

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



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## Rationale

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

We analyzed N=221 critically ill children who received RBC transfusions with or without cardiopulmonary bypass, using latent profile analysis to cluster patients into composite groups describing both baseline characteristics and responses post-transfusion.

Composite groups aligned very closely with whether patients received only transfusion (T) or both transfusion and cardiopulmonary bypass (T+CPB), highlighting the importance of fully characterizing the inflammatory profiles of these patients.

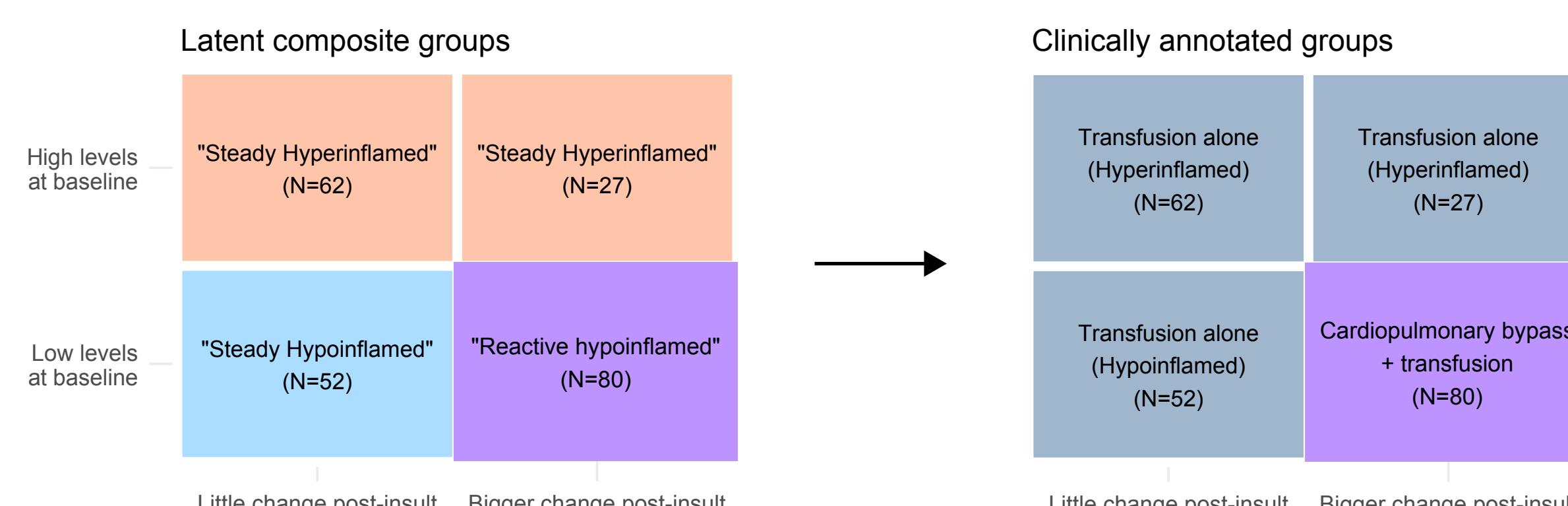
## Aims:

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

1. 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).

2. 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:

