**Title of the Study: Biomarkers of Lung Injury**

**NCT Number: Not Assigned Yet**

**Date of Submission: 30th May 2025**

**Date of Revision: 6th Aug 2025**

**STUDY PROTOCOL:**

Peripheral blood mononuclear cells (PBMC) will be isolated from human blood (obtained from ARDS subjects recruited for this study) using a Ficoll gradient (Ficoll-Paque Premium). The isolated PBMCs are the immune cells that will be used in this study. These immune cells will be studied in vitro. PBMC will be checked for activation (by monitoring ROS production). At the same time, clinical parameters of ARDS (ARDS severity and ARDS score that are routinely monitored daily for each patient) will be accessed and correlation analysis carried out to check if immune cell activation matches the ARDS severity. In separate in vitro experiments, the regulation of PBMC activation will be monitored by addition of novel agent PIP-2 to the immune cell culture.

**ANALYSIS PLAN:**

The association between the ROS, cytokine, oxidative stress levels with clinical parameters of ARDS (i.ARDS severity measures and ii. ARDS severity score) will be studied in two steps. As outcome variables associated with clinical parameters are both discrete and continuous, we will utilize multiple procedures to develop and test discrete, binary models and continuous models. In the first step, point biserial correlation will be used for dichotomous ARDS clinical parameters and Pearsons correlation coefficients or Spearman's rank correlation coefficients will be calculated for continuous outcome variables. Second, using multivariable linear and logistic regression procedures, several discrete, binary class prediction models will be developed to stratify the efficacy of PIP-2 on ARDS patients. Briefly, for each of the class prediction models, patients will be assigned to class 0, 1, or 2 based on ARDS severity (moderate, severe) and a weighted voting classification algorithm will be utilized in which each clinical parameter will contribute a vote, which will be summed to create a winning class. Different combinations of clinical parameters will be evaluated to determine combinations capable of stratifyingPIP-2 efficacy (low, moderate, high, very high responders to PIP-2) in immune cell samples from ARDS patients.