ABSTRACT# 39

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Title: Pulmonary Immune Profiling in Critically Ill Children with Acute Lung Injury
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Background: Acute lung injury (ALI) can be triggered by direct (e.g. bronchiolitis) or indirect (e.g. ischemia-reperfusion injury after cardiopulmonary bypass (CPB)) etiologies. Previous studies have investigated biomarkers and endotypes in ALI, but the cellular signaling across multiple cell types is not known. We attempt to comprehensively describe the signaling processes of immune and parenchymal cells in pediatric ALI by using single cell RNA sequencing (scRNAseq) and multiomic approaches to identify key pathways leading to the development and resolution of pediatric ALI.

Methods: We collected serial deep tracheobronchial lavage samples from intubated patients less than 18 years old with ALI secondary to severe viral infection or CPB. Samples were immediately processed for scRNAseq using 10x Genomics technology. Cell clustering, cell-type annotation and visualization were then performed to generate a graphical representation of cell clusters. The most differentially expressed genes among each cell type were identified and compared between timepoints. Metabolomic analysis was performed on the same samples using mass spectrometry.

Results: Preliminary analysis shows that we can reliably obtain a heterogeneous mixture of pulmonary parenchymal and immune cells. scRNAseq analysis of 7 patients who underwent CPB generated the UMAP in Figure 1. Differential gene expression analysis of these alveolar macrophages show significant up-regulation of HIF1α pathway genes and genes involved in mitigating the effects of ROS. Similarly, analysis of 5 paired viral infection samples, including patients with RSV, coronavirus NL63/HKU1, and SARS-CoV-2, generated the UMAP in Figure 2. Epithelial cells in the intubated sample from a patient with SARS-CoV-2 showed increased expression of proinflammatory chemokine genes and antiviral interferon-stimulated genes compared to the extubated sample. Metabolomic analysis has demonstrated differences in the metabolic fingerprint (Figure 3) when comparing samples at the peak of ALI and near resolution of ALI.

Conclusions: Children with viral-induced or CPB-induced ALI have distinct cell populations, transcriptional activity, and metabolism that change over the course of disease. Differentially expressed genes between serial samples may reveal common regulatory pathways that can then be used to identify potential therapeutic targets. Biomarkers identified through metabolomic analysis of serial samples can be identified and may predict disease trajectory.

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(data provided below)
**Figure 1:** UMAP of 7 patients’ samples in the cardiopulmonary bypass cohort across all time points (before bypass, 2 hrs after bypass, and 24 hrs after bypass).

**Figure 2:** A) UMAP of 4 patients’ samples in the viral cohort across all time points B) UMAP highlighting one patient with RSV bronchiolitis and superimposed MSSA pneumonia at the time of intubation in comparison to a control patient without lung disease C) UMAP highlighting one patient with RSV bronchiolitis and superimposed MSSA pneumonia at the time of extubation in comparison to a control patient without lung disease.

**Figure 3:** Metabolomic analysis of deep tracheobronchial lavage samples obtained from a patient with viral-induced lung injury secondary to bronchiolitis A) at the time of intubation and B) 10 days later on the day of extubation.