Leukocyte Immunobiology helps us predict Postoperative Risk using Preoperative Markers

Sumeet Deshmukh*, Srikanthi Ramachandrula*, Sunil C. Cherukuri*, Nitin Agrawal*, Galyna Bondar#, Mario Deng#

* Strand Life Sciences Pvt. Ltd, Bengaluru, India.
# David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.
Strand NGS enables a comprehensive and flexible RNA-Seq data analysis workflow consisting of Alignment, Quality Assessment, Filters, along with a multitude of analysis and visualization options that help in studying a variety of samples and answering long-standing biological questions. In this case study, we will introduce and discuss the application of available class prediction algorithms in Strand NGS for analysis of sequencing data in healthcare domain.

1. Overview

Heart failure is a serious medical condition wherein the weakened heart is not able to function fully and supply enough blood to the cells. Mechanical Circulatory Support (MCS) devices are a therapeutic option for Advanced Heart Failure (AdHF) patients with challenging clinical profiles. However, even after MCS surgery, some patients experience an increased mortality risk due to Multi-Organ Dysfunction (MOD) Syndrome associated with immune cell dysfunction. The ability to precisely predict postoperative risk for each AdHF-patient before surgery would therefore be paramount in clinical decision-making and management. Our goal is to demonstrate the use of biological information from sequencing studies to develop preoperative tests which can accurately predict postoperative outcomes in individual patients.

As a part of this pilot study, 29 patients undergoing MCS surgery were observed for a period of 9 days starting from a day before the surgery. During this period, blood samples were collected along with a comprehensive set of clinical variables to assess MOD. Based on medical records and clinician’s assessment, each of these patients was labeled as ‘high’ or ‘low’ risk. Peripheral blood mononuclear cells (PBMCs) were extracted from the blood samples of preoperative day and used for transcriptome profiling. For some of the patients, cytokine and flow cytometry data was available too. Data from each platform was imported into Strand NGS using the most optimal data transformation and normalization options. After quality assessments and filters, the high quality data points were used for statistical and fold change analysis. Statistical methods were employed to find the features that best captured trends in the data and differentiated low-risk patients from those of high-risk. Class prediction models were then built on patient data from each platform using Decision tree, and Support Vector Machine (SVM) algorithms. In order to leverage the potential of multi-dimensional data from different platforms, combinatorial models were built using features from across platforms.

Such models when constructed using a larger cohort could become a practicable option used to optimally predict the MOD risk to be faced by a new patient in case of MCS surgery.

2. Introduction:

Heart failure (HF) is a complex clinical syndrome that results from any structural or functional cardiovascular disorder causing a mismatch between demand and supply of oxygenated blood and consecutive failure of the body's organs. The death rate remains unacceptably high at approximately 50% within 5 years from the time of initial diagnosis.

Mechanical circulatory support (MCS) devices are increasingly being offered to advanced heart failure (AdHF) patients with challenging clinical profiles. After MCS-surgery, there is significant patient-to-patient variability for risk of adverse events, with some patients experiencing an increased mortality risk. Despite clinical expertise and validated prediction tools, it is not easy to predict this risk and, therefore, make recommendations about which method of therapy would benefit the individual patient most with respect to long-term survival. The ability to precisely predict the individual AdHF-patient’s postoperative risk before the surgery and the impact of this risk on the associated long term survival prognosis would be a very important component of clinical decision-making.

Since none of the current established clinical scoring and prediction tools integrate immune function parameters, they have the tendency to be imprecise in estimating risk among severely ill patients, making the therapeutic recommendation with the best survival result for the individual patient very difficult. We therefore decided to integrate the immune function parameter information and multi-dimensional molecular biomarker profiles (cell type populations, cytokine and gene expression levels) of the patients. Using supervised learning algorithm methods, the underlying preoperative biomolecular pattern of patients falling into high risk or low risk groups can be identified and used for prediction of postoperative outcome for a new patient.
3. Datasets:

A group of 29 patients referred to UCLA Integrated AdHF Program were studied as a part of this pilot study.

3.1. Clinical Phenotypes: Demographic variables were obtained for all patients. Starting from a day before the surgery, we collected 12 distinct parameters on a daily basis for time-dependent clinical phenotyping of the patient cohort. On each day, a comprehensive set of clinical variables to assess multiple organ dysfunction were recovered from patient records. Using combinations of these parameters, we also calculated two validated and commonly used composite organ dysfunction scores, the Sequential Organ Failure Assessment (SOFA)* score and Model of Endstage Liver Disease excluding International Normalized Ratio (INR) (MELD-XI)**.

