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Temporal Dynamics of the Plasma Microbiome in Recipients at Early Post-liver Transplantation

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Introduction

Immunosuppression during liver transplantation (LT) enables the prevention and treatment of organ rejection, but poses a risk for severe infectious diseases. Immune modulation and antimicrobials affect the blood microbiome. Thus, determining the impact of immunosuppression and antimicrobials on the microbiome may be important to understand immunocompetence, predict clinical adverse events after LT such as acute cellular rejection (ACR), and treat infectious diseases. In this study, we characterized the blood microbiome of LT recipients using next-generation sequencing (NGS). Moreover, NGS was evaluated as a detection tool of causative pathogens.

Conclusions

- *Anelloviridae* and *Enterobacteriaceae* abundance dynamically changed in the early phase of post-transplantation.
- The NMDS analysis revealed that antimicrobials are associated with the microbiome composition.
- *Enterobacteriaceae* abundance and high blood microbiome diversity may be an indicator for the development of ACR after LT.
- NGS is useful as a comprehensive diagnostic tool for pathogens in LT recipients.

Materials and Methods

Patients and Samples
Fifty-one LT recipients
Nagoya University Hospital
2016 to 2018

Plasma samples were collected according to the criteria below;
1) within a week after LT (46 samples)
2) 4 ± 1 weeks after LT (28 samples)
3) 8 ± 1 weeks after LT (29 samples)
4) within 2 days after a positive blood culture (16 samples)

Table. Characteristics of the patients

	Total (n = 51)
No. patients (pediatric/adult)	13/38
Age at LT [median (range)], pediatric adult	12 m (5–50 m) 50.5 y (18–66 y)
Sex (male/female)	19/32
Living donor/brain death donor	38/13
ABO-incompatible	8 (15.7%)

	n = 51
Underlying disease	
Biliary atresia	10 (19.6%)
Primary sclerosing cholangitis	9 (17.6%)
Primary biliary cholangitis	6 (11.8%)
Fulminant hepatic failure	6 (11.8%)
Others	20 (39.2%)
ACR	19 (37.3%)

Sample Preparation
- Extraction DNA from 140 µL of plasma
- Nextera XT Library Preparation Kit (illumina)
- Library quality assessment with Bioanalyser (Agilent) and Droplet digital PCR

Sequencing with NGS
- Hiseq (illumina) , 150bp Paired-end sequencing
- Target output was 10,000,000 reads/sample

Detection of Microorganism Genomes
- Metagenomic analyses were performed through the custom-made analysis pipeline PATHDET (PATHDET v1.0, https://pathdet.hgc.jp/)
- Relative abundance
- Simpson's diversity index and Shannon's diversity index

Results

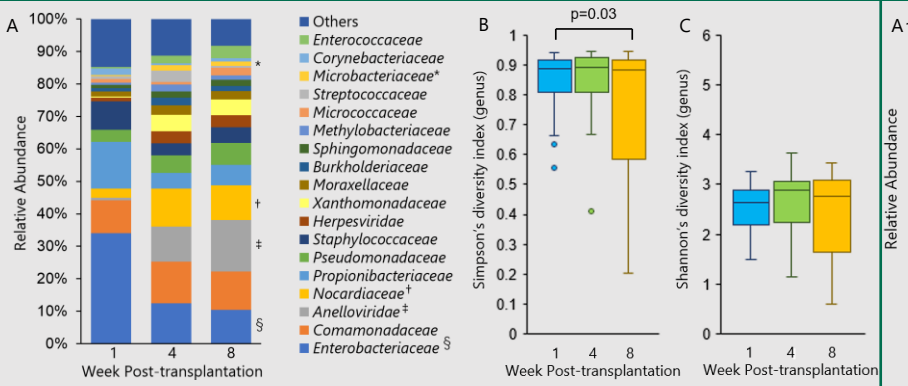

Figure 1.
(A) Change in the relative abundance of each microorganism at the family level
(B and C) Change in plasma microbiome diversity at the genus level

- *Enterobacteriaceae* (§) abundance decreased in samples collected at week 4 and 8 post-transplantation in comparison with samples collected at week 1 post-transplantation.
- Simpson's diversity index of samples collected at week 1 post-transplantation was significantly higher than that of samples collected at week 8 post-transplantation (0.85 ± 0.09 vs. 0.76 ± 0.23, respectively, p = 0.03).

