

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



Clove S. Taylor, Daniela Markovic, L. Nelson Sanchez-Pinto, Matt Zinter, Nithya N. Reddy, Christine Zhang, Matteo Pellegrini, Andreas Schwingshackl, Manish Butte, Anil Sapru on behalf of the TRAP-MODS investigators

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.

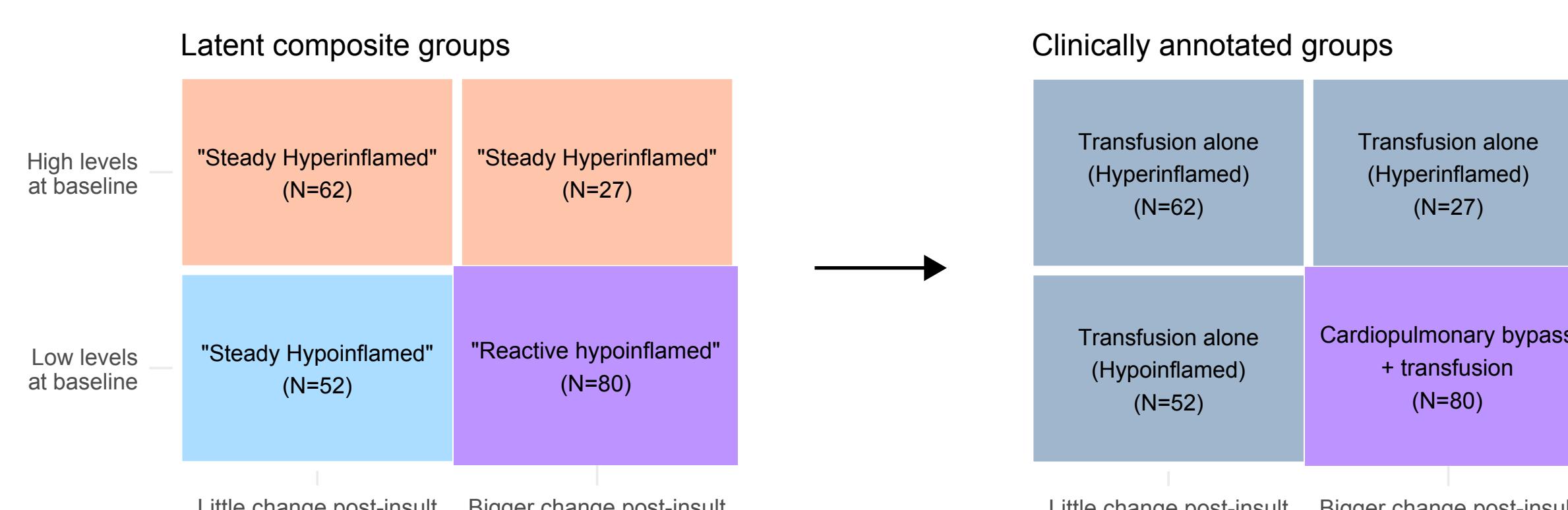
Aims:

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:

