Clinical Monitoring of Acute Respiratory Distress Syndrome Using Molecular Techniques and High-Throughput Sequencing

Claire Wingfield, Sara Hey, Joseph Natvig, Dayjah Whyte, Abigail Porras, and Inimary Toby, PhD
Department of Biology, University of Dallas, Irving, Texas

ABSTRACT

ARDS, Acute Respiratory Distress Syndrome, is a lung condition known for the collection of edema protein fluid in the lung's air sacs which subjects patients into a hypoxic state. Within the US, approximately 190,600 patients are affected by ARDS every year with a mortality rate of ~45%. Recently, ARDS has been shown to occur secondarily during SARS-CoV-2 infections. Although severe ARDS is considered a fatal disease, people who survive ARDS and recover from SARS-CoV-2 may have lasting pulmonary scarring. The experiment hypothesis was that ARDS patients exhibit immune specific T-cell populations and that these sequences could be identified using high-throughput sequencing strategies. To do this, we performed cellular experiments and utilized machine learning methods to assess viability of single cell ARDS patient samples. Next, we extracted genomic DNA from these samples followed by high-throughput sequencing using the Human T-cell receptor β (TCRβ) ImmunoSeq assay. Results from the cellular viability experiments showed that the range of viable cells across samples averaged between $1x10^6$ and $1x10^7$ cells per ul. Results from the immune sequencing experiments demonstrated that specific TCRβ genes could be tracked and utilized as markers to compare across samples. These genes could also be further quantified using their relative frequency which averaged from 4.12% to 33.98%. A total of 3,626 complementary determining region 3 (CDR3) sequences were extracted across samples for comparison. These findings represent an important crucial step towards the application of immune sequencing for clinical monitoring and risk stratification of ARDS patients.

BACKGROUND

- The first case of Acute Respiratory Distress Syndrome (ARDS) was identified in 1967.
- ARDS is an acute disorder that begins within 7 days of the inciting event. It is characterized by infiltrates in both lungs and severe progressive hypoxemia, as well as damage to the capillary endothelium and alveolar epithelium.
- Endothelial damage leads to the movement of fluid and macromolecules into interstitial and pulmonary air spaces, causing pulmonary edema.

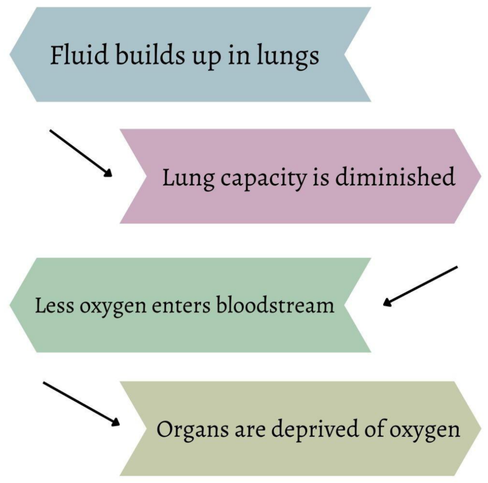

Figure 1: The proposed mechanism of ARDS pathogenesis and the downstream physiological effects.

METHODS

Figure 2a

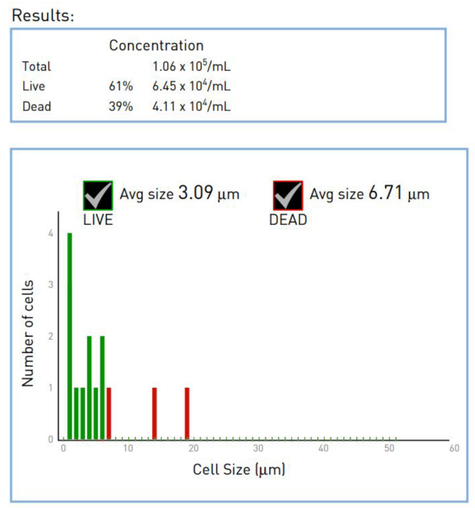

Figure 2b

Figure 2a: Overview of the ImmunoSEQ software. immunoSEQ is a multiplex PCR-based assay that uses high-throughput sequencing to measure the CDR3 variable chain of T-cell and B-cell receptors. This allows for the characterization of the diversity of the immune response and statistical data to help identify framework of disease and inform effective treatment.

