Evidence for Graft Colonization with Periodontal Pathogens in Lung Transplant Recipients

A Pilot Study

Keywords: Periodontitis, allograft, bronchiolitis, DNA probes

Introduction

Bronchiolitis obliterans (BO) and its clinical correlate bronchiolitis obliterans syndrome (BOS) is the major cause of late graft dysfunction in lung transplant recipients affecting up to 50–60% of patients who survive after surgery (Glanville et al. 1987). Although BOS is thought to be mediated by an allo-immunologic injury, it is likely that non-allo-immunologic inflammatory conditions also play a role. Bacterial, viral and fungal infections may increase the risk of acute rejection (Girgis et al. 1996) and, in some centers, it has been shown that cytomegalovirus infection was associated with chronic rejection (Kroshus et al. 1997). However, despite high clinical suspicion, data dealing with the impact of infection as a risk factor for BOS is scarce.

Periodontitis, a local inflammation in the supporting tissues of the teeth, is thought to be the result of a disruption of the homeostatic balance between the host response and pathogenic microorganisms (Haffajee et al. 1991). Prevalence and proportions of periodontal bacteria vary among patients with periodontitis and control subjects (van Winkelhoff et al. 2002). Aggregatibacter actinomycetemcomitans (Aa), Tannerella forsythia (Tf), Porphyromonas gingivalis (Pg), and Treponema denticola (Td) with the aid of a hybridization technique. No or only one periodontal pathogen (solitarily Pg) was found in the gingival plaques of five of the eight patients (group A). In three patients, two or more periodontal pathogens were detectable in the gingival samples (group B). Whereas group A also had not more than one periodontal pathogen in the lungs, group B had more than one species in the lungs. In group B, all patients suffered from BOS, whereas in group A only one patient was affected.

This is the first evidence for the presence of periodontal pathogens in the lungs of lung transplant recipients. Further studies with larger cohorts are required to elucidate potential links between periodontal infection, pulmonary colonization, and rejection.

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supra- and subgingival plaque was collected and only one site appropriate sites. To meet these concerns, a standardized manipulation was avoided as consequently as possible. There-fore, it was not possible to chart the periodontal probing pocket depths before the sampling procedures in order to choose the sampling sites. In this pilot study we investigated the presence or absence of putative periodontopathogenic bacteria in the oral cavity as well as the lungs of 8 lung transplant recipients.

Materials and Methods

This pilot study was approved by the local ethics committee (EK 1164, 24.1.2005). All patients gave written informed consent.

In our program surveillance bronchoscopies are performed on a monthly basis during the first six months after transplantation. Indication bronchoscopies are performed whenever clinically indicated, usually due to deterioration of lung function. In the current study eight chronologically sequential lung transplant recipients who were off antibiotic treatment for at least two weeks and were scheduled for either routine surveillance bronchoscopy or clinically indicated bronchoscopy underwent broncho-alveolar lavage, transbronchial biopsies, and endobronchial biopsies. Samples were routinely sent for microbiology, differential cell counts and histology. Routine microbiological analyses for broncho-alveolar lavage samples were performed using blood agar and macconkey agar. Oral flora was defined as a typical mixed flora. Rejection of the lung was graded according to the International Society for Heart and Lung Transplantation (ISHLT) Working Formulation (Yousem et al. 1996). With this system perivascular as well as peribronchiolar lymphocyte infiltration (for acute rejection) and bronchial obliteration (for chronic rejection) are described in a standardized fashion. In all patients, lung function tests were performed prior to bronchoscopy. The term BOS was used in accordance with the new Working Formulation of the ISHLT (Estenne et al. 2002).

