Salivary microbiome biomarkers predicting the response to albumin treatment

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ABSTRACT

It is already known that gut dysbiosis and bacterial translocation determine the development of decompensations in cirrhosis (1) and previous research has documented that bacteria isolated from circulating blood and other “sterile” compartments in patients with such condition are viable and this might be due to increased intestinal permeability (2). To date, albumin treatment is recommended in some specific clinical situations and only recently its immune modulatory role has been discovered in liver cirrhosis. Attempts to stratify patients for the use of albumin have failed and for this reason new tools to predict response to human albumin are urgently required.

The aim of this study is to investigate differences at inclusion between patients with decompensated cirrhosis responding (R, n=4) and not responding (NR, n=13) to albumin treatment in order to provide a list of potential salivary microbiome biomarkers for albumin response, through a quantitative metagenomics approach (Work Package 4 & Deliverable 4.7). Moreover, we found contrasts concerning the prevalence of species in saliva compartment focusing on the concept of tropism, the preferred niche of residence of microbial species, with NR carrying higher levels of species with gut and not determined (ND) tropism compared to R.

In conclusions, these observations might play an important role in deciphering the existing axis of interplay between saliva microbiome composition and albumin-related response in decompensation cirrhosis condition, that needs to be further investigated to depict the sophisticate mechanisms of such interaction.

METHODS AND RESULTS

The quantification of metagenomics species was carried out through the following pipeline:

- After extraction and quality control, DNA was sequenced using the Ion Torrent technology on Proton System;
- Generated reads were filtered for low-quality and human contaminants sequences prior mapping against the oral 8.4 million genes catalogue (3);
- Finally, the profile quantification and the taxonomical annotation of the Metagenomic Species Pangenome (MSP) was computed with the MSPminer software (4).

Despite no statistical differences between the two groups regarding the variation of whole microbial communities (β-diversity, PERMANOVA > 0.05), we found that NR had significantly higher microbial prevalence for species with gut and ND tropism compared to R group (**Figure 1**, X^2 pvalue < 0.05). Of note, the ND tropism gathers many species from disparate human niches, from skin or vagina, but also from environment, food and several opportunistic pathogens.

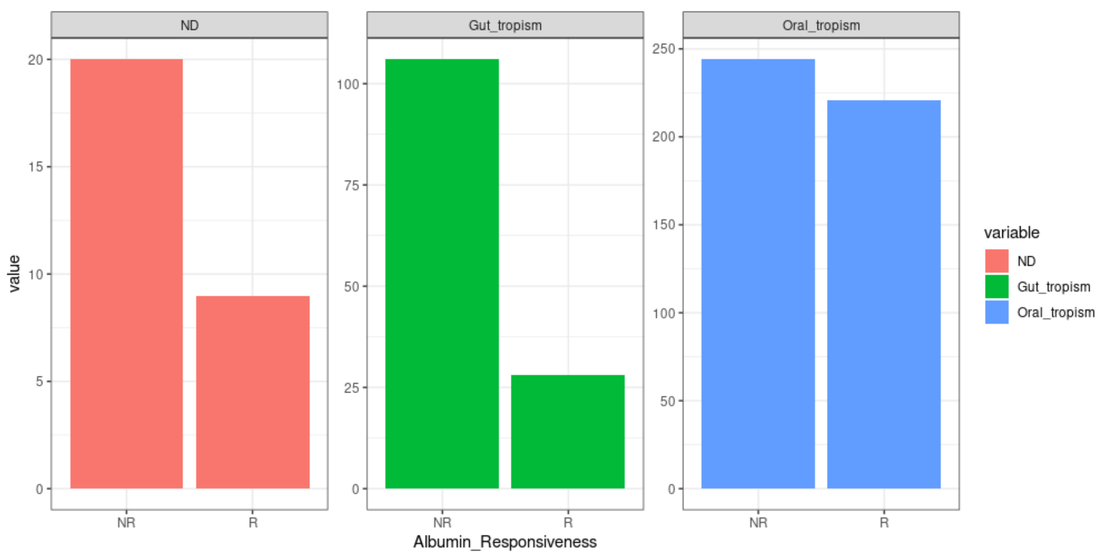

***Figure 1**. Microbial prevalence for different types of tropisms detected in the saliva compartment. R: Responders; NR: Not Responders; ND: Not determined (numerous opportunistic pathogens)*

