

Latent profile analysis of circulatory biomarkers indicates distinct pattern of inflammation post-transfusion with cardiopulmonary bypass in pediatric ARDS



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Background

Red blood cell (RBC) transfusions can trigger inflammation which can lead to or worsen multi-organ dysfunction (MODS).

In this study, we analyze patients who received RBC transfusions and used latent profile analysis to cluster patients into composite groups describing both baseline characteristics and responses post-transfusion.

We discovered that these composite groups very closely align with whether patients received only transfusion (T) or both transfusion and cardiopulmonary bypass (T+CPB), which is a more significant inflammatory insult with additional risks and variable outcomes.

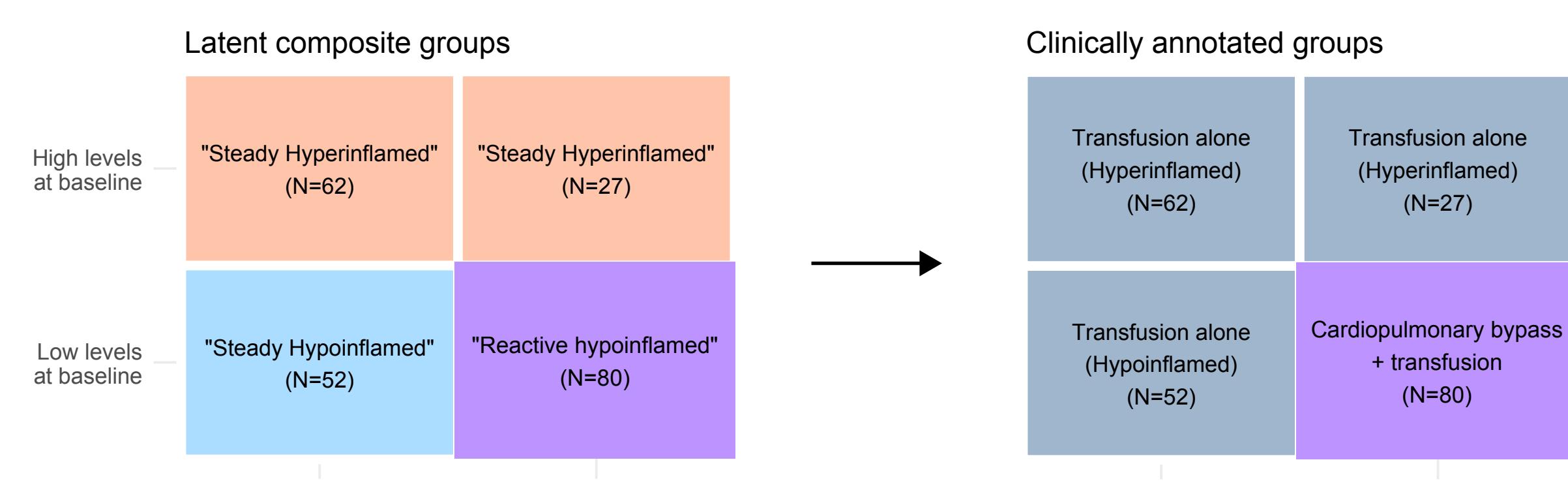
This work aims to address the following:

1. Use of latent profile analysis to discover meaningful groups of biological and clinical significance in pediatric MODS;
2. Correlation of latent groups with clinical characteristics, and interpretation of those groups in relevant clinical and biological context.

Methods

Protein biomarkers were assayed using a NULISA 250-marker panel on day 0 (pre- transfusion or T+CPB) and day 1 (post- transfusion or T+CPB).

1. Composite groups were determined using latent profile analysis on baseline levels and log-2 fold changes. Groups were identified by crosstabulation of the baseline and change profiles (Figure A) to form the following table:

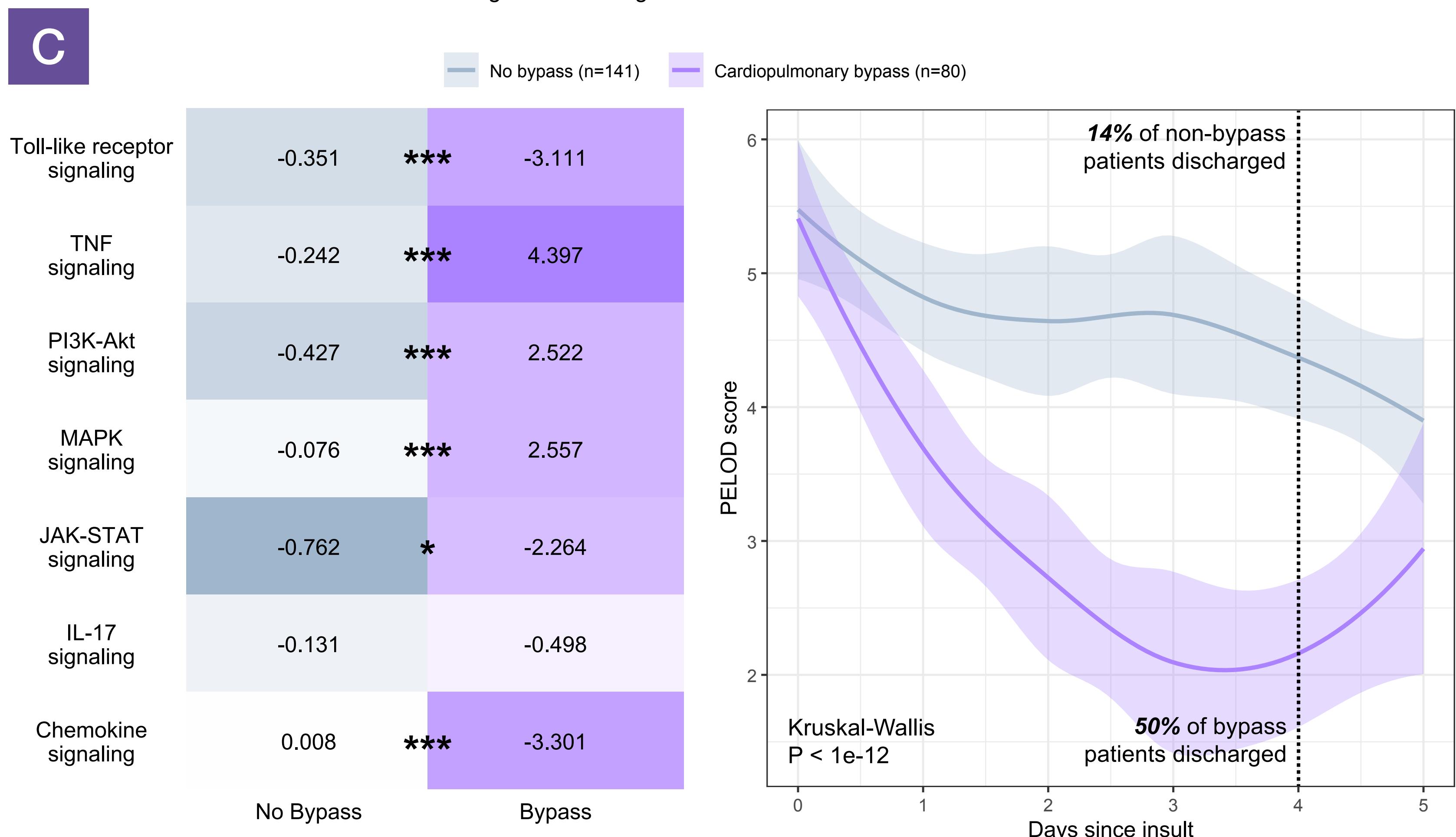
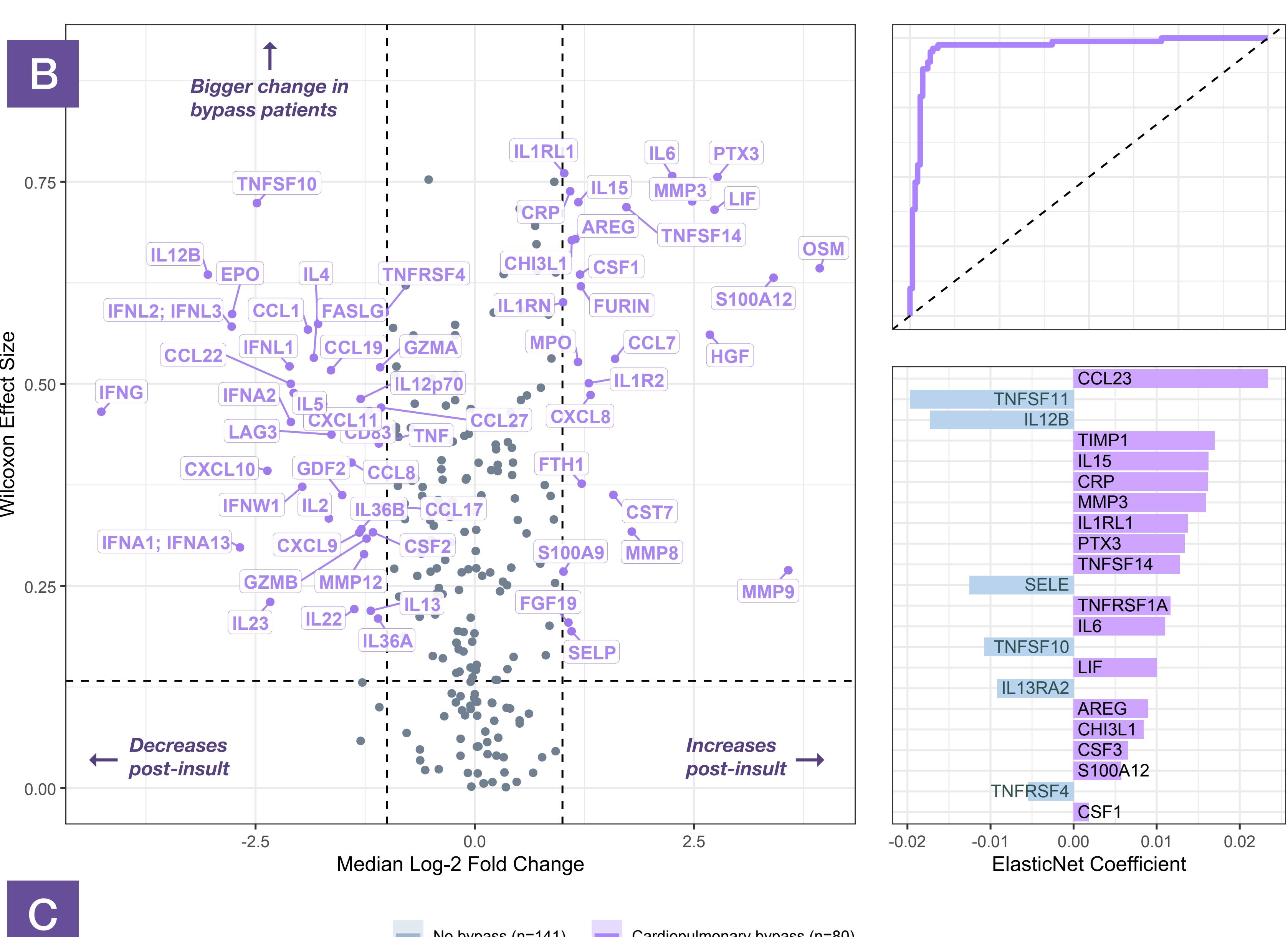