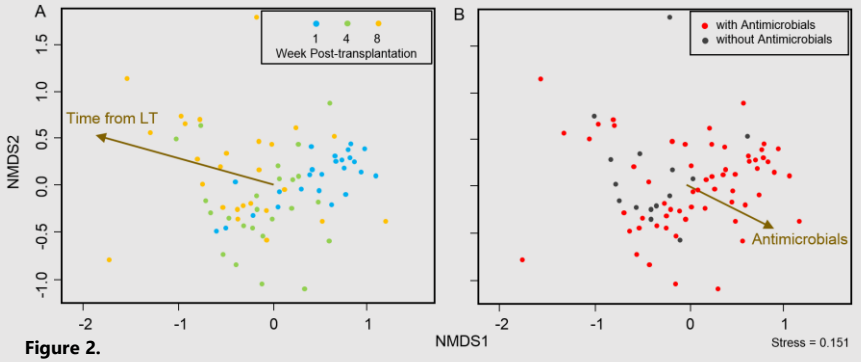

Figure 2.
Non-metric multidimensional scaling (NMDS) representation of plasma samples at each sampling point with envfit correlations
Arrows represent envfit correlations for (A) time from LT and (B) antimicrobials.

- The NMDS plot of the microbiome showed similarity of the microbiome among samples at the same time point and between samples with and without antimicrobials.
- Antifungals and antivirals were also associated with the microbiome (data not shown).

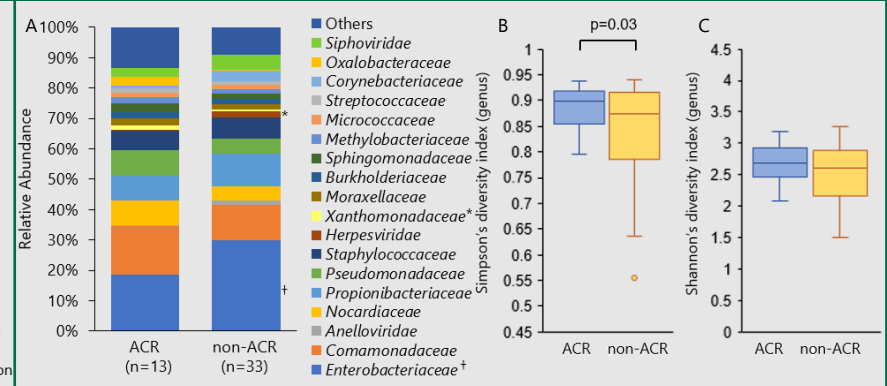

Figure 3.
(A) Comparison of the plasma microbiome at the family level in patients with and without ACR
(B and C) Comparison of plasma microbiome diversity at the genus level in patients with and without ACR

- *Enterobacteriaceae* (†) abundance was significantly decreased in the ACR group in comparison with the non-ACR group (p = 0.045).
- Simpson's diversity index in the ACR group were significantly higher than those in the non-ACR group (0.89 ± 0.04 vs. 0.84 ± 0.10, respectively, p = 0.03).

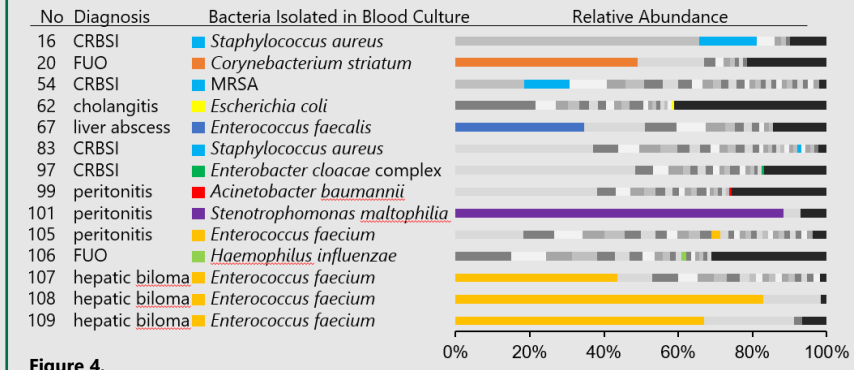

Figure 4.
Relative abundance of microorganisms at the species level in plasma from patients with positive blood cultures

- The same bacterial species isolated in blood culture were identified by NGS in 14 of 16 samples, and predominant pathogens in blood culture were identified in 8 samples.

CRBSI, catheter-related bloodstream infection; FUO, fever of undetermined origin; MRSA, methicillin-resistant *Staphylococcus aureus*.