Linking Stored Red Blood Cell Metabolism to Transfusion Recipient Iron Homeostasis Pathophysiology in Critically-Ill Children

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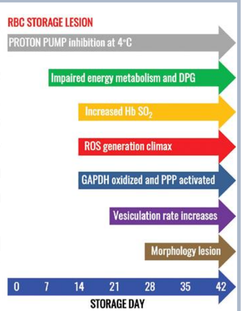
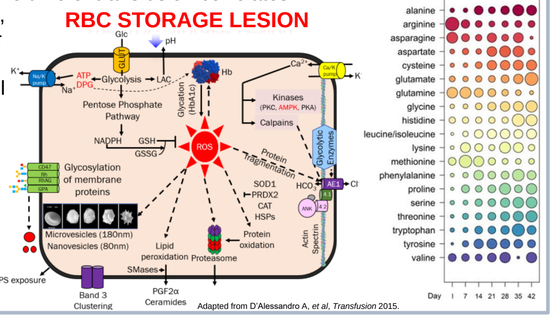
Background

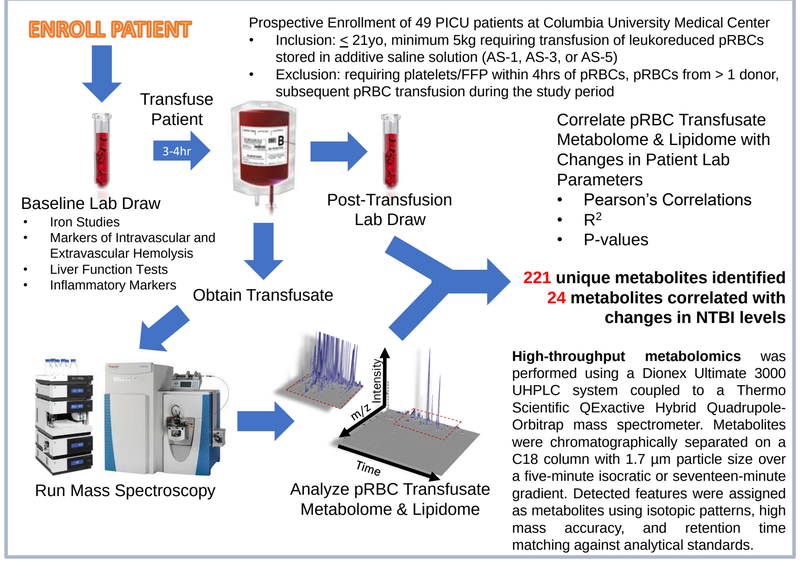
Transfusion of packed red blood cells (pRBCs) is a common, life-saving therapy for many critically-ill children. However, routine blood bank storage results in the progressive accumulation of a series of RBC modifications, termed the "storage lesion," that reduce RBC recovery *in vivo*, may affect transfusion recipient physiology, and may be implicated in transfusion-related adverse events. In particular, circulating non-transferrin-bound iron (NTBI) in recipients can be increased after pRBC transfusion. NTBI promotes bacterial growth, induces oxidative injury, and inversely correlates with post-transfusion recovery, all of which may increase the risk of sepsis, multiorgan failure, and death. We aimed to determine whether the metabolic state of donor pRBCs at the time of transfusion correlates with recipient NTBI levels, which serves as a marker of hemolysis and iron homeostasis in critically-ill pediatric patients.





Hypothesis: Markers of metabolically "older" pRBCs will correlate with NTBI levels in transfused pediatric intensive care unit (PICU) patients.

Study Design/Methods



Prospective Enrollment of 49 PICU patients at Columbia University Medical Center

- Inclusion: ≤ 21yo, minimum 5kg requiring transfusion of leukoreduced pRBCs stored in additive saline solution (AS-1, AS-3, or AS-5)
- Exclusion: requiring platelets/FFP within 4hrs of pRBCs, pRBCs from > 1 donor, subsequent pRBC transfusion during the study period

Correlate pRBC Transfusate Metabolome & Lipidome with Changes in Patient Lab Parameters

- Pearson's Correlations
- R^2
- P-values

221 unique metabolites identified
24 metabolites correlated with changes in NTBI levels

High-throughput metabolomics was performed using a Dionex Ultimate 3000 UHPLC system coupled to a Thermo Scientific QExactive Hybrid Quadrupole-Orbitrap mass spectrometer. Metabolites were chromatographically separated on a C18 column with 1.7 µm particle size over a five-minute isocratic or seventeen-minute gradient. Detected features were assigned as metabolites using isotopic patterns, high mass accuracy, and retention time matching against analytical standards.

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DISCLOSURES: SLS: Board of Directors for Hemanext, Inc. and consultant for Tioma, Inc. AD: Board of Directors for Hemanext, Inc. and founder of Omix Technologies, Inc.

REFERENCES: [1] D'Alessandro A, *et al*. *Transfusion.* 2015;55(1):205-19. [2] D'Alessandro A, *et al*. *Transfusion*. 2015;55(6):1155-68. [3] D'Alessandro A and Zolla L. *Expert Rev Proteomics*. 2017;14(3):243-252.

