2020

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Introduction

The complex biochemistry of human biological systems has been operationally broken down into a set of large molecular categories[1]. The metabolome, as it is termed, includes four classes of biologically active molecules including lipids[1]. Lipids are an integral structural component of cell membranes, play a significant role in energy storage, are involved in a variety of signaling pathways and intersect with other classes of compounds in the metabolome[1]. The lipidome has the ability to influence membrane mediated events[2]. Distinct lipid profiles have been identified in normal and pathologic conditions, and in response to specific therapeutic interventions. One such intervention is renal transplantation, the treatment of choice for End Stage Renal Disease (ESRD)[4]. In the United States, a shortage of suitable organ donors, and resultant organs, creates a marked supply and demand discrepancy leaving many patients on the waiting list for prolonged periods of time[4]. Current immunosuppression protocols result in a substantial decrease in T-cell mediated rejection at the cost of long term immunosuppression, with its resultant adverse effects including opportunistic infections[4], graft damage, and metabolic complications[5]. Additionally, these protocols do not have a significant effect on suppressing antibody mediated rejection (AMR), a major cause of graft loss[6]. Management of immunosuppression for individual patients is currently generalized based on protocols. Presently available biomarkers like donor-specific antibodies and degree of sensitization have proven to be inadequate to predict rejection[6]. Thus, there is an unmet need for biomarkers which could allow for better risk stratification to enhance the benefit and limit the risk of the immunosuppression therapy for individual patients.

Methods

The study population consisted of 16 consecutive patients who developed antibody-mediated rejection within 2 years of kidney transplant and 29 stable control (SC) patients who did not develop rejection at any point of post- transplant follow-up. Serial plasma samples are collected and stored at Time 1 (T1 - pre-transplant), Month 6 (T2) and Month 12(T3) and then yearly for all patient's post-transplant as part of an IRB approved biobank protocol at our institution. Indication biopsies were performed for acute allograft dysfunction defined as a rise in creatinine >20% above baseline, serum creatinine nadir ≥2.0 mg/dL post-transplant; or delayed graft function >21 days post-transplant. Surveillance biopsies were performed in patients with a positive flow-cytometric crossmatch (T or B >100 mean channel shifts) and/or presence of pre- formed donor-specific antibody [DSA; >5000 mean fluorescent intensity (MFI)] at 1 month and 6-month post-transplant. Biopsies were graded based upon the Banff criteria12. Patients with AMR were treated with 6-9 sessions of plasmapheresis with intravenous immunoglobulin (IVIG). Pre-transplant complement-dependent cytotoxicity (CDC) assays and three-color flow- cytometric cross matching (FCXM) were performed for all patients at the time of transplant. Donor-specific antibodies (DSA) were analyzed using the Luminex platform.

Results

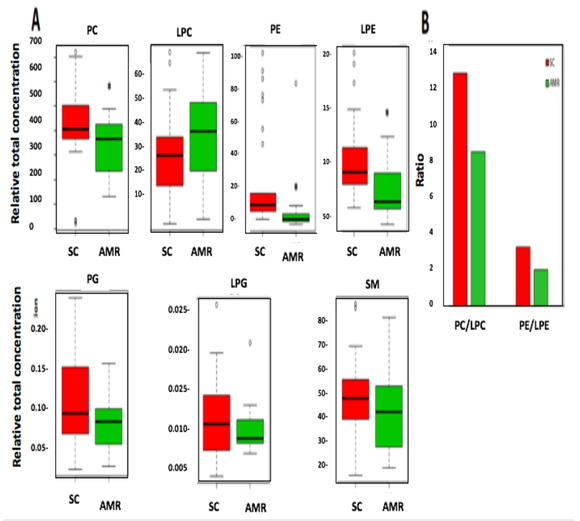

Figure 1: Significant differences are observed among phospholipids at T1 between SC and AMR. A) AMR group showed a significant lower concentration of PC, PE, and LPE. There was a trend towards higher levels of LPC in AMR. B) The ratio of PLs degradation to produce LPLs is an indication of PLA_2 activity with lower values suggesting higher activity. AMR group presented lower ratio for both more PC/LPC and PE/LPE. Suspected outliers are indicated by open circles in the box plot. Green rectangles represent AMR and the Red rectangles represent SC. * indicates significant differences with p<0.05.

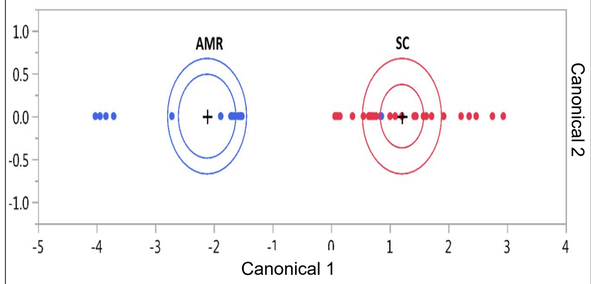

Figure 2: The RLDA model generated using 4 lipids and DSA demonstrate good separation between AMR and SC groups. The RLDA plot shows the clear separation of the patients in the two groups based on the Mahalanobis distance. This method determines whether the selected predictors can separate the distinct categories and reveals the presence of outliers in in the AMR and SC groups. Blue dot among the red dots indicates the one misclassified patient based in the predictive model. Internal ellipse indicates the 95% confidence region to contain the true mean of the group. External ellipse indicates the region estimated to contain 50% of group' population.

