The Molecular Fork in the Road: Defining the Upstream Regulators of Longitudinal Trajectories in Severe Trauma

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INTRODUCTION

- Severe traumatic injuries induce acute, multi-system physiological instability.
- Patient recovery after trauma varies widely, with trajectories separating into either uncomplicated recovery or a multicomplicated course marked by multiple complications.
- Transcriptomic profiling offers the opportunity to identify early molecular features that distinguish these divergent recovery trajectories.

Study objective: Define early regulatory signals and upstream transcriptional networks that distinguish uncomplicated and multicomplicated recovery trajectories.

METHODS

- **Study design and cohort:** A prospective cohort of 265 adult trauma patients recruited between 2015–2021 from the SC2i Tissue and Data Protocol (TDAP) repository.
- **Patient stratification**: Patients were partitioned into two groups representing divergent recovery trajectories:
  - **Uncomplicated**, 0 complications at discharge.
  - **Multicomplicated**, at least 1 complication at discharge.
- **Temporal matrix**: Whole-blood profiles binned into 5 post-injury windows (0–1, 2–3, 4–7, 8–10, and >10 days).
- **Bioinformatics pipeline**: DESeq2 for differential expression, VIPER (Virtual Inference of Protein-activity by Enriched Regulon) for transcription factor activity, and pathway enrichment using MSigDB Hallmark pathways.
- **Statistical modeling**: Trajectories calculated via linear mixed-effects models with temporal bins as fixed effects and patient ID as a random intercept.

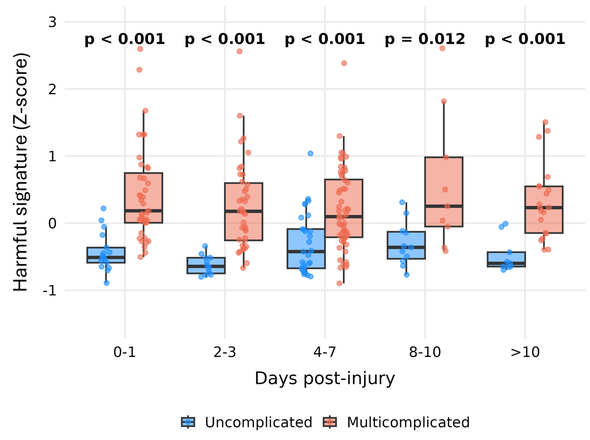
RESULTS

Table 1. Baseline demographics and clinical characteristics stratified by recovery group.

Group	Patients N	Samples N	Age (years) Median (IQR)	Male sex (%)	ISS Median (IQR)
Uncomplicated	90	105	34 (29-42.8)	86.7%	16 (9-19)
Multicomplicated	155	355	38 (26-54.5)	78.1%	27 (18-40.2)

Summary metrics detail age, sex, and Injury Severity Score (ISS) across recovery groups. Data are presented as medians with interquartile ranges (IQR) or as percentages (%).

Figure 1. Composite harmful gene signature over days post-injury by recovery group.



- The harmful gene signature, including MMP8 and ADAMTS3, was elevated and persistently activated in the multicomplicated group.
- The uncomplicated group showed sustained low expression of this signature.
- Nearly all between-group comparisons were statistically significant.

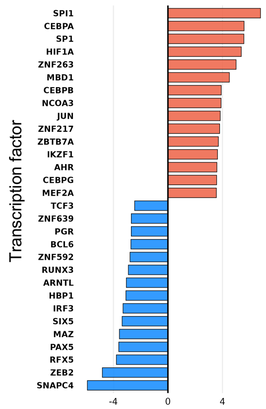
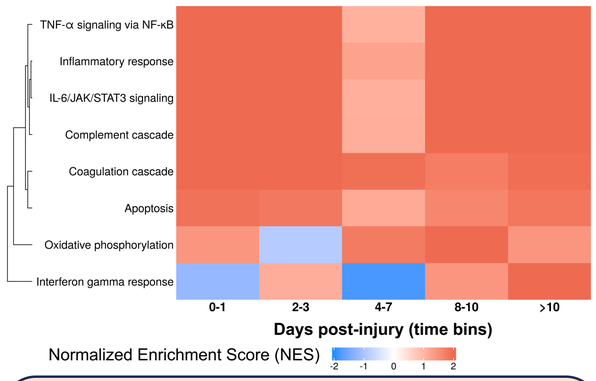


Figure 2. Upstream regulator activity (VIPER) assessed within 24 hours of trauma.

Higher activity in multicomplicated
Higher activity in uncomplicated

- Inferred transcription factor activity was measured using Normalized Enrichment Score (NES).
- Multicomplicated patients showed early activation of myeloid regulators, including SPI1 and CEBPA.
- Stress-response factors, including HIF1A and JUN, were also activated.
- Adaptive immune regulators, including PAX5 and SNAPC4, were repressed.

Figure 3. Pathway enrichment over time in multicomplicated patients.



- Pathway enrichment shows three main temporal patterns:
  1. Inflammatory, complement/coagulation, and apoptosis pathways show early and later enrichment in multicomplicated patients.
  2. Oxidative phosphorylation shows early suppression followed by later enrichment.
  3. Interferon gamma response shows relative repression, especially early after injury.
- Positive NES values (red) indicate higher activity in multicomplicated patients; negative NES values (blue) indicate relative repression.

Key results:

- **Baseline demographics (Table 1):** Multicomplicated patients had higher median ISS than uncomplicated patients.
- **Harmful signature trajectory (Figure 1):** Early transcriptomic analysis identified 230 tissue-degradation transcripts, including MMP8 and ADAMTS3. The harmful gene signature remained elevated through day 10+ in multicomplicated patients, but it stayed low in uncomplicated patients.
- **Upstream regulators (Figure 2):** VIPER identified early myeloid and stress-response activation, including SPI1, CEBPA, HIF1A, and JUN, with adaptive immune repression involving PAX5 and SNAPC4.
- **Pathway kinetics (Figure 3):** In multicomplicated patients, inflammatory and complement/coagulation pathways showed early activation, partial attenuation during days 4–7, and later renewed enrichment.

CONCLUSION

- **Transcriptomic polarization:** Multi-system complications are marked by early activation of myeloid and inflammatory pathways, occurring alongside suppression of adaptive immune signaling.
- **Resolution failure:** Multi-system complications are associated with early disruption of co-expression network structure, followed by sustained inflammatory and tissue-injury signaling that extends beyond the first week after injury.
- **Translational triage:** Early post-injury molecular signatures may provide objective markers to improve risk stratification and guide critical care decision-making.

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