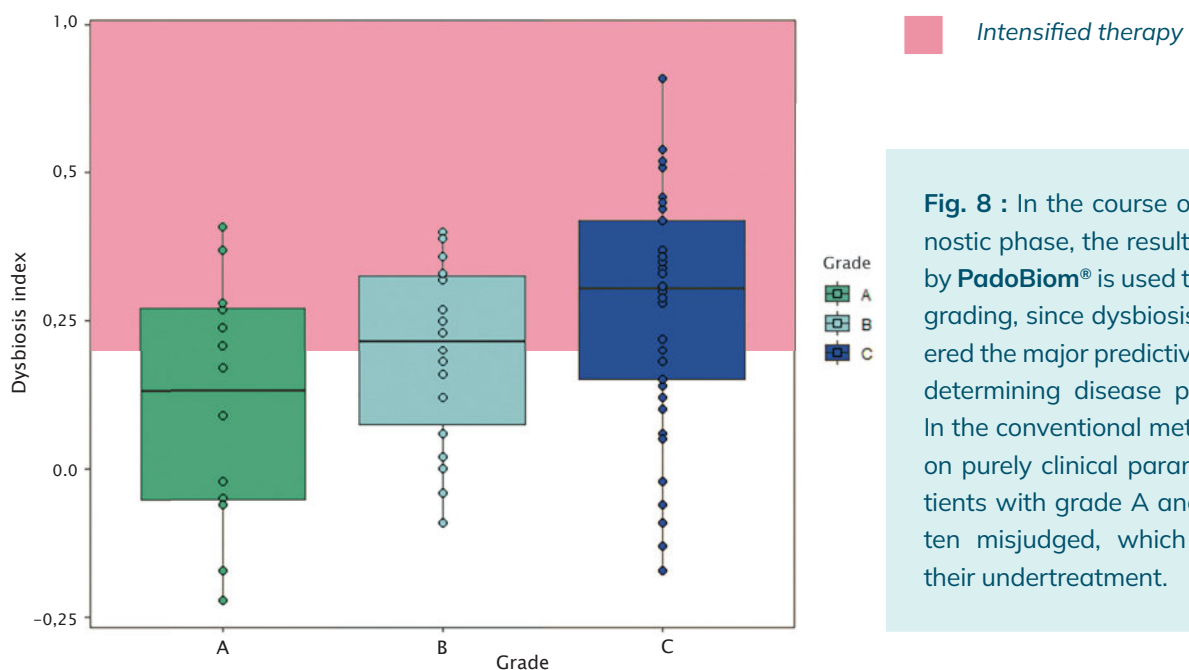


Fig. 8 : In the course of the diagnostic phase, the result generated by PadoBiom® is used to verify the grading, since dysbiosis is considered the major predictive factor for determining disease progression. In the conventional method based on purely clinical parameters, patients with grade A and B are often misjudged, which results in their undertreatment.

Pros and cons of antibiotics

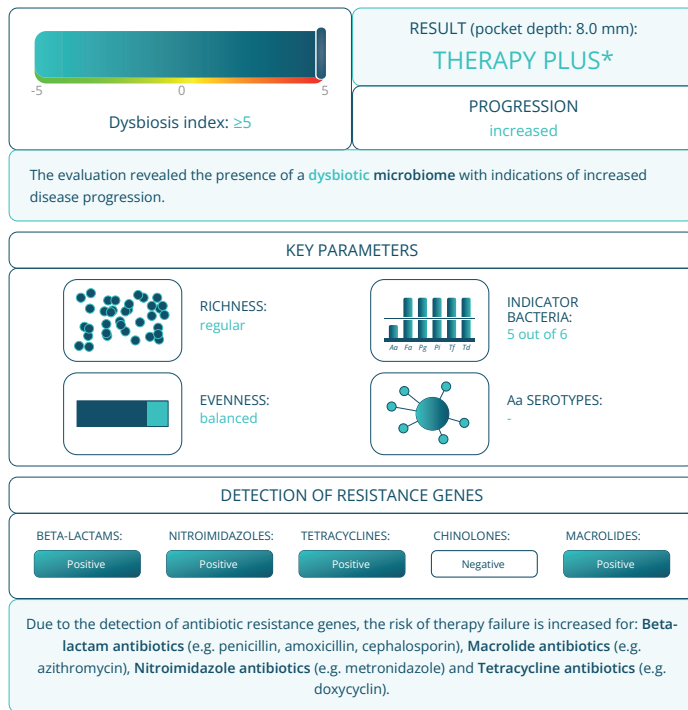
Systemic antibiotics as adjuvant in the AIT/CPT:

... should not be used routinely but only for aggressive course forms (according to the S3 Guideline)^[5].

