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JURA
Journal of Undergraduate Research in Alberta • Volume 5 • 2015

Predicting the Mortality of Pneumonia-Induced Direct Lung Injury Using Serum Metabolomics

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Acute respiratory distress syndrome (ARDS) has significant impact on the morbidity and mortality in the intensive care unit. It is estimated that there are 86.2 cases of ARDS per 100,000 people. This disease has an average mortality rate of approximately 38.5%[1]. Severe ARDS has a 49% mortality rate, with mortality increasing with age[1]. Currently, there are no viable specific treatment options in the intensive care unit for ARDS other than non-specific supportive care. As such, the purpose of this study was to use serum metabolomics to further characterize the mortality of patients with pneumonia-induced ARDS in the intensive care units at the Peter Lougheed Center, and the Foothills Medical Centre in Calgary, Alberta, Canada. It was hypothesized that using serum metabolomics would more accurately predict outcome in pneumonia-induced ARDS when compared to current used predictive indexes such as APACHE II or lung injury score.

The diagnostic criteria for ARDS include the acute onset of non-cardiogenic pulmonary edema, hypoxia, and presence of bilateral pulmonary infiltrates)[2]. Moreover, the state of patient hypoxemia and the fraction of inspired oxygen can be used to calculate a ratio of partial pressure of arterial oxygen to the fraction of inspired oxygen ratio (PaO_2/FiO_2 ratio). The ratio varies decreasing from in the 500s to less than 100, with a healthier lung having higher PaO_2/FiO_2 ratio. The specific PaO_2/FiO_2 ratio were used to clinically differentiate the severity of lung injury. The Berlin definition of ARDS is used to define the cohorts in this study[3]. Mild, moderate, and severe ARDS are described with PaO_2/FiO_2 ratios of 201-300, 101-200, and $\leq$ 100, respectively.

Metabolomics analysis is a systems-biology approach used to identify metabolites of organisms such as humans and plants[4]. This can be done by analyzing multiple biofluids (e.g. serum, plasma, urine) and utilizes spectroscopic methods such as proton nuclear magnetic resonance (^{1}H-NMR), gas chromatography mass spectrometry (GC-MS), and liquid chromatography mass spectrometry (LC-MS)[5]. In this study, we used ^{1}H-NMR.

Metabolomic profiling was completed to see if metabolomic fingerprints (biomarkers) can predict ICU outcome (death) in pneumonia-induced acute respiratory distress syndrome (ARDS).

Methods

Patient Selection

The selected population in the study consisted of adults admitted to the intensive care units at the

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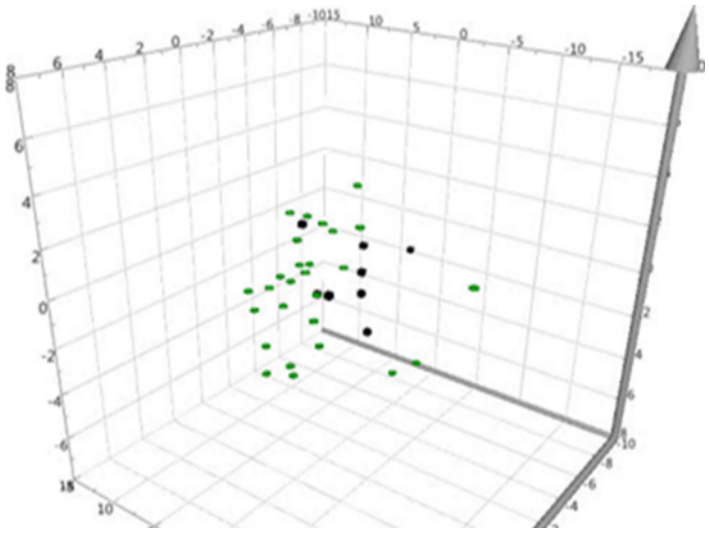

Figure 1:
PCA model of mild and severe pneumonia-induced ARDS. Model is based on 28 day mortality, and shows significant clustering between alive and dead outcomes. (27 alive, 8 dead, R^2Y=0.562, x-axis: prediction component). Green dots (alive), black dots (dead) at 28 days.

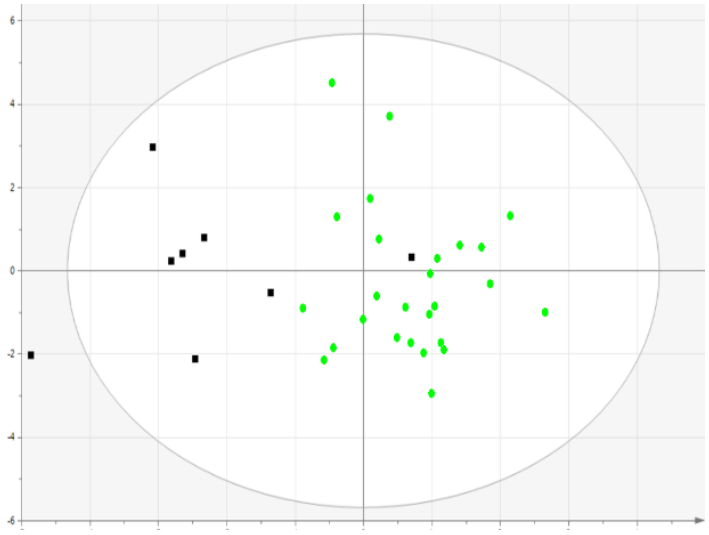

Figure 2:
OPLS-DA plot of mild and severe pneumonia-induced ARDS. Model shows distinct separation between alive and dead outcomes (27 alive, 8 dead, R^2Y=0.61, Q^2Y=0.458, p=0.004, x-axis: prediction component, y-axis: orthogonal component). Green dots (alive), black dots (dead) at 28 days.