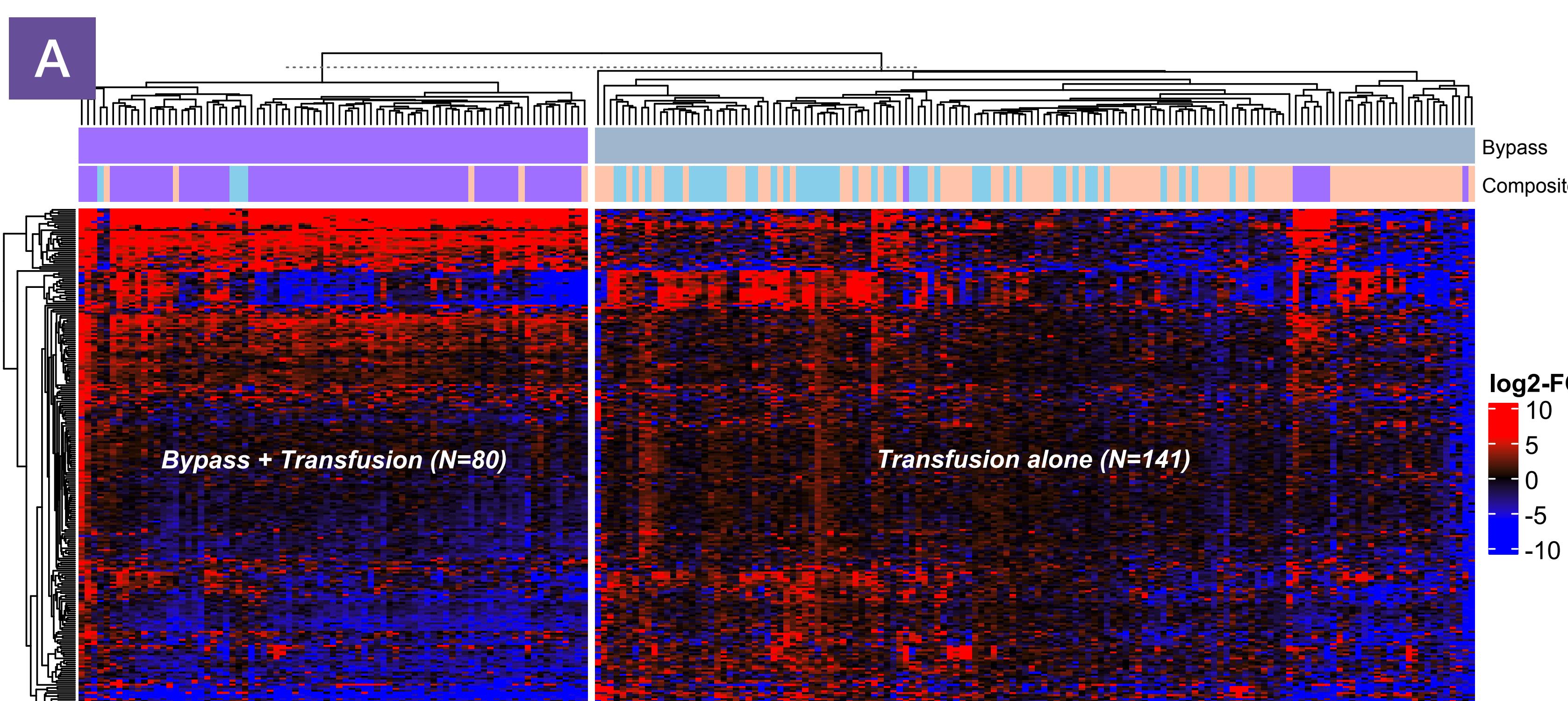


