Human Plasma Lipidome Variations in Active Tuberculosis

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Introduction

Tuberculosis (TB) is one of the oldest infectious diseases caused by the Gram-positive bacteria Mycobacterium tuberculosis (Mtb). Eradication of TB remains challenging with lengthy treatment durations.

As human pathogens continue to infect and acquire resistance to current anti-microbial drugs, recent focus has turned to potential host-directed therapeutics in search for novel treatment strategies (1).

Pathogens such as Mtb make use of host lipids as building blocks and influence the host cell physiology to enable their survival and replication (2). Hence, detection of changes in host lipidome during TB progression may enable novel and precise diagnostic tools as well as potentially providing insights into host-pathogen interactions

PROJECT AIM: To identify changes in the plasma lipidome of TB patients in comparison with that of healthy controls and latent individuals. Confirm the changes in longitudinal study with human plasma samples from active TB patients on established TB drug treatment over six months.

Experimental

235 human plasma samples were obtained from three different groups of volunteers: active TB patients (115), latent TB patients (45) and healthy controls (75). The ethnicity and gender ratios are represented in the pie charts. The age of the volunteers spans from 14 to 87 years old.

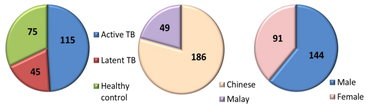

Figure 1: Pie charts illustrating the sample group, ethnicity and gender ratios

Experimental

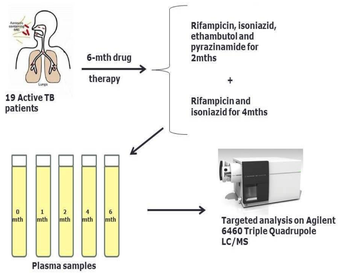

Figure 2: Longitudinal study where samples from 19 active TB patients who were on established TB drug treatment over six months were analysed

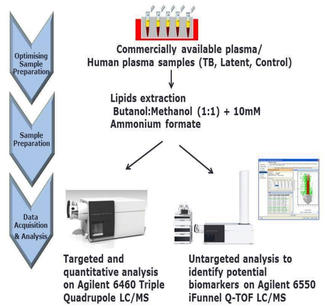

Figure 3: The research workflow

After lipid extraction optimization, analyses were performed by liquid chromatography mass spectrometry (LC/MS) using a targeted approach with the Agilent 1290 UHPLC 6460 Triple Quadrupole LC/MS mass spectrometer for the major classes of lipids. Potential biomarkers were identified.

Results and Discussion

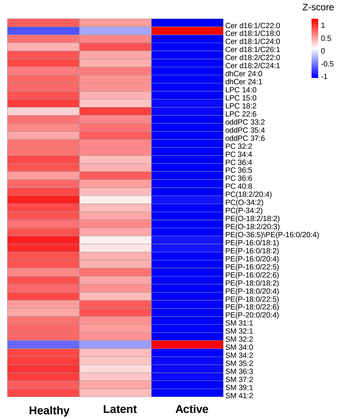

Figure 4: Heatmap of lipids showing significant differences between the sample groups

Using ANCOVA with Tukey HSD Post-hoc test and Linear model, the lipids that showed significant differences between active TB and healthy control/latent TB were illustrated in this heatmap.
47 out of ~285 measured lipids showed significant difference ($p < 0.05$).
These lipids were molecular species of ceramides (Cer), sphingomyelins (SM), plasmalogen and ether phosphatidylethanolamines (PE). The levels of these lipids were lower in plasma of active TB patients compared to non-active TB individuals.

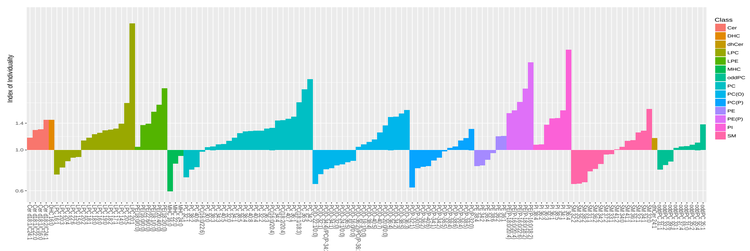

Figure 5: Index of individuality plot of lipid species measured in the longitudinal study

Data variation arises due to within-subject biological variation (CVI) and between-subject biological variation (CVG).
Index of individuality (II) is calculated as a ratio of CVI to CVG (3).
A low II value of less than 0.6 means that the analyte has marked individuality and a high II value of greater than 1.4 means that the analyte has little individuality.

Results and Discussion

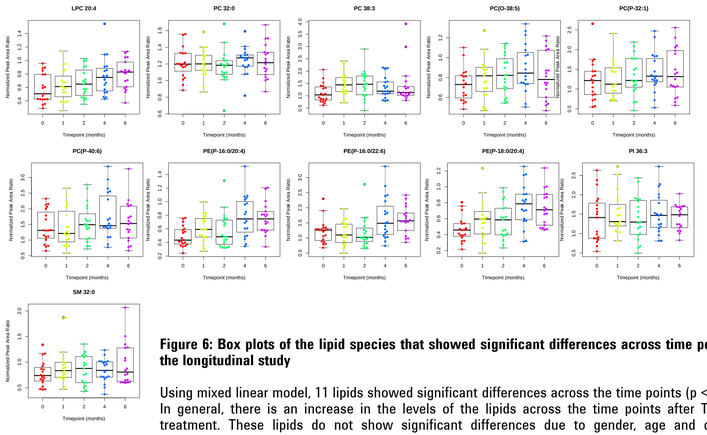

Figure 6: Box plots of the lipid species that showed significant differences across time points in the longitudinal study

Using mixed linear model, 11 lipids showed significant differences across the time points ($p < 0.05$). In general, there is an increase in the levels of the lipids across the time points after TB drug treatment. These lipids do not show significant differences due to gender, age and diabetic conditions.

References

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Acknowledgement

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Conclusions

PE(P-16:0/20:4), PE(P-16:0/22:6) and PE(P-18:0/20:4) are the lipids that showed significant difference between active TB patients and latent TB individuals/healthy controls (Figure 4) and at the same time, showed significant difference across the time points (Figure 5) with II values above 1.4 in the longitudinal study.

The identified plasmalogen PE species could be useful TB biomarkers if we can substantiate it with further studies measuring their natural variation in healthy controls.