Foothills Medical Centre and Peter Lougheed Centre in Calgary, Alberta, Canada with pneumonia-induced direct lung injury between 2009 and 2014. Blood was drawn from these patients on day 1 of ICU stay. Of the 868 adult patients enrolled in the Critical Care Epidemiologic and Biologic Tissue Resource (CCEPTR) a total of 97 patients were selected to be enrolled in the study. By applying the specific inclusion and exclusion criteria, patients were grouped into two groups based on severity of ARDS and age- and sex-matched to minimize variable confounders: Mild ARDS (n=18), Severe ARDS (n=18) using the Berlin definition of ARDS.

REDCap Initialization and Data Entry

Upon selection of the 97 patients into their respective cohorts a REDCap (Research Electronic Data

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Capture) epidemiologic relational database was used to enter patient data.

Sample Preparation NMR Analysis

Each of the 54 serum samples (200 μl) were defrosted from -80°C to 4°C. 3 kDa NanoSep microcentrifuge filters were prewashed five times with ddH2O, reducing preservative contamination. Then, the samples were filtered via centrifugation at 11963 x g for 1 hour at 4°C using the pre-washed 3 kDa filters. The filtrates were rinsed with 100 μl of D2O. The filtrates were collected into clean 1.5 ml vials. The samples were adjusted to 400 μl with 80 μl of phosphate buffer (0.5 M NaH_2PO_4 buffer solution at pH 7.0) containing 2.5mM 2,2-dimethylsilapentane-5-sulfonate (DSS, final concentration 0.5 mM) as an internal reference compound, 10 l sodium azide (1M NaN_3) to prevent bacterial growth, and D_2O. The pH of the samples was adjusted to 7.0 $\pm$ 0.04 at room temperature. ^{1}H-NMR data for all the samples were generated on a 600 MHz Bruker Ultrashield Plus NMR spectrometer (Bruker BioSPin Ltd., Canada), without bias by the use of an automated sample changer. Bruker 1D proton spectroscopy pre-saturation pulse sequence (noesypr 1d) were used to acquire one dimensional spectra, where an optimal water suppression program and mixing time of 100 ms were used. ^{1}H-NMR spectra were analyzed using the ChenomX NMR Suite 7.1 software (Chenomx Inc., Edmonton, Alberta, Canada) for metabolite identification and quantification using the targeted profiling approach in the profiler module.

Results

The unsupervised PCA model showed significant clustering in the 3-dimensional model for alive- and dead- outcomes for the patients in the study (Figure 1). Eight patients with a dead outcome were clustered together, and denoted by black squares (Figure 1). The patients with an alive outcome showed clustering in their respective coordinates, and were represented by green squares (Figure 1). 2 and 3-dimensional OPLS-DA models were generate and revealed statistically significant separation for predicting the alive and dead outcomes for the patient cohort on day one of OCU admission. As the black squares (dead) and green squares (alive) are positioned on separate portions of the orthogonal component (x-axis), a separation is observed between the two states (Figure 2).

Discussion and Conclusions

The purpose of this study was to determine if there was a statistically significant metabolomics difference in 28-day mortality for patients with ARDS. The use of ^{1}H-NMR to establish a metabolic distinction in ICU outcome (dead or alive) for patients with pneumonia-induced ARDS was needed ICU stay. This study was successful in determining a model to separate 28 day mortality in mild vs severe pneumonia- induced ARDS patients from samples taken on the first day of ICU admission.

An unsupervised PCA model of the 55 metabolites detected by ^{1}H-NMR dataset was based on 28 day mortality for patients with pneumonia-induced ARDS. The PCA plot revealed statistically significant data clustering between groups, demonstrating that alive patient outcome can be separated from the dead outcome (Figure 1). In the OPLS-DA model, significant and predictive separations were observed in the alive and dead outcomes (Figures 1, 2). R^2Y values measure the differences within the group, and typically give the degree of variation explained by the model. The R^2Y value determined from the OPLS-DA plot was 0.61, suggesting reliable statistical variation in the data. The OPLS-DA model revealed a $Q^2Y = 0.458$ and p-value of 0.004. The Q^2Y is a measure of model predictability and this value suggests good predictability of ICU outcome. An excellent separation between the serum profile for the alive and dead outcomes in pneumonia-induced ARDS patients was observed.

References

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