We wanted to circumvent any antibiotic treatment before the bronchoscopy was performed due to impaired bacteriologic yield of the transplanted lung under antibiotic treatment. On the other hand, due to the immunosuppressive regimen intermediate antibiotic prophylaxis before periodontal probing is standard care in our transplant program requiring subgingival manipulations. Hence, due to this severe status of immuno-suppression of these patients, any generalized gingival manipulation was avoided as consequently as possible. Therefore, it was not possible to chart the periodontal probing pocket depths before the sampling procedures in order to choose appropriate sites. To meet these concerns, a standardized simplified and minimal-invasive sampling protocol was used and supra- and subgingival plaque was collected and only one site was manipulated: A paper point was used to collect a combined supra- and subgingival plaque at the mesial surface of the first lower right molar (if not existing of the nearest available tooth mesially) for ten seconds and placed into a stabilizing guani-dinium buffer two hours before bronchoscopy.

The endobronchial biopsy sample was obtained from the upper lobe carina, one transbronchial biopsy sample and 1 ml of broncho-alveolar lavage fluid were also transferred to guanidinium buffer. Hybridization was done. The probes that were used are synthetic DNA oligonucleotide probes directed against the small subunit ribosomal RNA (SSU rRNA) of four of the most pathogenic periodontic bacteria namely Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Porphyromonas gingivalis, and Treponema denticola (IAI PadoTest4.5®). This test avoids amplification steps in order to facilitate exact quantification. Unlike polymerase chain reaction based approaches the hybridization technique used in this study allows a quantita-tive assessment of different bacterial species. Quantification is performed by using plasmid-cloned copies of the ribosomal RNA gene of each tested species. After hybridization with the probe, the samples are compared to the standard and the bacterial number is computed by assuming that 10,000 ribosomal RNA copies are equivalent to one bacterium. Furthermore, the total bacterial load of the sample was determined by a universal probe. The quantitative results of the DNA-rRNA hybridization are given as a proportion of the determined species compared to the total bacterial load.

From lung specimens, the sample with the highest number of pathogenic species detected was taken into consideration. Subjects were further evaluated depending on the number of these pathogens found in their gingival samples. Based on the latter findings, we arbitrarily defined a group A, where only one or none of the four marker bacteria was identified in the oral cavity, whereas patients were attributed to a group B, which was defined by the detection of two or more species in the oral sample.

Results

Eight bilaterally lung transplanted patients underwent fibre-optic bronchoscopy either for routine surveillance (5 patients, 63%) or for diagnostic work up (3 patients, 37%). The clinical characteristics of these patients and the results of the bacterial examination are shown in Table 1.

In five of the eight patients one or less of the four above mentioned periodontal pathogens was found in the gingival plaque (group A), the other patients had two or more of the periodontal pathogens (group B). In two of these three patients all bacteria were also detected in the transplanted lungs, whereas patients harboring only one of these periodontal bacteria in the gingival collecting area had also not more than one pathogen in the lungs.

Figure 1 shows the fraction of each of the four bacterial species (expressed as a percentage of the total bacterial load) of both groups in the gingiva and in the lungs, respectively.

In the pulmonary compartment of the three patients of group B the extent of bacterial colonization varied between broncho-alveolar lavage, endobronchial and transbronchial biopsies. In one patient, dental pathogen species were exclusively found in the transbronchial biopsy specimen. In the other two patients, the pathogens were found in three and two lung specimens, respectively.

The cytology of the broncho-alveolar lavage, the histology of the transbronchial biopsy and the sum of the dental patho-
gens of the two groups are shown in Table 2 (as medians with interquartile ranges, % for categoric items). By conventional culture techniques, representatives of an oral flora were found in the broncho-alveolar lavage in one patient of each group (Table 2).

One patient in group A fulfilled the clinical criteria for BOS, whereas every patient in group B at least suffered from BOS stage 0-p (Table 1).

