Measurement of Cell-Free RNA Signatures After Severe Trauma in a Porcine Model Supports Future Platelet Transcriptomic Profiling

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Introduction

- Thromboinflammation – a complex dysregulation of coagulation, inflammation, and immune function – drives downstream complications following combat and major civilian trauma.
- Platelets play a central role in thrombosis and inflammation. Emerging evidence from platelet transcriptomics demonstrates that platelets harbor dynamic, disease-specific RNA signatures that reflect and actively modulate thrombinflammation.
- Platelet-based functional assays have shown inconsistent associations with thrombinflammatory outcomes after trauma, limiting utility in biomarker development and therapeutic discovery.
- Evaluation of platelet RNA signatures in clinical practice is challenged by logistics - including timing of sample collection, lack of pre-injury assessment, and heterogeneity of injury mechanism intervention. Multi-day translational research studies offer a unique opportunity for protocolized investigation.
- ~30% of plasma cell-free RNA (cfRNA) is platelet-derived, offering a unique opportunity to interrogate platelet-related molecular responses.

Objective and Hypothesis

Objective: Interrogate plasma space for platelet-related cfRNA in serial samples in a combat-relevant swine polytrauma model with 48-hour follow-up.

Hypothesis: cfRNA profiles demonstrate consistent time-dependent changes following trauma.

Methods

- Yorkshire swine (*sus scrofa domesticus;* n=10, 54±2 kg) were anesthetized, mechanically ventilated, and instrumented.
- Following baseline data collection, swine encountered traumatic brain injury, bilateral pulmonary contusion, and controlled hemorrhage; followed by 30-min shock period.

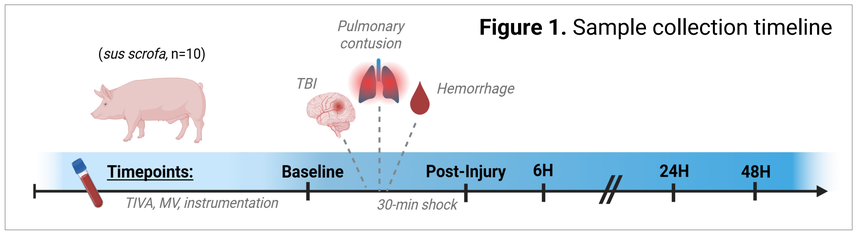


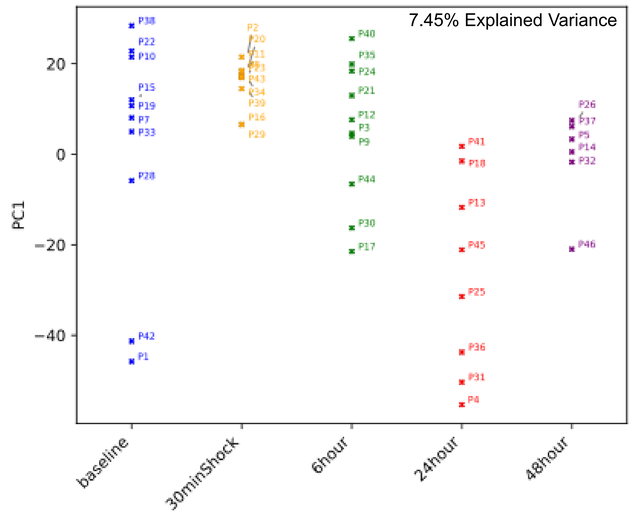
Figure 1. Sample collection timeline

Methods

- Citrated plasma was prepared at discrete timepoints (**Figure 1**) and flash frozen (-80 ºC).
- cfRNA was extracted and subjected to next-generation sequencing (n=43 passed QC; ~1M reads / sample). Fibrinogen was quantified (Stago CompactMax).
- EdgeR was used for differential gene discovery. Principal component analysis (PCA) was performed on normalized counts per million to examine patterns of gene discovery between individuals and timepoints.
- ANOVA or paired T-tests were used to assess differences in principal components at timepoints ($p<0.05$ for significance).
- Pearson's correlation was performed on principal components and fibrinogen level.

Results

- cfRNA profiling revealed consistent platelet-related genomic response following polytrauma, despite baseline inter-animal variability (**Figure 2**).
- Initial post-injury timepoint showed strongest agreement between samples, suggesting initial trauma perturbation drives samples to similar projection status (**Figure 2**).



	p-value vs Baseline	p-value vs Post-Injury	p-value vs 6 H	p-value vs 24 H
Post-Injury	0.14	---	---	---
6H	0.67	0.03	---	---
24H	0.019	.0017	0.016	---
48H	0.56	0.04	0.27	0.015

Figure 2. PCA showed trauma-related genomic patterns (PC1). Paired T-tests highlight significantly different timepoints.

Results

- One genomic pattern (PC10) was identified with significant changes between timepoints (ANOVA p=0.003) suggesting a stereotyped early response to injury, with modulation over 48 hours. This pattern was correlated with fibrinogen levels (**Figure 3**).
- Cumulative findings suggest that trauma induces a structured, reproducible cfRNA signature in circulation, and is correlated with other physiological measures.

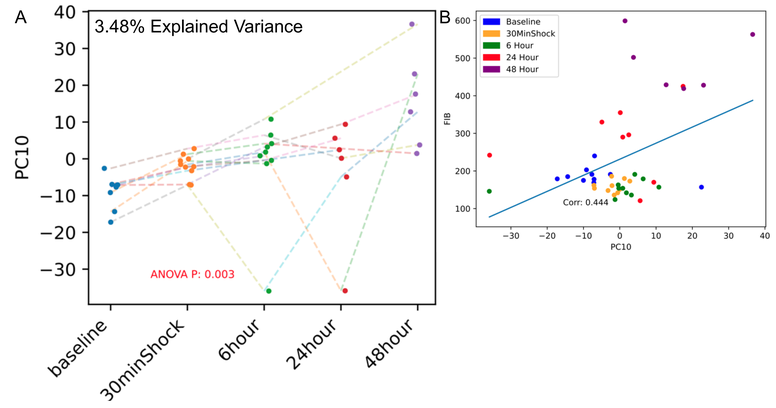


Figure 3. (A) Principal component 10 differed significantly (p=0.003) over time. (B) PC10 was correlated with fibrinogen level (Pearson's correlation coefficient 0.444).

Conclusions

- Pilot data demonstrate that platelet-related cfRNA captures coherent, time-dependent molecular responses to severe trauma in a translational model.
- cfRNA PCA analysis may discover different cell types undergoing lysis, restoration, and adaptation over the injury course – necessitating future analysis of cell-resolved analysis of gene expression changes in acute trauma.
- These findings support isolating platelets for dedicated platelet transcriptomics, enabling novel biomarker identification and therapeutic target discovery for military trauma care.

Acknowledgments

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