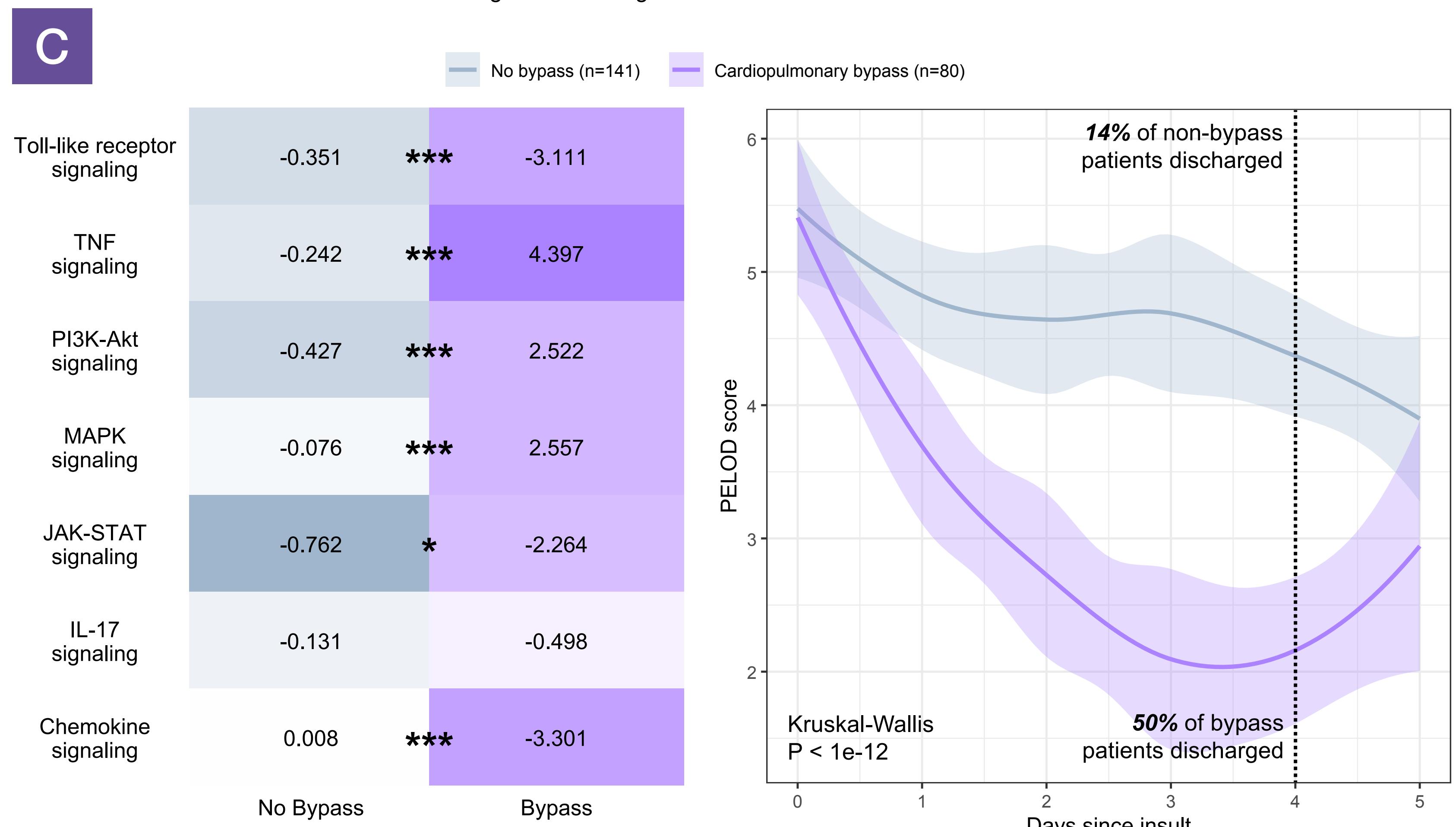
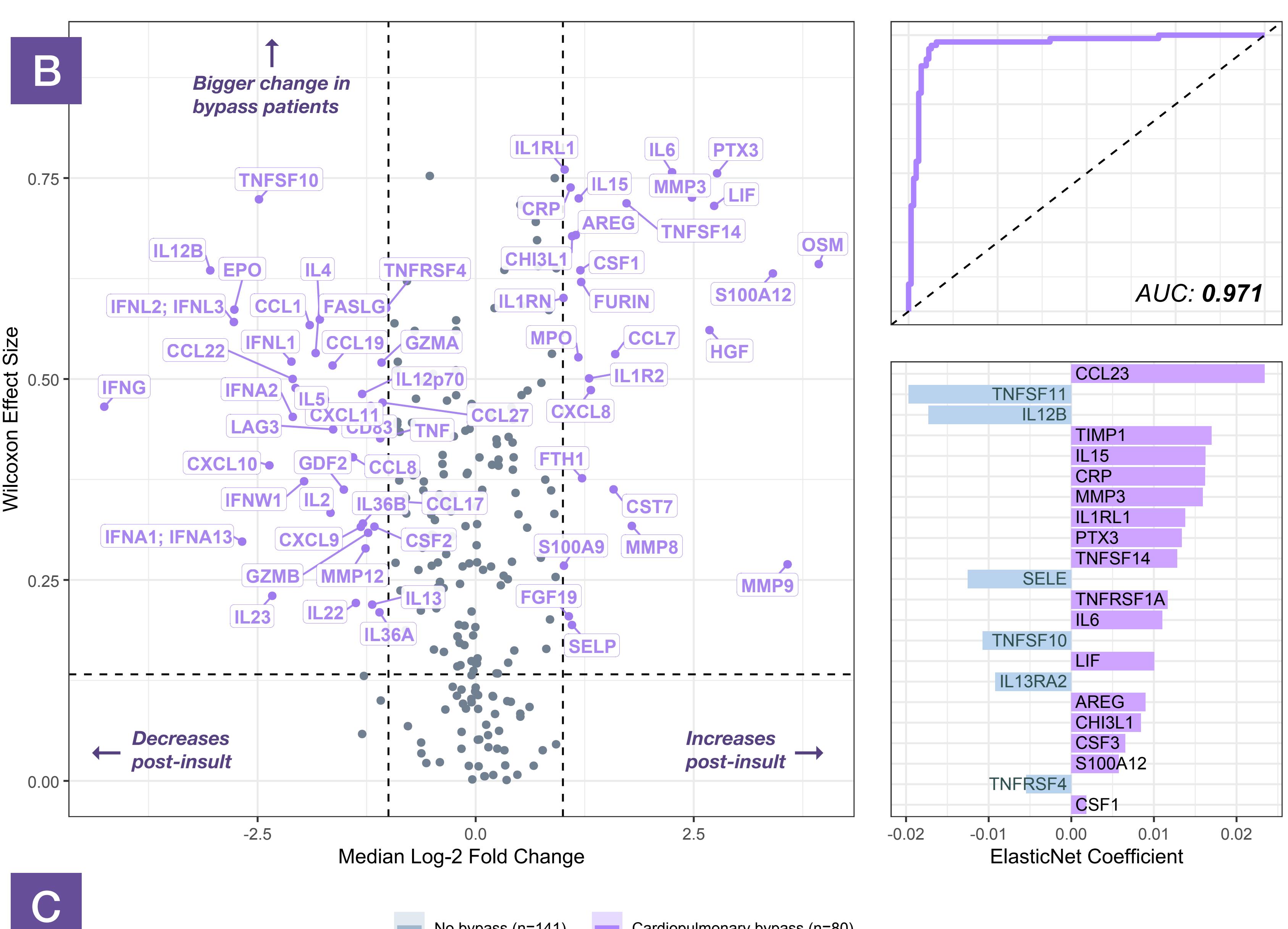