Discussion

The risk of infection after lung transplantation is considerably higher as compared to other solid organ transplant recipients and more than 70% of infections involve the respiratory tract (Kramer et al. 1993). Whereas bacterial bronchopneumonia caused by Gram-negative species is a well described entity (Speich & van der Bij 2001), little is known about colonization or infection of the transplanted lung with the host’s dental flora. Using DNA probes directed against SSU rRNA of selected dental pathogenic species (Aggregatibacter actinomycetemcomitans, Tannerella forsythia, Porphyromonas gingivalis, and Treponema denticola) we identified these bacteria not only in the supra- and subgingival biofilm of lung transplant recipients but also in their lungs. Since RNA’s are quickly degraded once the bacteria are removed from their natural medium, these molecules are particularly suitable for detection of vital microorganisms. We have demonstrated that pathogenic gingival microflora can also be found in the transplanted lung. We feel...
that these findings are remarkable. With conventional culture techniques dental pathogens are often underestimated due to their anaerobic growth and not differentiated but only mentioned as “oral flora” and unappreciated as putative contaminants of bronchoalveolar lavage. In our study, only in one patient of each group were microbiological results reported as “oral flora”. In one of the three patients with lungs positive for more than one pathogen exclusively the transbronchial biopsy specimen was positive. This makes simple contamination unlikely and argues for colonization or infection. A further argument against contamination is the fact that in all but one case (Case No 2) endoscopy was performed through the nasal route. Most of all, the hybridization technique which we have used in the current study strongly argues against a simple contamination of the endoscope and other instruments.

All patients with \( \geq 2 \) pathogens in the gingival plaque (arbitrarily referred to as group B) met the criteria for BOS (at least stage 0-p) whereas only one of five patients of group A did. Because of the small number of patients no statistical comparison could be made but an association between pulmonary colonization or infection with pathogenic gingival flora and BOS is conceivable. Primary infection with consecutive BO or secondary colonization in a lung with preexistent BO are both possible scenarios. The latter is a well known phenomenon probably due to architectural damage and over-immunosuppression. Since periodontal disease and BO share some phenomenological aspects (i.e. inadequate inflammatory responses to different injuries and a specific individual genetic predisposition) the first hypothesis (pulmonary infection with dental pathogens leading to BO) needs further attention. Considering the significance of the clinical problem additional studies are highly warranted and as in many other fields an interdisciplinary approach seems ideal and promising.

One critical point of this study is the simplified oral detection method for bacteria. Under normal conditions, selection of the deepest pocket in each quadrant has been shown to be the most efficient method of sampling (Mombelli et al. 1991a). However, due to the severe status of immunosuppression of these patients, any additional gingival manipulation was avoided under stringent conditions as suggested by the internal clinical guidelines and the ethical protocol for safety reasons, despite a lack of evidence concerning lung transplant patients. Taking these concerns into consideration, only one site was scheduled for epi- and subgingival manipulation. In addition, given the potentially high number of samples needed to reliably detect the presence of P. gingivalis and other oral pathogens, the simple method of collecting supra- and subgingivally at only one specific site, presented an inferior, but adequate solution for screening the patient in a standardized manner. Mombelli and co-workers (1991a) found that in patients with moderate to advanced chronic periodontitis three distinct patterns of distribution and relative proportion of P. gingivalis were recognized. In one group of patients, the organism was not cultured. In a second group, few positive sites with low proportions of P. gingivalis were present. A third group of patients yielded high frequencies and proportions of P. gingivalis. Sampling of only one site could potentially lead to inadequate or underestimation of the bacteria found in the sample. However, it was shown that in the molar region, frequencies and mean proportions of P. gingivalis increased, which makes this area a potentially appropriate site (Mombelli et al. 1991b). Due to uneven and even cluster like distribution of positive samples in certain areas of the dentition, potential difficulties even remain when sampling more than one site. In this study, seven of the eight patients revealed P. gingivalis in the oral samples, which is a high frequency given the limitation of just one arbitrary sample site. On the other hand, one can speculate that a prevalence of \( \geq 2 \) bacteria in a single site may argue for an increased contamination potential of the whole oral cavity and therefore a higher overall oral and gingival contamination, which may more easily spread in the body. Therefore that arbitrary allocation to a “high” (\( \geq 2 \) bacteria) and “low” colonization group may be justified and explain our preliminary, but clinically relevant findings in the lung parenchyma.

Despite this particular shortcoming of this pilot and feasibility study, the presented findings and results are the first to show that pathogenic oral bacteria can be detected in the lung parenchyma of lung transplant recipients, irrespective of the arbitrary colonization type pattern in the oral cavity.

