Systems Analysis of Influenza Vaccine Responses in Chronic Dialysis Patients Reveals Altered Metabolism at Baseline

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Aims

- ✓ **Compare innate and adaptive immune responses to influenza vaccine in pediatric chronic dialysis patients vs healthy children**
- ✓ **Evaluate baseline differences in immune function and metabolism between chronic dialysis patients and healthy children**

Background

Chronic kidney disease is a life-threatening condition that affects over 11,000 children in the United States. CKD can lead to end stage renal disease (ESRD) and dependence on chronic dialysis (CD) therapy in the form of peritoneal dialysis or hemodialysis. Metabolic disarray resulting from ESRD causes impairments in the innate and adaptive immune system, the mechanisms of which are incompletely understood. CD patients have higher rates of complications from many infections compared to healthy children, including influenza. Vaccination remains the best protection against influenza infection, but there is mixed evidence as to whether adult CD patients mount protective immune responses against influenza and no studies have assessed this question in pediatric populations. ***We hypothesized that CD patients exhibit dysregulated immunometabolism at baseline pre-vaccination and impaired innate and adaptive immune responses to influenza vaccination compared to healthy children (HC).***

Methods

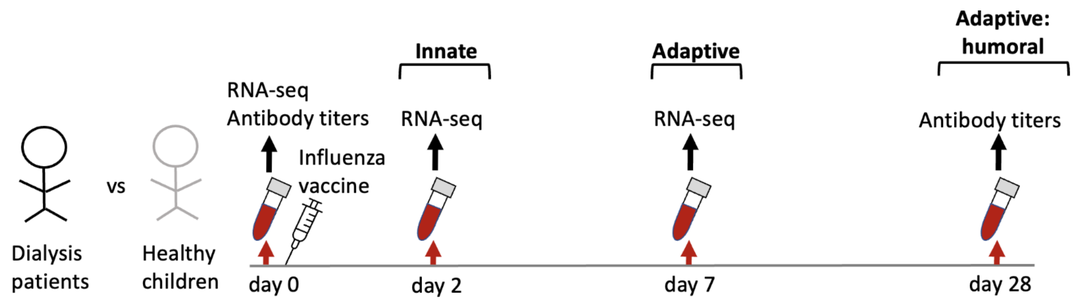

Figure 1: Schematic of the timeline of influenza vaccination and peripheral blood collection in CD and HC patients at days 0, 2, 7, and 28 post vaccination.

To test this hypothesis, we recruited 7 chronic dialysis patients and 14 healthy children (6 months to 18 years) during the fall and winter of 2023-2024. We immunized patients using the quadrivalent influenza vaccine and collected peripheral blood samples on days 0, 2, 7 and 28, measured from the time of vaccine administration (Figure 1). 2.5ml of peripheral blood was collected on days 0, 2, and 7 into PAXgene tubes that was used for total RNA isolation, library preparation, and RNA sequencing. An additional 1ml of peripheral blood was collected on days 0 and 28 for antibody titer measurement using a hemagglutination inhibition assay (HAI). The DESeq2 R package was used to calculate fold changes in gene expression at at post-vaccination timepoints compared to baseline and to analyze enrichment in previously described blood transcription modules (BTMs), calculated as normalized enrichment scores.

Post-vaccination increase in antibody titers is similar between chronic dialysis patients and healthy controls

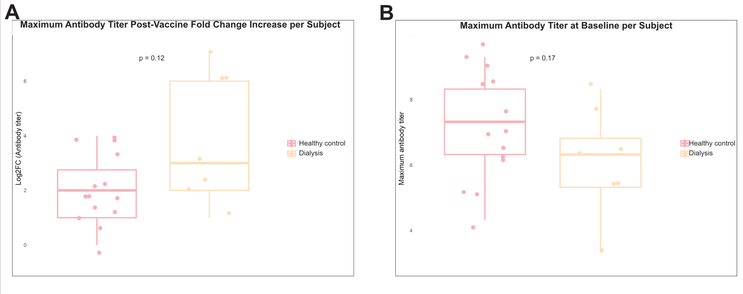

Figure 2: Antibody titers were measured at baseline day 0 and at day 28 post vaccination from each of 3 influenza strains matching vaccine strains. (A) Fold changes in antibody titers were measured for each subject for each strain, and the maximum fold change for each subject was plotted for healthy children and chronic dialysis patients. (B) The maximum antibody titer of all strains per subject at baseline was plotted. p-values were calculated using the Wilcoxon signed-rank test.