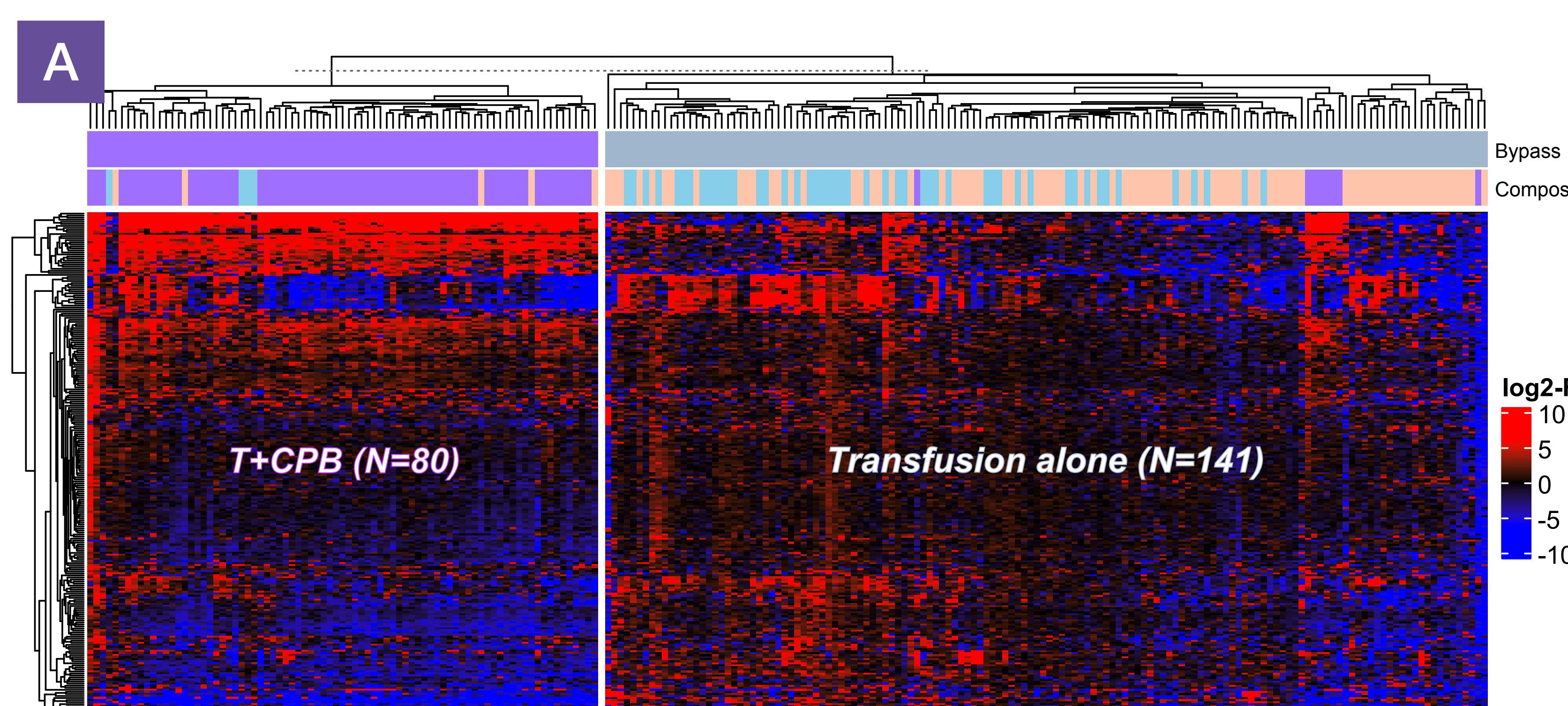


3. Changes between groups were compared using Wilcoxon effect sizes, and changes across time were compared by log-2 fold changes pre- and post-insult (Figure B, left).

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).