Other limitations of this study lay in its cross sectional nature and in the small number of study patients included. Furthermore, the frequent use of antibiotics in this population hampers diagnostic approaches to a certain degree. Since antibiotic treatments are necessary so frequently in lung transplant recipients a maximum time period off antibiotics of only two weeks could be applied.
However, if these results are confirmed in larger studies they may well lead to new diagnostic and treatment strategies for better allograft survival.

Zusammenfassung


Résumé

Le syndrome de la bronchiolite oblitérante (BOS) est une cause fréquente d’une dysfonction retard de la greffe chez les transplantés pulmonaires. 50 à 60% de survivants long terme après une transplantation pulmonaire sont touchés par cette complication. Le BOS est la principale cause de décès de ces patients. La pathogénie du BOS est encore mal comprise. Outre les conditions alloimmunologiques, la réaction inflammatoire non-alloimmunologique provenant d’une infection bactérienne, virale ou fongique a été récemment discutée comme facteur de risque d’une réaction de rejet aigu ou chronique. La cavité buccale représente un réservoir potentiel de bactéries pathogènes à cause de sa proximité anatomique. Peu d’informations existent sur la présence des pathogènes parodontaux dans les poumons, en particulier chez les patients greffés des poumons. C’est surtout dans cette population que les propriétés inflammatoires des ces bactéries peuvent potentiellement avoir une grande importance clinique. Cette étude pilote analyse pour la première fois la présence de germes pathogènes parodontaux dans les poches parodontales et les poumons de patients greffés des poumons.

Huit patients transplantés des poumons, qui n’avaient pas reçu d’antibiotiques depuis au moins deux semaines, ont subi un lavage broncho-alvéolaire ainsi qu’une biopsie trans- et endobronchique. Ces examens ont été réalisés au moyen d’une bronchoscopie flexible et sous légère séduction. En plus des examens de routine (microbiologie, histologie et cytologie différentielle), des prélèvements des poumons et de plaque sous-gingivale ont été analysés pour déterminer la présence des bactéries Aggregatibacter actinomycetemcomitans (Aa), Tannerella forsythia (Tf), Porphyromonas gingivalis (Pg) et Treponema denticola (Td) en utilisant une technique d’hybridation de l’ADN. Les prélèvements de plaque sous-gingivale ont été pris avec des pointes de papier sur la surface mésiale de la première molaire inférieure deux heures au maximum avant la bronchoscopie.

Aucun ou seulement un marqueur de germe parodontal (exclusivement Pg) a été trouvé dans les prélèvements sous-gingivaux chez cinq des huit patients (groupe A). Pour les trois autres patients, deux ou plusieurs bactéries ont été identifiées (groupe B). Dans les prélèvements pulmonaires, jamais plus d’une espèce bactérienne n’a été trouvée pour le groupe A, tandis que pour le groupe B au moins deux espèces bactériennes étaient identifiées. Dans ce dernier groupe, toutes les bactéries sous-gingivales étaient également détectées au niveau des prélèvements pulmonaires chez deux patients sur trois. Au moyen d’une culture bactérienne conventionnelle, la flore buccale était détectée dans le lavage broncho-alvéolaire d’un patient de chaque groupe. En outre, tous les patients du groupe B souffraient d’un BOS, alors que dans le groupe A seul un patient en était atteint.

Cette étude pilote montre pour la première fois l’évidence que des bactéries parodontales peuvent être trouvées dans les poumons des patients greffés des poumons. Des études supplémentaires avec des cohortes plus grandes sont néanmoins nécessaires pour démontrer les possibles relations entre maladies parodontales, infections pulmonaires et la réaction de rejet de la greffe pulmonaire. Si les données présentes sont confirmées au niveau d’une population plus grande de patients.
transplantés des poumons alors la relation pathogénique entre la présence de germes pathogènes dentaires et l’inflammation locale pulmonaire serait du plus grand intérêt. Des nouvelles et importantes stratégies à valeur diagnostique et thérapeutique pourraient potentiellement en résulter dans la lutte contre le BOS.

References