Figure 2b: Illustration of the cell viability quantification process. The countess instrument is a machine learning based device utilized in this study. It quantifies and qualifies cell colonies based on the presence of fluorescence-tagged cells using laser beams. The light absorbed by the fluorescence-tagged cells is then reported to the main computer in the device and displays the percentage of cell viability and cell density counts using machine learning.

RESULTS

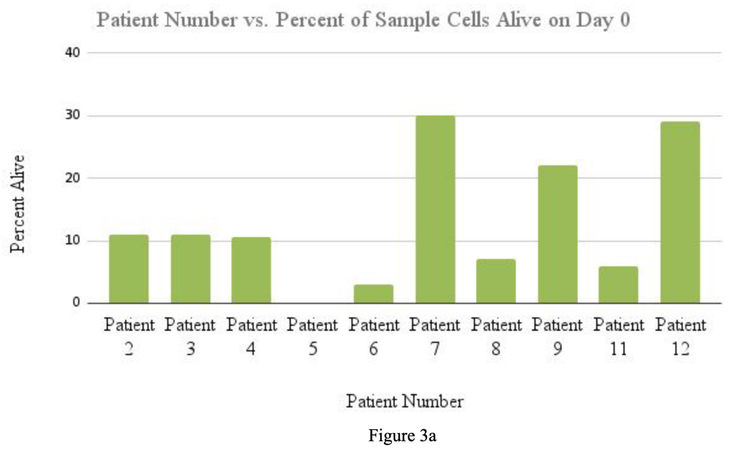

Figure 3a

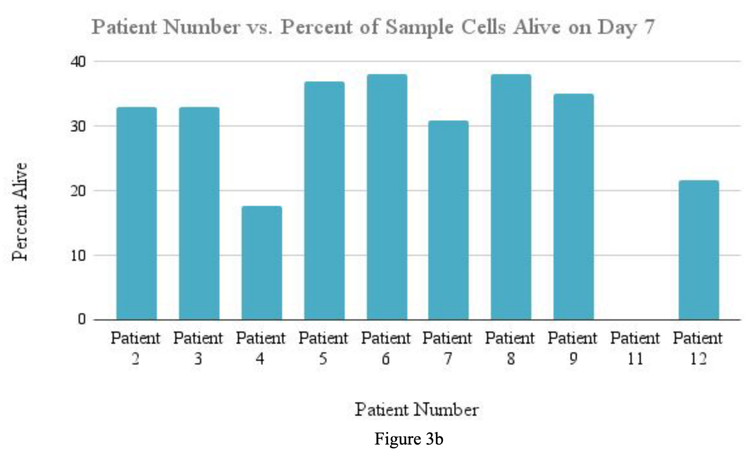

Figure 3b

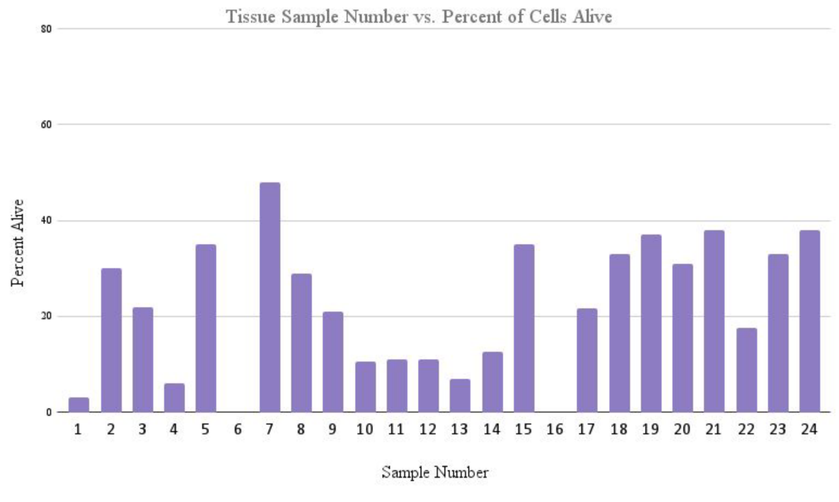

Figure 3c

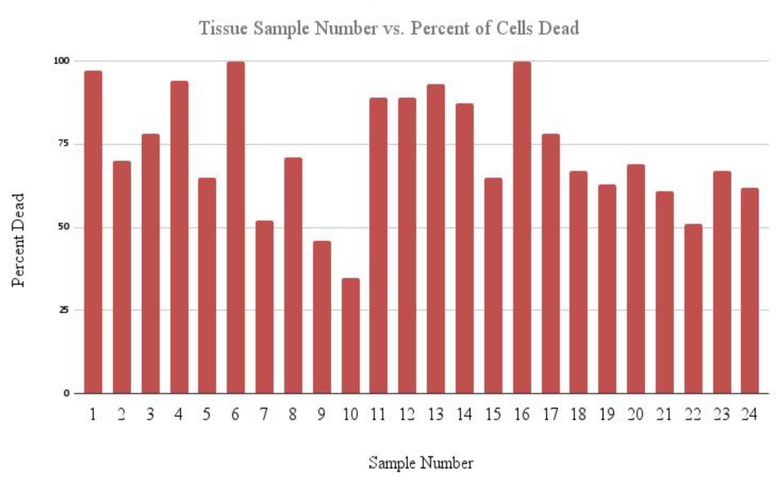

Figure 3d

Figures 3a-3d: Patient or Sample Number Versus Percent Viability graphs. Figure 3a depicts the percentage of live cells in each patient sample taken on Day 0. Figure 3b depicts the percentage of dead cells in each sample taken from the same patients on Day 7. Interestingly, there are overall more live cells in the samples from Day 7 than from