In addition, by applying a Wilcoxon test and filtering for the Cliff delta effect size metric we identified several salivary species (n= 16) contrasting the R to NR group and the results thus obtained are summarized in the **Table I**. To further validate such findings, we performed the same analysis in recovery/not recovery groups independently of albumin intake. Hence, this explored approach led us identifying and removing some species from the list not directly associated to albumin response (labelled as “YES”). Interestingly, when we explored the correlation of the remaining MSP to score disease measure through the CLIF-C AD value, we observed that the relative abundance of *Lacticaseibacillus paracasei* (enriched in the NR) was positively correlated to the severity disease. Moreover, negative associations to such index were retrieved for *Prevotella nanceiensis* and *Rothia mucilaginosa* species (Spearman’s ρ = -0.51 and -0.4, respectively), both enriched in the R group and with oral tropism. Despite a limited number of samples, our findings highlighted potential biomarkers predicting the response to albumin treatment.

***Table I**. List of potential Albumin-related response biomarkers.*

Cliff Delta	Taxa	Status	MSP	Tropism	Recovery biomarker independently of albumin	Correlation with CLIF-C AD score	OR (95% CI, pvalue)
-0.66	*Prevotella nanceiensis*	R	msp 2228	Oral		-0.51 (p<0.05)	36.00 (2.40 – 1529.14, p = 0.021)
-0.66	*Unclas. Saccharimonadaceae*	R	msp 2357	Oral			
-0.63	*Gemella haemolisans*	R	msp 2916	Oral	YES		
-0.61	*Actinomices sp.* ICM47	R	msp 2227	Oral			
-0.61	*Corynebacterium durum*	R	msp 2239	Oral			
-0.56	*Gemella sanguinis*	R	msp 0974c	Oral	YES		
-0.56	*Rothia mucilaginosa*	R	msp 2240	Oral		-0.4 (p = 0.1)	16.50 (1.37 – 458.28, p = 0.043) (*)
-0.55	*Unclas. Pauljensenia*	R	msp 2892	Oral		-0.64 (p = 0.01)	
-0.53	*Oribacterium sinus*	R	msp 0446c	Oral	YES		
-0.50	*Eubacterium brachy*	R	msp 2451	Oral			
0.5	*Dialister pneumosintes*	NR	msp 1193	Oral			
0.5	*Streptococcus mutans*	NR	msp 1477	ND			
0.5	*Actinomices oris 2*	NR	msp 2300	Oral			
0.51	*Actinomices israelii*	NR	msp 2334	Oral			
0.52	*Prevotella maculosa*	NR	msp 2397	Oral			
0.53	*Lacticaseibacillus paracasei*	NR	msp 1089	ND		0.48 (p=0.05)	

R: Responders; NR: Not Responders; ND: Not determined OR: Odds Ratio.

CONCLUSIONS

Decompensated cirrhosis patients not responding to albumin treatment might have a pronounced gut dysbiosis condition associated to a greater intestinal permeability. This might be reflected into a larger prevalence of taxa with gut tropism and opportunistic pathogens into the oral microbiome compared to albumin responders.

A novel paradigm of the bidirectional exchange of host bacteria from different sites could be investigated (from oral to gut and *vice versa*). The extent of knowledge in such contest might open new encouraging paths for novel and potential microbiome-based biomarkers, involved in the responsiveness to albumin treatment for compromised cirrhosis and progression to ACLF, delivering guidance in a personalized medicine approach.

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