Peripheral Blood Mononuclear Cells (PBMCs) were extracted from blood samples collected a day before the surgery, and used for the following analysis:

3.2. Transcriptome: Total RNA extracted from PBMCs of the 29 patients was sequenced on Illumina HiSeq 2500.

3.3. Flow Cytometry: Immuno-phenotyping of PBMCs from 21 patients was performed with 12-color monoclonal antibody panels to identify the subpopulations.

3.4. Cytokines: Assessment of plasma cytokine/chemokine levels in 12 patients was performed using a custom multiplex Luminex array (Millipore).

4. Methods:

4.1. Transcriptome Analysis: Raw data (fastq files) was imported into Strand NGS v2.9. Pre-alignment quality checks were followed by alignment to transcriptome (hg19 - UCSC Gene model). Reads with average base quality >35 were used for quantification and normalized using DESeq. Genes showing <20 percentile expression in one or both groups were discarded as noise. The retained genes were checked for fold change (FC) in expression across low risk and high risk groups. Expression patterns were visualized using hierarchical clustering. Genes showing FC >2 were tested for significance using Mann-Whitney unpaired test (p-value ≤0.05). The list of significant genes was used for Principal Component Analysis and Support Vector Machine based prediction modeling.

4.2. Clinical Variables, Flow Cytometry, and Cytokine Data Analysis: Each of these data sets was imported into Strand NGS in a spreadsheet format using the ‘Externally Quantified Data’ option. Statistical tests and visualizations were used for pattern assessment. The significant list of features from each platform was used for prediction model building exercises.

5. Results and Discussion:

5.1. The prominence of clinical information

Dr. Deng’s lab at UCLA has been studying a number of patients with AdHF and observing their recovery process. With the patients’ consent, they have recorded a large number of these observations including vital clinical parameters on a daily basis for a period of 9 days during the MCS surgery. They further derived organ function recovery scores and stored them. This rich collection of information forms the basis of our analysis.

From Figure 1, it can be seen that SOFA and MELD-XI, the two organ function scores from a day before the surgery correlate weakly and show no potential to predict the survival status of the patient, a year after the surgery. This led us to an important aspect of predictive analytics in healthcare, namely, a quantifiable phenotype. For any prediction analysis to be meaningful, the phenotype or trait definition has to be evidence-based, quantifiable, and reproducible. We realized that while the vast resource of clinical variables was highly useful, none of them could serve the purpose of a quantifiable trait that translates into an outcome.

Dr. Deng and team then defined the concept of risk as the probability of improvement of organ dysfunction wherein -

(a) A low risk patient would show improvement in both SOFA and MELD-XI scores from day [-1] to day [8].
(b) A high risk patient would not show improvement in either or both SOFA and MELD-XI scores from day [-1] to day [8].
Given that the goal is to be able to classify a new patient before the surgery, we identified the need to be able to work with day [-1] data alone.

When the 12 measured clinical variables and 2 derived organ function recovery scores from day [-1] were correlated with 'risk,' respiratory rate, heart rate and SOFA score showed promise. We then built a decision tree model using these three parameters. While the model showed potential, the accuracy was only 77% and called for better measures.

![Figure 1: A scatter plot showing the weak positive correlation between SOFA and MELD-XI scores recorded from patients a day before MCS surgery. The color-coding indicates the survival status of the patient a year after undergoing the surgery.](image)

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![Figure 2: A wizard page from Strand NGS showing the decision tree formula for predicting risk using the clinical information - respiratory rate, heart rate, and SOFA score.](image)

**Figure 2:** A wizard page from Strand NGS showing the decision tree formula for predicting risk using the clinical information - respiratory rate, heart rate, and SOFA score.

### 5.2. Transcriptome Data Analysis

As observed from the accuracy of clinical variable based decision tree, we recognized the need for a more robust set of features that better reflect the individual's biological state and ability to recover from MCS surgery. Since PBMCs best reflect the immunological state, we decided to look for Gene Expression Profiles (GEP) that differentiate low risk patients from high risk ones.

Upon alignment, quantification, and statistical analysis of the transcriptome data, we arrived at a list of genes that were differentially expressed between low and high risk patients. Gene Ontology analysis of the gene list revealed that it was enriched for genes involved in immune response, chemokine activity, and fever generation. The pathway analysis further confirmed the immunological basis of difference in response to surgery. Most genes that were differentially expressed between low and high risk groups also had prominent roles in Cytokine and Inflammatory Response pathways.
Figure 3: Gene expression profiles in preoperative PBMCs samples from low and high risk patients seen in conjunction with their disease information and organ dysfunction scores.

Figure 4: Principal Component Analysis view showing the separation achieved between high risk and low risk patients using the list of significantly differentially expressed genes between the two groups.
We then used this list of 24 genes that were most significantly differentially expressed between the two groups for building a SVM-based class prediction model. This model showed an accuracy of 91%, an improvement over the clinical variable based decision tree. We further validated this model using a set of 5 patient samples - four of them were correctly predicted, one sample was misclassified.

