Metabolomic and flow cytometry analyses of platelet concentrates spiked with *Staphylococcus aureus* reveal significant modulations in platelet functionality

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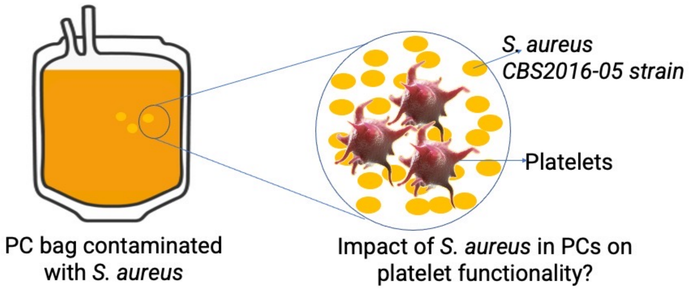
Background and Aim of the Study

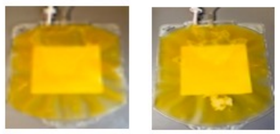
- Platelet concentrates (PCs), used to treat bleeding patients, get occasionally contaminated with *S. aureus*, which can escape detection during PC screening and can cause septic transfusion reactions.

- *Staphylococcus aureus* is an important opportunistic human pathogen that causes skin and bloodstream infections and is also known to be leading cause of morbidity and mortality.



- We performed targeted metabolomic and flow cytometry analyses on PCs inoculated with *S. aureus* CBS2016-05, a strain involved in a septic reaction event.

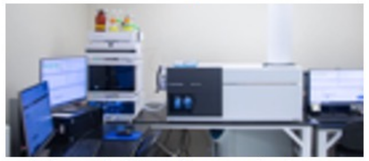
Study Methodology



Non-spiked and spiked PCs (stored at 20-24°C /agitation).
Spiked PCs were exposed to two different CBS2016-05 strain concentrations viz. 1E+06 CFU/bag and 0-10 CFU/bag .
Samples for analysis were collected at 0h, 24h, 48h, 72h and 144h

Metabolomics

Spiked and non-spiked PC samples were processed in extraction buffer 1:1:1 (MeOH:H2O:ACN) using bead beating method.



Agilent 6470A tandem quadruple mass spectrometer with ultra-high-performance LC

Metabolites quantified by using external standard calibration curves with Mass Hunter Quant

Flow cytometry

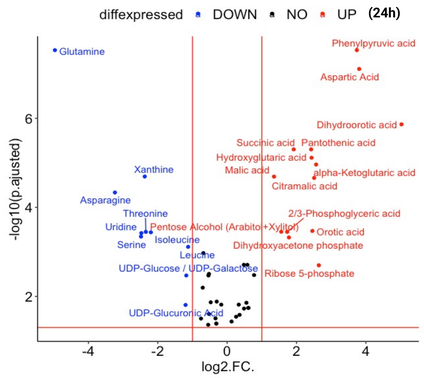
Spiked and non-spiked platelets were targeted for phycoerythrin (PE)-labeled CD62P (activation marker), CD41a (platelet glycoprotein IIb receptor) and Annexin V (externalization of phosphatidylserine)

5ul, 10ul and 1.5ul of CD41a, CD62P and Annexin v were added to diluted (10-40 x 106 platelets/mL) PC samples and incubated at room temperature in dark for 20 min.

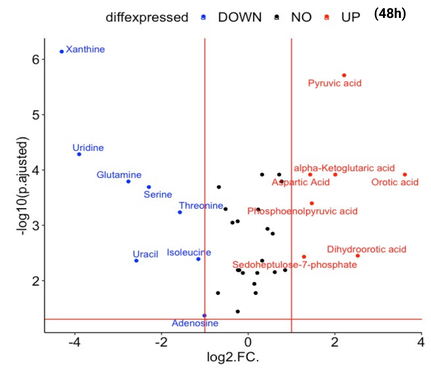
Attune acoustic focusing cytometer

Results

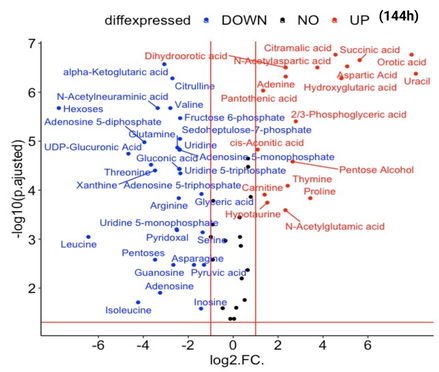
Metabolomics Results- Significant downregulation and upregulation of 10,14, and 8,7, and 30,18 metabolites at 24h, 48h and 144h, respectively, in spiked PCs vs non-spiked units (log2fold-change ≤ or ≥ ±1, p<0.05).



(a) Spiked vs non-spiked- Initial inoculum was 7E+05 CFU/mL and at 24h it was 7E+08

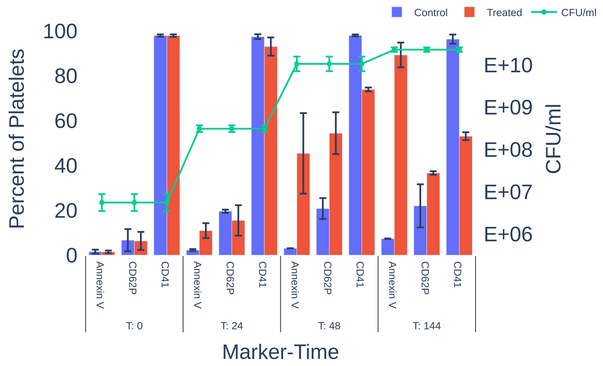


(b) Spiked vs non-spiked- Initial inoculum was 1-10 CFU/bag and at 48h CFU was 1E+07

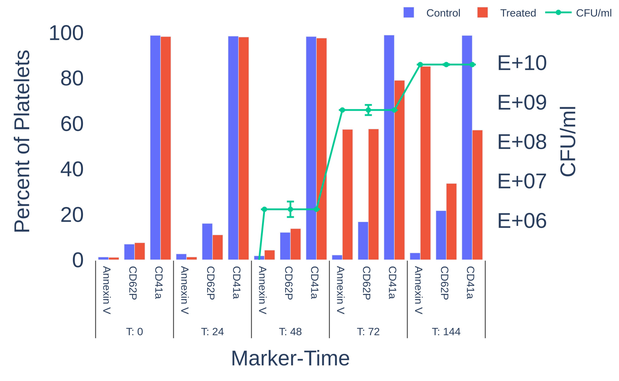


(c) Spiked vs non-spiked- Initial inoculum was 1-10 CFU/bag and at 144h CFU was 5E+10

Flow Cytometry Results- 2.6-fold and 14.6-fold increase in the percentage of CD62+ and phosphatidylserine+ platelets in spiked PCs (1E+06 CFU/mL), respectively at 48h whereas 3.5-fold and 27.3-fold at 72h (spiked 0 CFU/bag).



(a) Initial inoculum was 1E+06 CFU/mL



(b) Initial inoculum was 0 CFU/bag

Conclusions and Future Work

- *S. aureus* seems to induce substantial changes in the platelet metabolite content, alteration in platelet activation and loss of expression of GPIIb possibly causing dysfunction and apoptosis of platelets.
- Further studies include measurement of mitochondrial mass/membrane potential using Mitotracker/TMRE staining, respectively.

Acknowledgements

- Volunteer blood donors
- netCAD Blood4Research Facility- blood collection & PC production
- Canadian Blood Services for providing funding
- Ken McTaggart for providing sterile Terumo Blood Bags

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