2018 Critical Care Center Research Award Final Progress Report

Identification of Novel Biomarkers via Examination of miRNA Profiles of Pediatric Patients with ARDS

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Summary Statement

The overall goal of this project was to identify circulating plasma microRNA (miRNA) that may promote pathology in Acute Respiratory Distress Syndrome (ARDS) in pediatric patients. We selected 72 plasma samples (61 different patients with 11 technical replicates) from pediatric patients diagnosed with ARDS. Due to a limited number of samples from patients who did not survive, we attempted to match two survivors were matched to every non-survivor. The samples were otherwise matched for mechanism of lung injury, gender and age within 1 month for ages below one year, within 6 months for ages 1-2 years, within 12 months for patients aged 5-10 years and within 24 months for ages over 11 years. This sample cohort consisted of 47 survivors 25 non-survivors. Nanostring nCounter Human miRv3 Assay was used as the platform for miRNA profiling. Differential expression analysis was performed using DESeq2.

Our first aim was to examine whether there is differential miRNA expression through miRNA profiling in pediatric patients with ARDS who survive versus those who do not. With the false discovery rate set at 10%, we found six miRNA which were significantly differentially-expressed. miR-223-3p, miR-106a-5p + miR-17-5p, miR-126-3p, miR-127-3p, miR-191-5p, miR-29a-3p were differentially expressed between survivors and non-survivors after Benjamini-Hochberg correction. Of the six miRNA, miR-223-3p was noted to be the best predictor. In our particular cohort, the Pediatric Risk of Mortality Score (PRISM) was not a good predictor of mortality, with an AUROC of 0.61 (95% CI 0.45-0.76). When this six miRNA panel (samples drawn on Day 1 of PICU admission), AUROC for mortality was 0.81 (95% CI 0.72-0.91). In a model containing both the six miRNA panel and PRISM score, the AUROC was 0.83 (95% CI 0.74-0.92). Adding in age and gender marginally improved the prediction model, with an AUROC of 0.87 (95%CI 0.79-0.95).

Our second aim was to examine whether or not differential miRNA expression in pediatric patients with ARDS who have pulmonary triggers for ARDS versus those who have extra-pulmonary triggers for ARDS is the same in survivors and non-survivors. For this outcome, we found that miR-574-5p was differentially-expressed between pediatric patients with pulmonary vs. non-pulmonary causes of ARDS after controlling for survival status and gender. Expression of miR-574-5p in patients with non-pulmonary causes of ARDS was four times higher than those with pulmonary causes of ARDS. Interestingly, one other publication in literature has noted an association of increased miR-574-3p expression with mortality in sepsis.

The third aim was to validate the clinical significance of differentially-expressed miRNA by identifying their potential target genes. The six miRNA found to be differentially expressed were confirmed using quantitative real-time PCR. miR-223-3p appeared to be the most promising candidate for further study. In a search using databases of predicted and experimentally confirmed targets of miR-223-3p, MMP-2 and MMP-9 were uncovered. These MMPs belong to a class of zinc-dependent endopeptidases that are best known for their involvement in the degradation of the extracellular matrix39 and are regulators of the extracellular tissue signaling network.40 They have widely been cited in literature for their involvement in ARDS.41-43 However, the role of miR-223-3p on MMPs in ARDS has been less well studied. The next step will be to evaluate the role of miR-223-3p on MMP2 and MMP9 in a murine model of ARDS.

Positive and Negative Results Considered Significant

- **Six miRNA found to be differentially expressed between pediatric survivors and non-survivors of ARDS.**
  - miR-223-3p
  - miR-106a-5p + miR-17-5p
  - miR-126-3p
  - miR-127-3p
  - miR-191-5p
  - miR-29-3p
- **One miRNA differentially expressed between pediatric patients with pulmonary vs. non-pulmonary causes of ARDS**
  - miR-574-3p

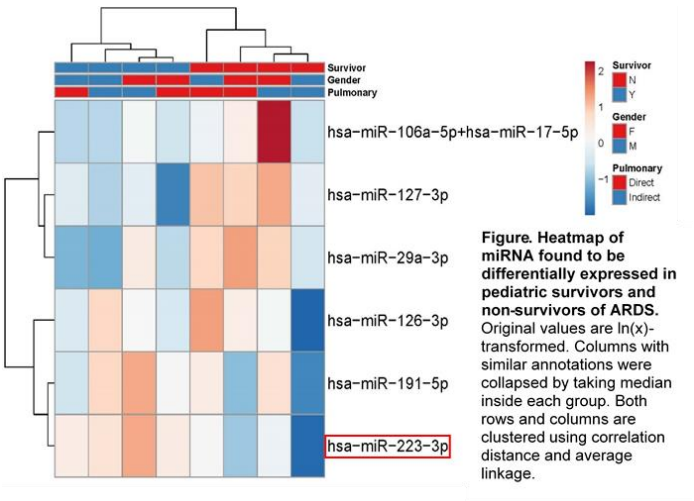

Figure. Heatmap of miRNA found to be differentially expressed in pediatric survivors and non-survivors of ARDS. Original values are ln(x)-transformed. Columns with similar annotations were collapsed by taking median inside each group. Both rows and columns are clustered using correlation distance and average linkage.

- **In our particular cohort, the Pediatric Risk of Mortality Score (PRISM) was not a good predictor of mortality; The six miRNA panel was a better predictor of mortality.**
  - PRISM Score – AUROC of 0.61 (95% CI 0.45-0.76)
  - Six miRNA panel – AUROC 0.81 (95% CI 0.72-0.91)
  - Six miRNA panel + PRISM Score – AUROC 0.83 (95% CI 0.74-0.92)
  - Six miRNA panel + PRISM Score + Age + Gender – AUROC of 0.87 (95%CI 0.79-0.95)

Future Plans

The results from Aim 1 of this project were submitted as an abstract to the American Society of Anesthesiologists Annual Meeting in October 2019. A review manuscript on the role of miRNA in ARDS is currently being drafted. The results obtained using funding from this grant have formed the preliminary data that I needed in order to apply for KL2 funding. I also plan on applying to the Foundation for Anesthesia Education and Research (FAER) Mentored Research Grant this August, after further development of an animal model for testing new hypotheses.