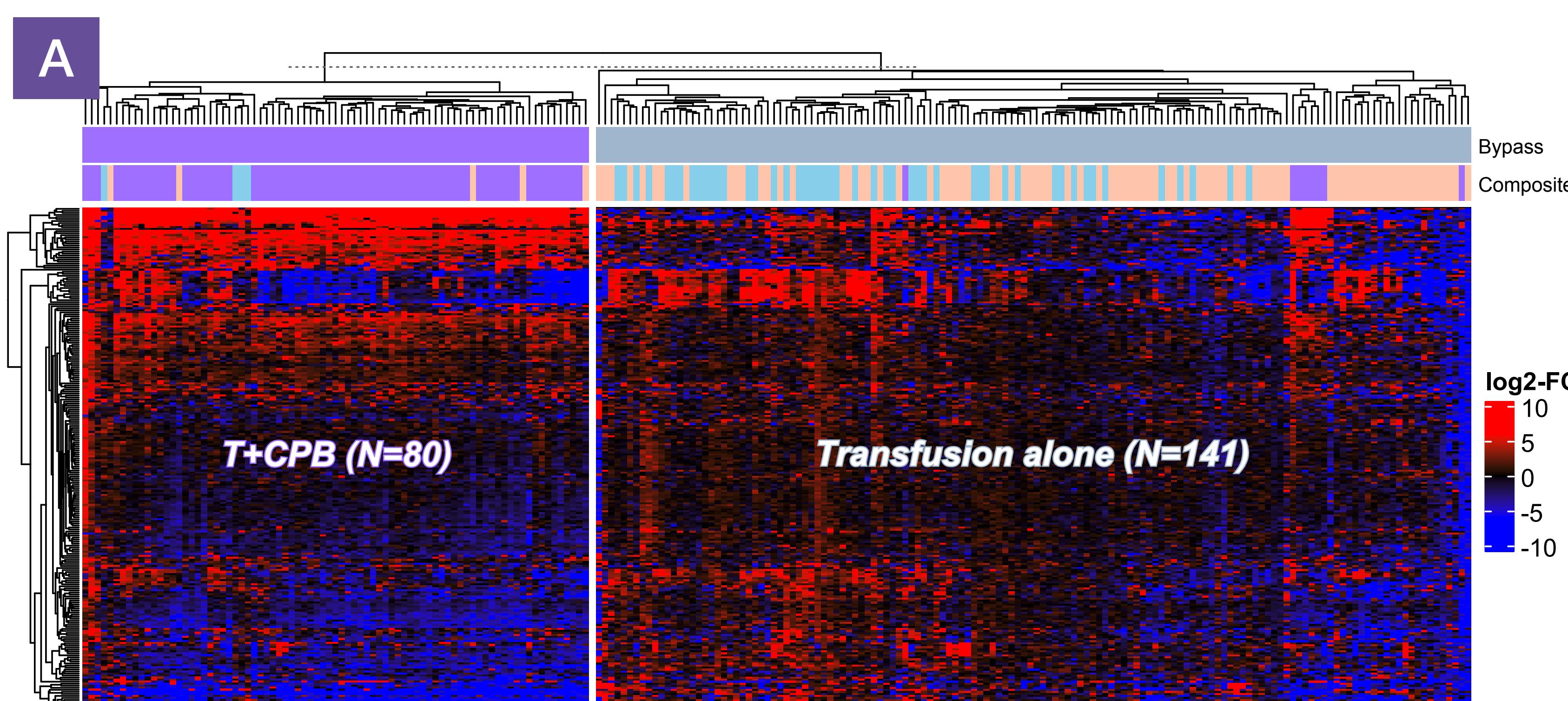