While the correct prediction of four unknown samples is encouraging, the wrong prediction of one new sample emphasizes the need for more reliable prediction models, especially, in a sensitive domain like healthcare. Ideally, a good prediction model would be accurate, robust, scalable and most importantly, unambiguously interpretable. All this goodness should be achievable within the pragmatic confines of measurability.

5.3. Towards Multi-dimensional molecular biomarkers

In order to increase reliability of the prediction model, we further included flow cytometry and cytokine measurements during model building. The idea was to increase the diversity of measurements and therefore, overcome any platform-specific biases. However, fewer samples were studied on all three platforms - 7 of the original training set of 23, and all 5 of 5 from the validation set were measured on all platforms. While the sample that was misclassified by the transcriptome model was misclassified by the combinatorial model too, it could be correctly predicted using the flow cytometry model.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Clinician’s Assessment</th>
<th>Transcriptome</th>
<th>Combinatorial</th>
<th>Weighted</th>
</tr>
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<tbody>
<tr>
<td>MODE_001_0022_01</td>
<td>Low Risk</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>MODE_001_0023_01</td>
<td>High Risk</td>
<td>High</td>
<td>High</td>
<td>High</td>
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<tr>
<td>MODE_001_0026_01</td>
<td>High Risk</td>
<td>High</td>
<td>High</td>
<td>High</td>
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</tbody>
</table>

Table 1: Risk prediction using Transcriptome only, Combinatorial, and Weighted SVM models. Wrong prediction is highlighted in dull red.

6. Conclusion

Strand NGS offers an end-to-end solution for class prediction challenges in healthcare domain. Starting from the import of the raw data, followed by alignment, analysis and biological contextualization of features, Strand NGS provides all tools that would be pre-requisites for mining sequencing data to build reliable class prediction models that are suited for varied machine learning goals. Partial Least Square Discrimination Analysis, Support Vector Machine models, Decision trees, Naïve Bayesian classifiers and Neural Networks can be built, retrieved, validated, and used for prediction in Strand NGS. Further, these models can be employed in isolation or in combination for improved accuracy and confidence.
Table 2: Possible ways to increase accuracy and confidence of prediction models in healthcare.

<table>
<thead>
<tr>
<th>Model Type</th>
<th>Description</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual</td>
<td>Built using data from a single technology (e.g., Decision tree using clinical information, SVM using Transcriptome data)</td>
<td>Economical</td>
<td>Limited feature space</td>
</tr>
<tr>
<td>Combinatorial</td>
<td>Built using data combined from multiple platforms (e.g., SVM using clinical information, flow cytometry, cytokine and transcriptome data)</td>
<td>1. Increased feature space 2. Better separation leading to higher accuracy</td>
<td>1. Data collection is expensive 2. Error in capturing data from a particular source may adversely impact the prediction accuracy</td>
</tr>
<tr>
<td>Weighted</td>
<td>Built using a combination of multiple individual prediction models (e.g., SVM using Transcriptome data and SVM using Flow Cytometry data)</td>
<td>1. Chance of error reduces 2. Combined probability of two models giving incorrect results is minimal</td>
<td>Data collection is expensive</td>
</tr>
</tbody>
</table>

Appendix:

*SOFA score is routinely used in Intensive Care Units to assess the rate of organ failure. A lower score implies that there are fewer chances for organ failure in a given patient. Intensive Care Med. 1996 Jul;22(7):707-10.

**MELD-XI score can identify high-risk patients with right heart volume overload, higher pulmonary arterial pressure and multiple organ failure associated with heart failure. High scores are associated with poor prognosis. PLoS ONE 9(6): e100618.

Acknowledgement:

We thank Dr. Mario Deng and team from UCLA for this collaboration and readily agreeing to showcase their study for elucidating the use of machine learning in genomics and healthcare. We, at Strand Life Sciences, have used the conceptual framework of this case study and took a cautious approach to showcase generic ideas only and not publish the actual findings of this research study in this Application Note.
About Strand

A History of Innovative Genomic Research

Strand Life Sciences is a global genomic profiling company and leader in precision medicine diagnostics, aimed at empowering cancer care and genetic testing for inherited diseases. Strand works with physicians and hospitals to enable faster clinical decision support for accurate molecular diagnosis, prognosis, therapy recommendations, and clinical trials. The Strand Center for Genomics & Personalized Medicine is India's 1st and only CAP & NABL accredited NGS laboratory.

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Strand Life Sciences Pvt. Ltd
5th Floor, Kirloskar Business Park, Bellary Road, Hebbal, Bangalore 560024
Phone:+91-80-40 (787263) Fax: +91-80-4078-7299