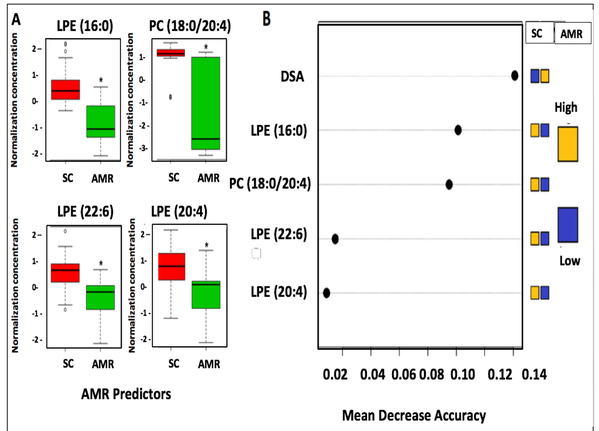

Figure 4: Lipids predict potential for AMR on the day of transplant. A) Box plot of normalized concentration shows that AMR group has lower concentration of the lipids predictors. Suspected outliers are represented as open circles that appear outside the whiskers. The validation method showed that the predict model can discriminate SC and AMR in the day of transplant with 0.022 OOB error. The mean Decrease Accuracy method shows that DSA is the more important predictor, followed by LPE (16:0) and PC (18:0/20:4) and they independently could be used as biomarkers. The analysis also reveals that when considering these predictors as biomarkers, the inclusion of LPE (20:4) and LPE (22:6) does not add any predictive power, and rather must be use to compose the RLDA model. * indicates significant differences with p<0.01.

Model	Predictors	R^2	Misclassification	AUC
Only lipids	PC (16:0/22:6) PC (18:0/20:4) PC (18:1/20:4) LPE (16:0) LPE (16:1) LPE (20:4) LPE (22:6)	0.63 (0.40 – 0.80)	8.9% (3.3 – 18.6)	0.95 (0.84 – 0.98)
Only clinical	cPRA DSA	0.36 (0.16 – 0.57)	15.9% (7.4 – 29.2)	0.80 (0.66 -0.90)
Merged models	PC (18:0/20:4) LPE (16:0) LPE (20:4) LPE (22:6) DSA	0.81 (0.49 – 0.96)	2.3% (0.1 – 12.1)	0.97 (0.88 – 1.00)

Table 1 – Predictors of Rejection at the Time of Transplant. Bootstrap validation with 95% Confidence intervals is included for RLDA estimates and area under the curve (AUC). cPRA: Calculated Panel Reactive Antibody; DSA: donor specific antibodies; GFR: Estimated glomerular filtration rate (mL/min/1.73m2); SC: Stable Controls; AMR: Antibody-mediated Rejection; *statistically significant.

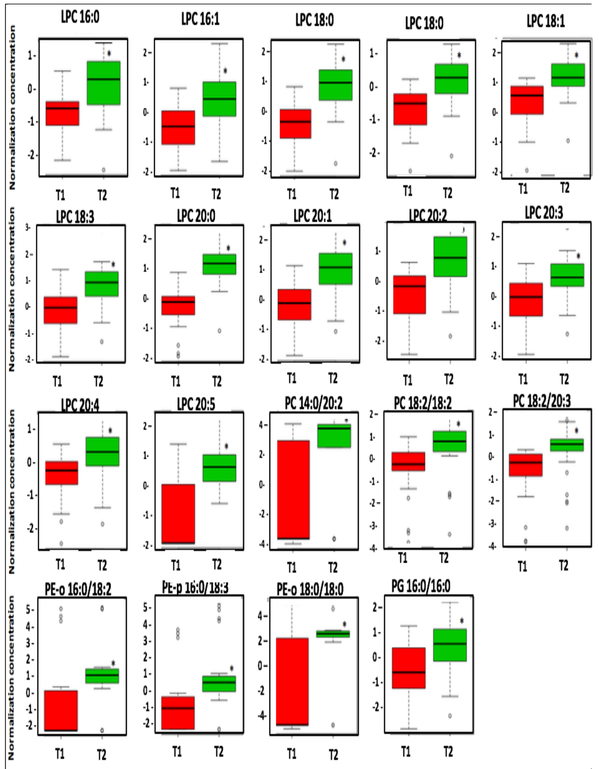

Figure 5: Specific lipids characterize the difference between T1 and T2 among SC patients.

Conclusions and Future Directions

Our study for the first time, identify the lipid differences pre-transplant and post-transplant. Additionally, we identify a pre transplant lipid signature that distinguish kidney transplant patients with favorable transplant outcomes (SC) and a major form of non-favorable transplant outcomes (AMR). We further demonstrate that unlike SC patients that demonstrate a dynamic longitudinal lipid change, AMR patients maintain a relatively unchanging lipid profile over time with respect to the measured lipids. Finally, we demonstrate for the first time the potential for risk stratification of kidney transplant patients on the day of transplant with respect to the potential for onset of AMR. Following validation in a larger cohort, these findings have the potential to alter the current paradigm of post-transplant monitoring and treatment of these patients via an evidence based risk stratification strategy and thereby vastly improving the success of kidney transplantation.

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