Post-vaccine responses in innate and adaptive immune system transcriptional pathways are similar in dialysis patients and healthy children

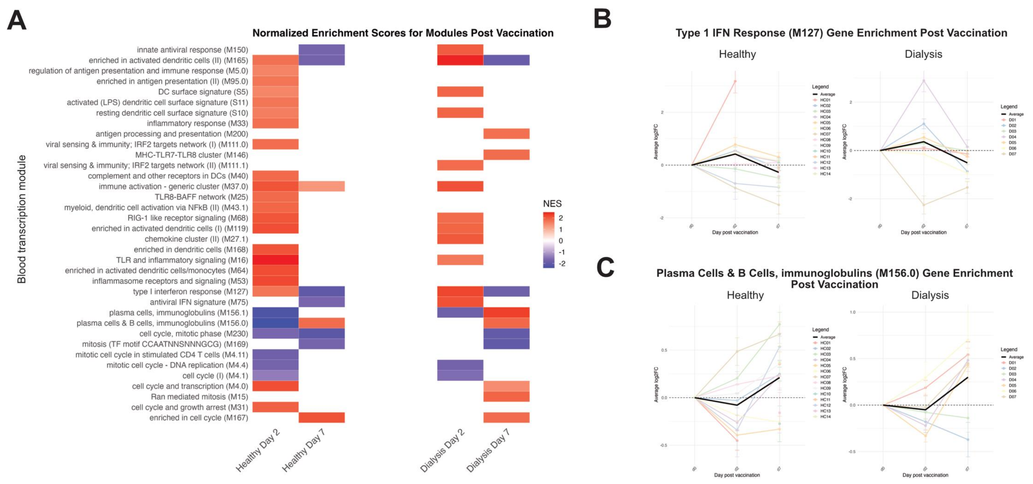

Figure 3: (A) Blood transcription modules (BTMs) involved in interferon responses, innate immunity, plasma cells, or cell cycle that are significantly enriched at days 2 and 7 in healthy children or dialysis patients. (B) and (C) Individual subject average log2FC and overall average log2FC +/- SD of genes in modules M127 or M156.0, respectively.

Metabolism-associated gene pathways are dysregulated in dialysis patients compared to healthy children at baseline

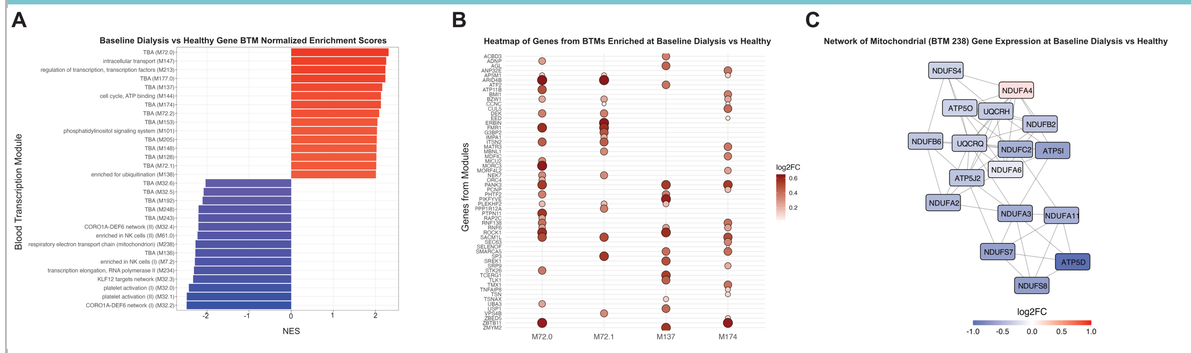

Figure 4: (A) Normalized enrichment scores for positively enriched or negatively enriched BTMs in dialysis patients compared to healthy children, padj < 0.05. (B) Heatmap of enrichment of common genes among 4 poorly described "to be announced" BTMs containing genes involved in metabolic regulation and protein turnover, including PANK3, SACM1L, RNF6, BZW1. (C) Network map of genes included in a respiratory electron transport chain BTM (238) that is negatively enriched in dialysis vs healthy at baseline with log2FC differences coloring nodes.

Future Directions

- ✓ Assess functional differences in metabolism between healthy children and dialysis patients by performing an extracellular flux assay (Seahorse assay) with isolated PMBCs
- ✓ Use experimental data from healthy children to define pediatric influenza blood transcription modules

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