Respiratory tract microbiome modifications after lung transplantation and its impact in CLAD

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EXTENDED ABSTRACT

Survival after lung transplantation is limited in large part due to the high incidence of chronic lung allograft dysfunction (CLAD). Infection is a recognized risk factor for the development of CLAD, and both acute infection and chronic lung allograft colonization with microorganisms increase the risk for CLAD. The aim of our study was to analyze respiratory tract microbiome modifications after lung transplantation, with a focus on its relationship with CLAD. Here we used 16S rRNA metabarcoding, observing specific microbiome profiles for both upper and lower respiratory tract and observing lower beta diversity between healthy subjects and patients with NO-CLAD as well as differentially abundant features associated with CLAD.

A. INTRODUCTION

Lung transplantation is an increasing procedure for end-stage lung disorders patients. Most of the mortality within the first year after the transplant is due to infections and development of chronic lung allograft dysfunction (CLAD) [1]. The microbiome is the set of microbial organisms (microbiota) including its genes and metabolites, which inhabit a certain niche. Several studies have recognized its essential role in the regulation of metabolic and physiological processes [2]. Two of the most widely used strategies to study the microbiome are: 16S ribosomal RNA amplicon sequencing, and whole-genome shotgun (WGS). The 16S subunit ribosomal gene has nine hypervariable regions that can be amplified with the use of universal PCR primers [3]. Sequencing of these amplicons and comparison to rRNA sequence databases allows assessing what taxa are present in a sample, and their relative abundances.

In the present work we described the microbiome of lung transplant candidates with different lung disorders and assessed the changes in their microbiome longitudinally after the transplant, and the impact of these changes on the outcome.

B. MATERIALS AND METHODS

This was a longitudinal study in which we analysed the microbiome in both the upper (nasopharyngeal swab (NP) and lower (Lung tissue, Bronchial swab and Bronchoalveolar lavage fluid (BALF)) respiratory tract from 68 lung transplanted (LT) patients the day of the transplant, when patients were discharged and 2-5 months and 12 months after the transplant. We also analysed NP swab samples from 10 healthy subjects. CLAD was assessed after two years of follow-up. DNA was extracted from the samples and the hyper-variable region (V4) of the 16S ribosomal RNA gene was amplified by standard PCR. Amplicons were purified and sequenced in barcoded pools using Ilumina MiSeq technology. Raw sequence reads were demultiplexed by using idemp. The resulting single-end reads were processed using Dada2 [4] pipeline obtaining an amplicon sequence variant table (ASV) to which taxonomy was assigned. Data was normalized by transforming the raw counts to centered log-ratios (clr). Alpha diversity, including both Shannon and Observed indices and beta diversity metrics such as the Aitchison distance, were calculated.

The effect of the clinical variables on the overall composition was evaluated by using the adonis test from the Vegan R package. Differential abundance at the day of the transplant and one year after the transplant was assessed by applying a linear model considering as fixed effect the CLAD variable and as possible source of batch effect the sequencing run. We also performed a differential analysis longitudinally to assess differential trajectories according to CLAD by using the clr of the top25 genus and the function permuspliner of the Splinectome [5] R package.

B. RESULTS AND DISCUSSION

Alpha diversity metrics were calculated for the different types of samples at the day of the transplant observing a significantly increased Observed index, but a decreased Shannon index (although not significantly) in NP swab samples compared to both lung tissue (Lung_Tissue) and bronchial swab samples (B_Swab) of the recipients. Therefore, our analyses suggested more richness but less evennes in upper respiratory tract (NP) as compared to the lower respiratory tract (Lung_Tissue and B_Swab) maybe due to their dynamism and higher influence of external factors.

The type of the sample also influenced the overall composition as suggested by beta diversity such as the Aitchison distance (Adonis test, p-value = 0.001). We detected clustering of the samples according to their source (Fig. 1), observing less distance between lower respiratory tract samples (lung tissue and bronchial swabs) and a composition derived mainly of Actinobacteria at the upper tract as compared to the lower tract in which we observed high presence of Proteobacteria, Firmicutes and Bacteroidetes phyla.