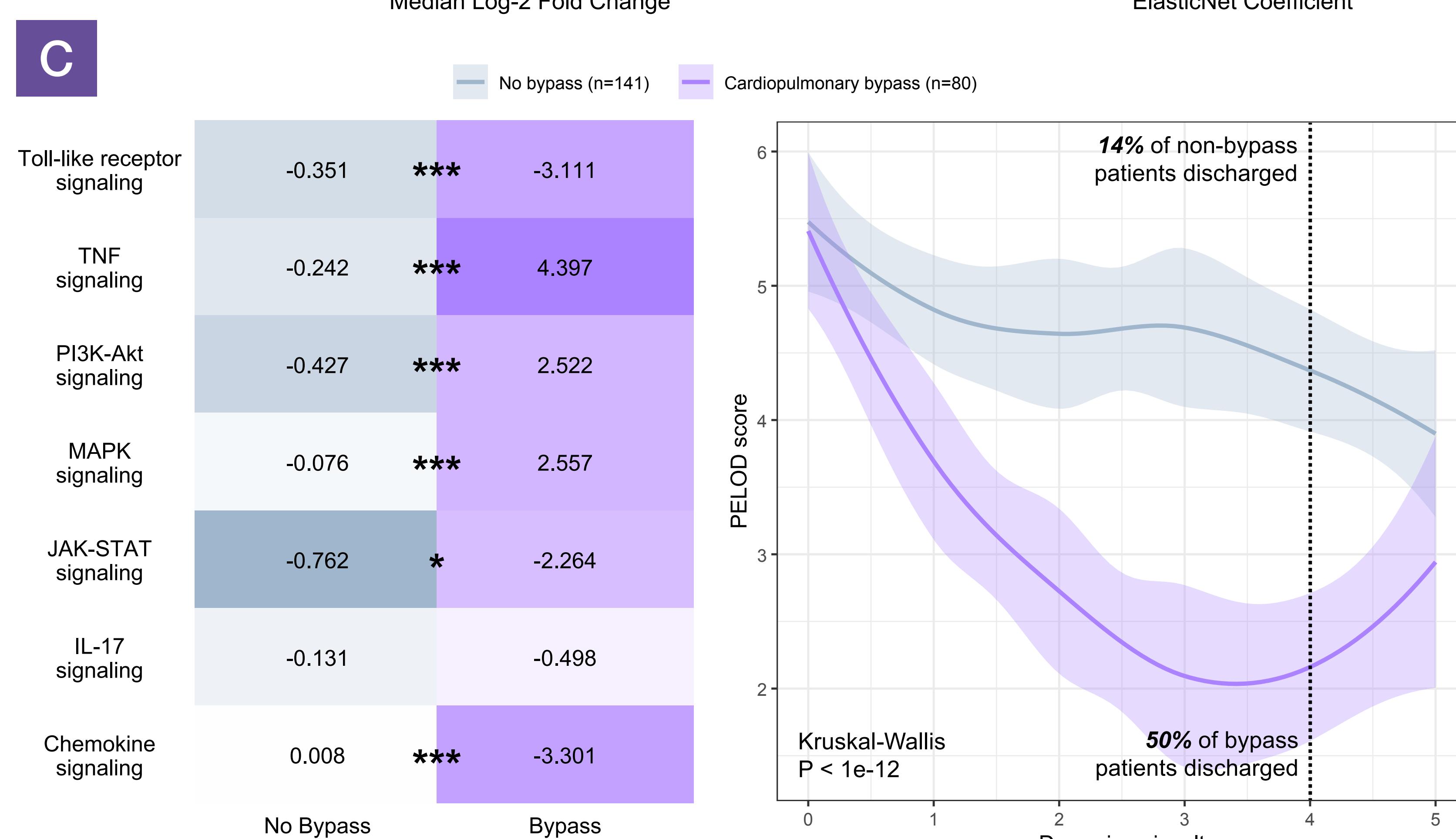
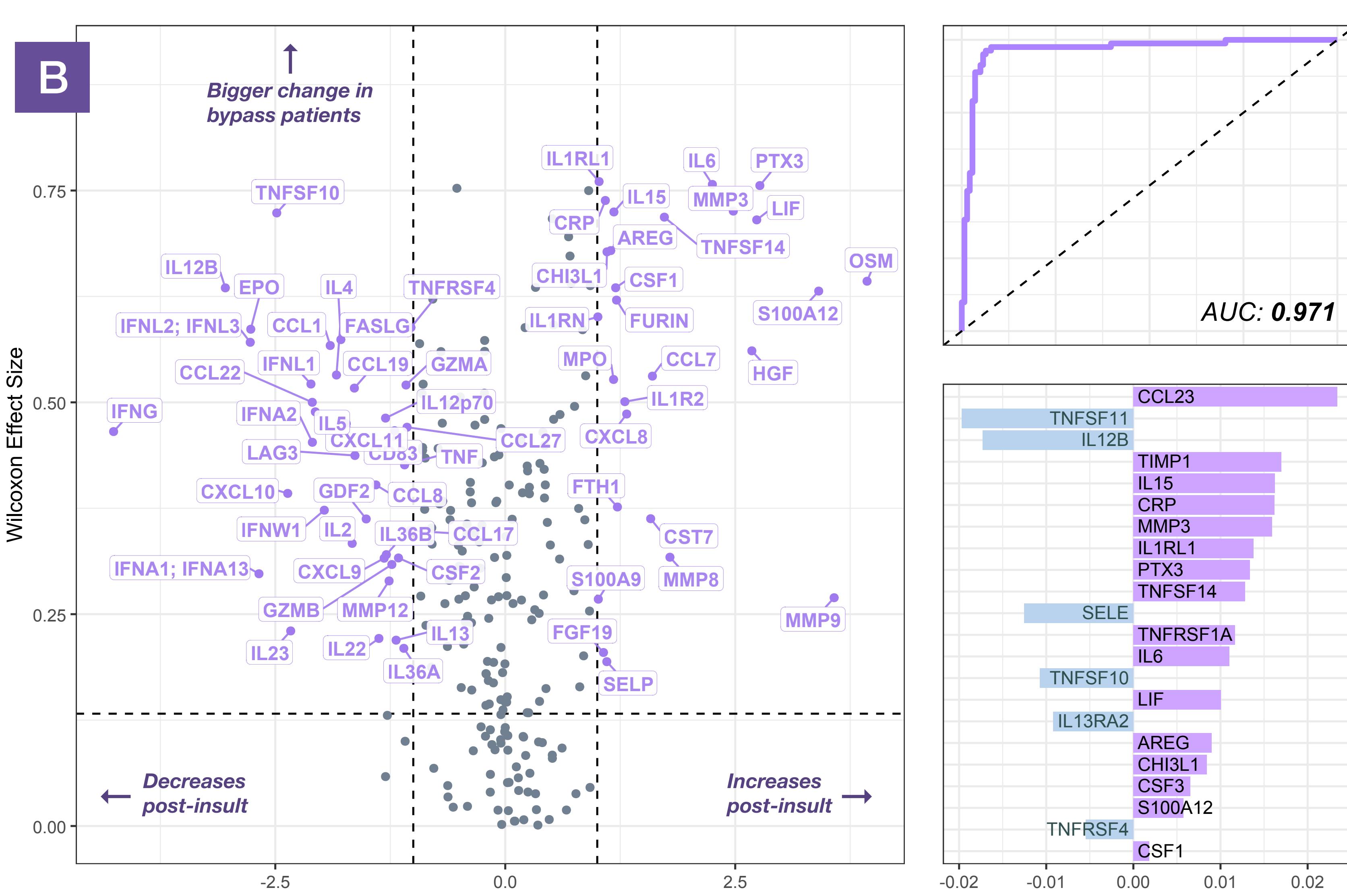
Results

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 $P < 1e-10$)

Discussion

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

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.