Day 0. Figure 3c depicts the percentage of live cells from Day 0 and Day 7 samples combined, while the corresponding Figure 3d demonstrates the percentage of dead cells with the same parameters.

RESULTS

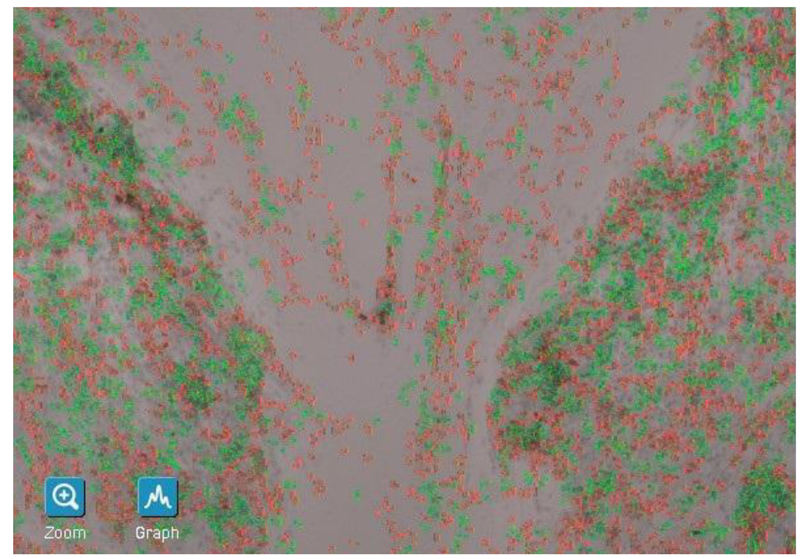

Figure 4: Representative image showing the parameters assessed by the Countess II for our samples. Cell regions outlined in green indicate the presence of live cells while cell regions outlined in red indicate the presence of dead cells.

CONCLUSIONS

- High-throughput sequencing multiplex PCR-based assays and artificial intelligence are both viable techniques for assessing the viability of cell samples.
- The statistical data derived from these techniques can be utilized to track immune specific T-cell populations in ARDS patients, facilitating a greater understanding of the disease and its potential treatment.
- The experimental techniques employed in this project can be utilized in the analysis of other human diseases.

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