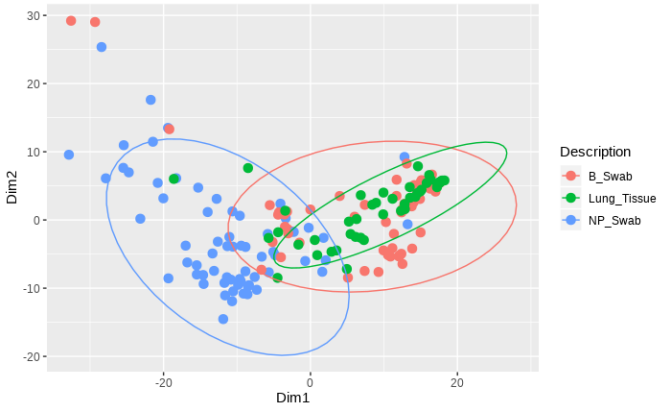

Fig. 1 Overall composition of the samples according to their source. Multidimensional (MDS) plot representing the Aitchison distance. Samples are colored according to the type of the sample: Lung tissue (Lung_Tissue), Bronchial swab (B_Swab) and Nasopharyngeal swab samples (NP_Swab).

One year after transplant a lower beta diversity, as indicated by the Aitchison distance, was observed between healthy subjects and patients with no CLAD. We confirmed a significant effect of the CLAD on the stratification of the samples according to the overall composition (Adonis test, p-value=0.02, R2 (explained variability)=0.049).

Our differential analysis detected 5 and 11 genus as differentially abundant according to CLAD in both the day of the transplant and one year after the transplant, respectively.

We also made a longitudinal follow up of the 25 top genus, detecting significant differences of 2 trajectories between CLAD and Non-CLAD patients: *Veillonella* (p=0.006) and *Neisseria* (p=0.048). Looking more in depth in the particular time points we observed a significant increase in the CLAD group of *Veillonella* 2-5 months after the transplant as compared to Non-CLAD and Healthy subjects (Fig.2).

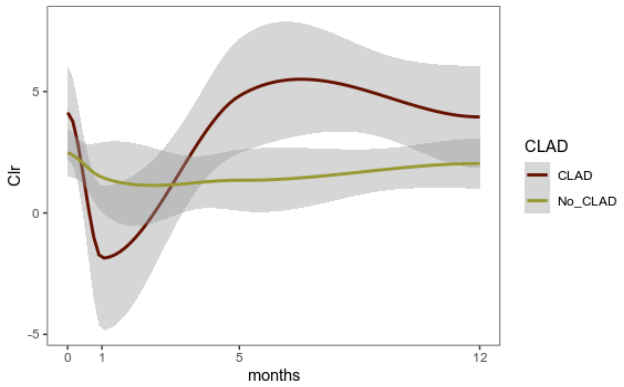

Fig. 2 Veillonella clr over the time distinguishing CLAD and Non-CLAD groups.

We assessed the influence of the donor on the outcome by computing the Aitchison distance between bronchial swab donor samples and distinguishing those distances within donors with patients that ended up with CLAD or not and also between donors of CLAD patients and donors of non-CLAD patients observing more distance within CLAD donors but not clear separation between CLAD and Non-CLAD donors. These suggested no influence of the donor on the outcome of the patient.

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Author biography

Olfat Khannous Lleiffe was born in Vic, Spain, in 1996. She received the BSc in Biotechnology from University of Vic in 2018 and the MSc in Bioinformatics for Health Sciences from University Pompeu Fabra (UPF) in 2020. She is currently doing a PhD in Biomedicine at Toni Gabaldon's Comparative Genomics group with the "Formación de profesorado universitario (FPU)" fellowship from the Spanish ministry of universities. She is also a teaching assistant in both University of Barcelona and ESCI UPF.