3. Changes between groups were compared using Wilcoxon effect sizes, and changes across time were compared by log-2 fold changes pre- and post-insult (Figure B, left).

4. 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).

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

6. Clinical outcomes were compared using PEdiatric Logistic Organ Dysfunction (PELOD) scores using nonparametric tests (Figure C, right).



## Results

**Composite latent profile analyses discovered unique changes in transfusion + cardiopulmonary bypass (T+CPB) patients.**

- Latent profile analysis accurately classified clinical groups by inflammatory markers alone (92.3% accuracy, 0.94 sensitivity, 0.89 specificity).

**T+CPB patients had more, larger changes in inflammatory markers post-insult (B).**

- Significant increases in 40 markers (16%, LFC > 1)
- Significant decreases in 27 markers (11%, LFC < -1)
- Greater change in most markers in T+CPB patients compared to T alone (n=198/250, FDR < 0.05)

**T+CPB inflammatory changes included upregulation of TNF, PI3K-Akt, and MAPK signaling, and downregulation of TLR, JAK-STAT, and chemokine signaling (C).**

- Significant enrichment of many inflammatory KEGG pathways (FDR < 0.05, n=65)
- Median fold changes for pathway markers significantly greater in T+CPB compared to T alone (Wilcoxon P < 0.05)

**T+CPB patients experienced faster illness improvement and departure from ICU (C).**

- Significantly lower PELOD scores in T+CPB for the first 5 days (Kruskal-Wallis P < 1e-12)
- Lower cumulative PELOD score in T+CPB (12 vs. 34, Wilcoxon P < 1e-9)
- Lower time to discharge from ICU in T+CPB (4 vs. 10 days, Wilcoxon P < 1e-10)

## Conclusion

Through our analysis, we are able to:

- Effectively isolate the inflammatory effects of cardiopulmonary bypass from transfusion and other constant sources of confounding;
- Identify potential markers & pathways of CPB-induced inflammation for further investigation with the largest known panel applied to these patients.

From a broad inflammatory panel, we found a number of potential biomarkers of bypass-induced inflammation including **CCL23, TNFSF11, IL12B, TIMP1** and others.

Future directions will include:

- Incorporating bypass times and other relevant covariates as they become available
- Incorporating more longitudinal measurements (days 1-3, 3-5) to strengthen relevant markers
- Mediation analysis of identified biomarkers in clinical outcomes.