2. Changes between groups were compared using Wilcoxon effect sizes, and changes across time were compared by log-2 fold changes pre- and post-insult (Figure B, left).

3. Biomarkers most highly enriched in T+CPB were predicted using an ElasticNet model (*glmnet*) and the ROC curve and resulting coefficients were measured (Figure B, right).

4. Significant markers with $\text{abs}(\text{LFC}) > 1$ were tested for enrichment using KEGG biological pathways (Figure C, left).

5. Clinical outcomes were compared using PEdiatric Logistic Organ Dysfunction (PELOD) scores, using Kruskal-Wallis tests of 5-day scores following insult and rank-sum comparisons of cumulative PELOD score and time-to-discharge (Figure C, right).



Results

Patients receiving cardiopulmonary bypass (T+CPB) experienced substantial changes in circulating inflammatory markers, and broadly better clinical outcomes.

Composite latent profile analyses discovered unique changes in transfusion + cardiopulmonary bypass (T+CPB) patients. Latent profile analysis on the n=250 markers discovered groups very similar to transfusion vs. transfusion + cardiopulmonary bypass.

T+CPB patients had more, larger changes in inflammatory markers post-insult. Patients in the T+CPB group experienced significant increases in 40 biomarkers (16%, LFC > 1) and significant decreases in 27 biomarkers (11%, LFC < -1). Most markers (n=198/250, FDR < 0.05) had significantly larger change in the T+CPB group compared to transfusion alone.

T+CPB inflammatory changes included upregulation of TNF, PI3K-Akt, and MAPK signaling, and downregulation of TLR, JAK-STAT, and chemokine signaling. Significant KEGG pathways (FDR < 0.05, n=65) are shown in Figure C, with Z-scored median fold changes of proteins annotated to each pathway. Significance annotations are determined using Wilcoxon tests between scaled fold changes.

T+CPB patients experienced faster illness improvement and departure from ICU. PELOD scores between the two groups were highly significant (Kruskal-Wallis $P < 1e-12$) for the first 5 days, and both cumulative PELOD score (12 vs. 34, Wilcoxon $P < 1e-9$) and time to discharge (4 vs. 10 days, Wilcoxon $P < 1e-10$) were significantly lower for bypass patients. PELOD score differences remain significant beyond day 5 (Kruskal-Wallis $P < 1e-10$).

Discussion

Cardiopulmonary bypass is a significant insult that incurs substantial proinflammatory effects in circulation. However, the patients undergoing CPB are often healthier before admission, and experience quick and significant reductions in illness scores and length of stay.

Our results build on existing evidence that cardiopulmonary bypass incurs significant inflammatory change in circulation, far exceeding that of transfusion alone.

From a broad inflammatory panel, we found a number of potential biomarkers of CPB including **CCL23, TNFSF11, IL12B, TIMP1** and others. These markers may be useful targets for understanding postoperative complications.

Our work is limited as a preliminary study of broad inflammation, and future steps will incorporate additional bypass variables as they become available to provide a more comprehensive picture.