

David Gordon Louis Daniel Foundation Quarterly Research Progress Report

Grant Title: Development of a diagnostic biomarker panel for Kawasaki disease

Number of pts studied (if applicable): 60

Number of assays performed (if applicable): 5,350

Description of any statistical analyses performed: Continuous variables were analyzed by Mann-Whitney U and unpaired t test. Correlations were evaluated by Spearman's test.

Description of any scientific presentations submitted or delivered: See attached word document (SPRabstract_112009).

Description of any publications submitted or accepted:

Manuscript in preparation: "Markers of cardiomyocyte injury in acute Kawasaki disease." Yuichiro Sato, BS, Adriana Tremoulet, MD, MAS, Chisato Shimizu, MD, Virginia Watson, MD, Brookie Best, PharmD, MAS, James Snider, PhD, Jeffrey Frazer, MD, Delaram Molkara, MD, Alan Maisel, MD, and Jane Burns, MD.

Estimated date of completion of project: 12/1/2010

Synopsis of progress:

To identify plasma proteins that might differentiate KD from other pediatric rash/fever syndromes, we measured 89 analytes in inflammatory pathways using the Luminex antibody-coated bead system (HumanMap, version1.6, RBM Inc.) in 30 acute KD subjects and 30 age- and sex-matched febrile control children. Drs. Ling and Cohen, our collaborators at Stanford University identified a nine-analyte panel that effectively classified KD from FC using algorithms of nearest shrunken centroid for biomarker feature selection, 10-fold cross validation analyses, and Gaussian linear discriminant analysis for classification analyses. Overall, the nine-analyte biomarker classifier correctly classified 83.3% KD and 93.3% FC subjects (P value = 1.42×10^{-9} , ROC AUC=0.948). When all 30 KD and 30 FC samples were clustered by unsupervised analysis (Figure 1 A) of their analyte abundance, all but 4 KD samples co-clustered, and all but 9 FC patients clustered separately from the KD sample cluster (P value = 1.606×10^{-5}). Given that our 9 analyte panel was selected from an 89-analyte set, we needed to address the multiple comparison problem. Permutation-based false discovery rate (FDR) analysis (Figure 1 B), comparing our nine-analyte panel to the ones "falsely discovered" through 500 permutations to construct random data sets, NSC feature selection of top nine analytes, and supervised discriminant modeling for KD classification, estimated overall FDR as 2.4%, supporting the notion that the discovery of this nine-analyte panel is unlikely to be the outcome of random discovery. To evaluate the nine-analyte classification performance and gauge the potential unexpected bias in the training set, we bootstrapped the data set 500 times for ROC analysis. The plotted ROC curve (Figure 1 C) is the average of the 500 bootstrapped runs, and the boxes and whiskers plot the vertical spread around the average (the area under the curve of ROC, 98%), indicating the high sensitivity and specificity of the nine-analyte panel in KD classification.

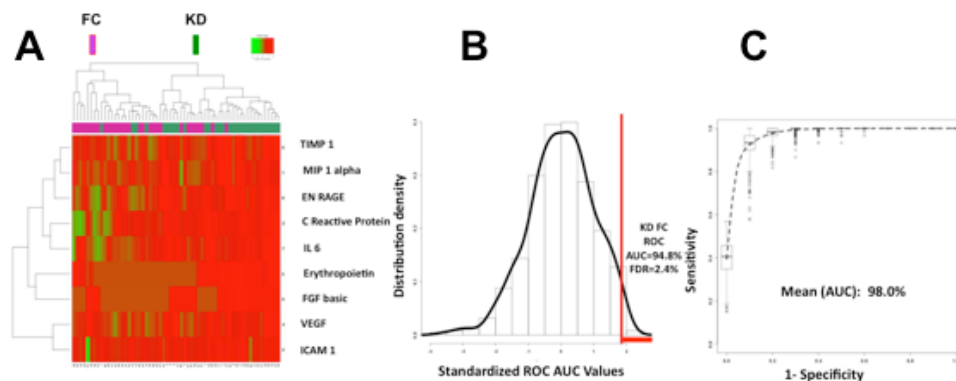


Figure 1. A nine-analyte biomarker panel to distinguish KD from FC. (A) unsupervised clustering analysis (B). Distribution of the standardized ROC AUC values of 500 falsely discovered panels. (C). Bootstrapping based ROC analysis.

The next phase of our analysis will be to apply statistical methods to incorporate clinical and demographic data with the analyte data to see if we can achieve superior prediction with a smaller analyte panel. We will also be performing assays on additional cohorts of KD and febrile control subjects to see if we can validate these results.

Grant applications using these preliminary data have been submitted to NIH and AHA with the hopes of being able to pursue a nanowire biosensor device that can simultaneously measure this panel of analytes on a single drop of blood.