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Shared microbiome in gums and the lung in an outpatient population

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Summary *Objectives:* Whether periopathogenic bacteria occur in the lung and gums simultaneously and what impact periodontitis has is unknown.
Methods: In consecutive outpatients scheduled for bronchoscopies we performed a periodontal screening index. PCR to determine four periopathogens and seven less pathogenic species in both the periodontal pocket and bronchial protected specimen brush was used. Activated MMP8 in saliva and bronchial fluid was measured.
Results: Periopathogens were detectable in gums and in the bronchial protected specimen brush in 75 (80%) and 27 (30%) of the cases, respectively. The concentration of activated MMP 8 was above 40 ng/ml in the saliva and in the bronchial fluid sample in six and 31 subjects, respectively. Significant agreement between the periodontal and bronchial compartment was found in three out of the four periopathogens. Patients with periopathogens in the lung suffered from periodontitis more frequently ($p = 0.01$). In patients with periopathogens detectable in the lung the concentration of aMMP8 tends to be more frequently above 40 ng/ml in the bronchial fluid ($p = 0.09$).
Conclusions: Agreement between periodontal and bronchial microbiome is more distinct for periopathogens than for less pathogenic species. Periodontitis itself represents a risk factor for pulmonary colonization with certain periopathogens. Pulmonary colonization with periopathogens seems to be associated with increased local inflammation.

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Introduction

So far few studies have investigated the lower respiratory tract (LRT) microbiome, mainly in patients suffering from chronic obstructive pulmonary disease (COPD),[1–3] Asthma[4] or lung cancer.[5] Data from healthy individuals concerning LRT microbiome are particularly scares.[6]

Periodontitis is a highly prevalent human infection of the gingival tissue affecting to some extend the majority of adults.[7] Periodontitis is an inflammatory disorder of the supporting tissue of the teeth and is thought to be the consequence of a disturbance of the homeostatic balance between pathogenic microorganisms and the consecutive host response.[8] The microorganisms detected in the subgingival plaque of affected patients predominantly consist of Gram-negative fastidious anaerobic rods.[9]

Periodontal inflammation has been associated with several lung diseases such as COPD,[10–15] airflow limitation[16] or pneumonia.[17] In a pilot study we were able to detect periodontal pathogens in the lungs of lung transplant recipients in spite of the frequent use of antibiotics in this specific population.[18] To our knowledge no study addressed the association of the clinical and microbiological periodontal status with simultaneously taken LRT specimen so far.

Therefore, it was the aim of the current study to test the hypothesis that in an unselected population similar pattern of bacterial species can be detected in the periodontal pocket and the bronchial compartment. In addition, we intended to compare the clinical data (particularly periodontal health) of subjects with and without pathogenic species detectable in the bronchial compartment. Finally, we aimed to measure activated MMP8 in both saliva samples and bronchial samples. Activated MMP8 is a key mediator of tissue damage related to periodontitis[19] and has also been shown to be elevated in bronchoalveolar fluid of patients with bacterial pneumonia.[20]

Materials and methods

Subjects

This prospective study included adult outpatients attending the Clinic of Pulmonary Medicine of the Cantonal Hospital Aarau, Switzerland, scheduled for bronchoscopy. Exclusion criteria were use of antibiotics within the last six weeks, less than four remaining teeth or relevant coagulopathy. In each patient clinical data were accessible and body plethysmography (MasterScreen, Jaeger, Hoechberg, Germany) was performed within one month before bronchoscopy in a clinically stable situation. The study was approved by the local ethics committee (EK 2012/018) and written informed consent from the patients was obtained.

Intraoral sample collection and assessment of periodontal disease status

Before manipulating the gingiva, saliva samples were obtained to determine activated MMP-8[21] according to the manufacturer's instruction using a commercially available and validated chair-side test (aMMP-8 Rapid Test Kit, dentognostics GmbH, Jena, Germany). It was interpreted positive only when a clear blue line was visible, which implied activated MMP-8 concentration of more than 40 ng/ml.[22]

Bacterial samples were obtained by inserting sterile paper points in the line angle of the mesio-buccal aspects of each first molar (micro-IDent, heico Dent, Wolfhausen, Switzerland). If the molars were not present, the next tooth located mesially in each quadrant was selected for the sampling procedure at the respective site. Thereby, a standardized pooled sampling protocol was ensured without previous determination of the pocket depths.[18] All samples were then placed into a transport vial and the following bacteria were determined: *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), *Tannerella forsythia* (Tf), *Treponema denticola* (Td), *Prevotella intermedia* (Pi), *Parvimonas micra* (Pm), *Fusobacterium nucleatum* (Fn), *Campylobacter rectus* (Cr), *Eubacterium nodatum* (En), *Eikenella corrodens* (Ec) and *Capnocytophaga spec.* (Cs). A commercially available and validated[23] PCR test was used (micro-IDent test, Hain Lifescience, Nehren, Germany) for semi-quantitative analysis of the eleven bacteria: code 0: $<10^4$ bacteria detected, code 1: $\approx 10^4$ bacteria detected, code 2: $>10^4$ and $<10^5$ bacteria detected, code 3: $\geq 10^5$ and $<10^6$ bacteria detected and code 4: $\geq 10^6$ bacteria detected, except for Aa that is given in the same classes with ten times lower concentrations. DNA was extracted using a DNA extraction kit system (High Pure PCR Template Preparation Kit; Roche, Mannheim, Germany). The multiplex PCR amplification was performed in a reaction volume of 25 μl containing 2.5 μl of template DNA and 22.5 μl reaction mixture. For the subgingival specimens the cycling conditions were as follows: initial denaturation step at 95 °C for 5 min; 10 cycles at 95 °C for 30 s and at 58 °C for 2 min, followed by 20 cycles at 95 °C for 25 s, at 53 °C for 40 s and at 70 °C for 40 s; a final extension step was performed at 72 °C for 8 min. Due to the reduced bacterial burden of the lung specimens the PCR protocol was adjusted: 95 °C for 5 min; 10 cycles at 95 °C for 30 s and at 58 °C for 2 min, followed by 25 cycles at 95 °C for 25 s, at 53 °C for 40 s and at 70 °C for 40 s; a final extension step was performed at 72 °C for 8 min. In a subsequent reverse hybridization the biotinylated amplicons were denaturated and incubated with hybridization buffer and strips coated with specific probes and 2 control lines at 45 °C. Unspecifically bound DNA was removed and the hybridization products were visualized by addition of a streptavidin-conjugated alkaline phosphate system. The results were obtained on the basis of developed bands. As mentioned before the results were considered semi-quantitative in the case of the subgingival