... must only be used after the diagnosis and with the narrowest potential spectrum.

(according to 'Antibiotic Stewardship')

... contribute to the improvement of the oral microbiome^[3].



THERAPIE PLUS*

(PA patients, dysbiosis, increased progression) As in Therapy, but:

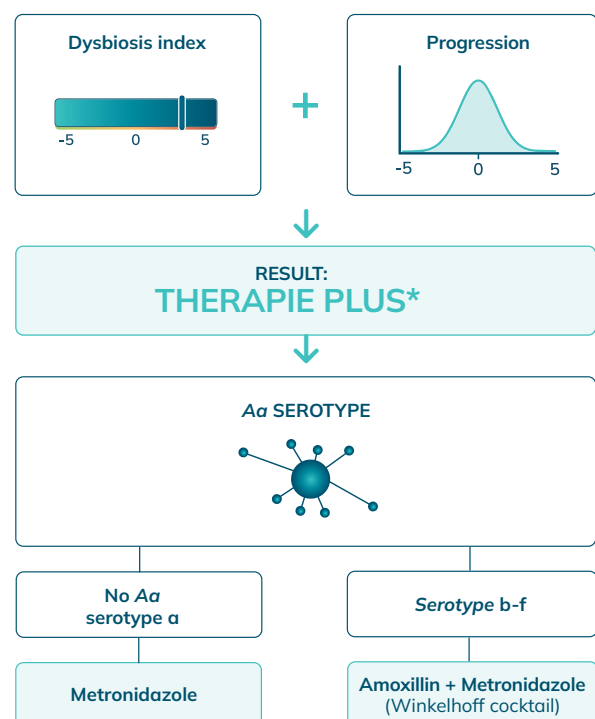
- Anti-infective therapy (AIT) measures **with adjuvants/antibiotics recommended**
- Depending on the clinical picture, surgical periodontal therapy **with adjuvants/antibiotics recommended**
- Highest SPT frequency

PadoBiom® ...

- Recommends systemic antibiotics only when dysbiosis and elevated progression are present
- Aligns the selection of active substances with the diagnosis and/or serotyping of *Aggregatibacter actinomycetemcomitans* while applying the minimum principle^[4,10]
- Considers the presence of resistance genes against the agents normally used within the framework of the PA therapy and consequently minimises the risk of therapy failure as well as of the spread of resistances

Presence of resistance genes







If the active substance associated with the respective resistance gene is used, there will be an increased risk of therapy failure. It is recommended to closely monitor the effectiveness.



How can the practitioner benefit from PadoBiom®?

 <p>Disease prevention through early detection in the reversible stage</p>	 <p>More comprehensive assessment of the clinical picture</p>	 <p>Treatment planning adapted to the progression</p>	 <p>Periodontitis prevention in clinically healthy patients</p>
 <p>Early detection of reinfections and monitoring of at-risk patients</p>	 <p>Added value for dental practises through more efficient scheduling</p>	 <p>Higher treatment quality and elevated patient satisfaction through better therapeutic success</p>	 <p>Demand-oriented UPT planning through adapted intervals</p>

How can the patient benefit from PadoBiom®?

 <p>Prevention of periodontitis and associated secondary diseases</p>	 <p>Cost savings by avoiding unnecessary therapies and/or the development of periodontal diseases</p>	 <p>Optimal, personalised treatment</p>
 <p>Faster and safer achievement of the treatment objective</p>	 <p>Increased patient motivation through better understanding of the disease</p>	 <p>Reduced risk of under-treatment and unnecessary antibiotics</p>

Literature

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