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National Center for Research Resources, National Institutes of Health

Baseline Characteristics

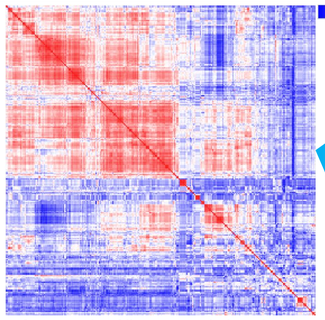
Patient Data

Patient Characteristic	Value	Patient Characteristic	Value
Age, years (mean ± SD)	8.4 ± 6.6	Primary Diagnosis, no. (%)	
Weight, kg (mean ± SD)	32.1 ± 27.3	Infection	9 (18)
Gender, no. (%)		Respiratory Failure or ARDS	6 (12)
Male	33 (67)	Liver Failure/Transplant	4 (8)
Female	16 (33)	Renal Failure/Transplant	3 (6)
Race/Ethnicity, no. (%)		Hematologic/Oncologic	4 (8)
White	19 (39)	Post-Op Cardiac Surgery	6 (12)
Black	12 (24)	Post-Op Neurosurgery	2 (4)
Asian	5 (10)	Post-Op Spinal Surgery	4 (8)
Hispanic	13 (27)	Post-Op Other Surgery	6 (12)
Patient ABO Type, no. (%)		Other Diagnosis	5 (10)
A	15 (31)	Sepsis, no. (%)	
B	7 (14)	Yes	15 (31)
AB	3 (6)	No	34 (69)
O	24 (49)	Requiring CVVH, no. (%)	
		Yes	3 (6)
		No	46 (94)

pRBC Data

pRBC Characteristic	Value
Volume Transfused, per kg (mean ± SD)	10.3 ± 5.3
Transfusion Duration, h (mean ± SD)	2.8 ± 0.7
Irradiated, no. (%)	
Yes	31 (63)
No	18 (37)
Storage Solution, no. (%)	
AS-1	14 (29)
AS-3	34 (69)
AS-5	1 (2)

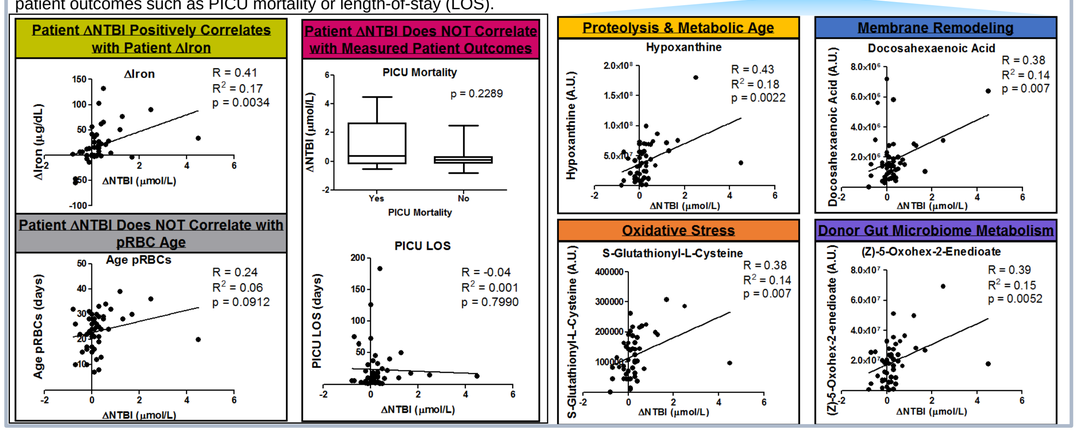
Results



Relative Metabolite Level Data Correlating with ΔNTBI

Metabolite Groupings	Compound Correlating with ΔNTBI	R	R^2	p-value
Markers of Proteolysis & Metabolic Age	Hypoxanthine	0.43	0.18	0.0022
	L-Tyrosine	0.38	0.14	0.0073
	L-Phenylalanine	0.36	0.13	0.0119
	L-Leucine	0.32	0.10	0.0237
	L-Tryptophan	0.32	0.10	0.0269
	L-Valine	0.31	0.10	0.0278
	Lactate	0.29	0.09	0.0419
Markers of Membrane Remodeling	Docosahexaenoic Acid	0.38	0.14	0.0070
	Docosapentaenoic Acid	0.34	0.12	0.0160
	Arachidonic Acid	0.33	0.11	0.0202
	Methylmalonylcarnitine	0.31	0.10	0.0289
Markers of Oxidative Stress	S-Glutathionyl-L-Cysteine	0.38	0.14	0.0070
	5-Oxoproline	0.30	0.09	0.0391
	D-Glucono-1,5-Lactone	0.29	0.08	0.0453
Markers of Possible Donor Gut Microbiome Metabolism	(Z)-5-Oxohex-2-enedioate	0.39	0.15	0.0052
	Indole	0.34	0.12	0.0160
Other Metabolites	Poly-γ-D-Glutamate	0.35	0.13	0.0127
	N-Acetylneuraminate	0.34	0.12	0.0161
	2-Hydroxyglutarate	0.31	0.10	0.0301
	Cytidine	0.30	0.09	0.0334
	N6-Methyl-L-Lysine	0.30	0.09	0.0362
	Allantoate	0.29	0.08	0.0426
	Creatinine	0.28	0.08	0.0473
	sn-Glycero-3-Phosphoethanolamine	0.28	0.08	0.0476

Top-Left: A matrix of linear correlations between metabolite levels and all continuous measured patient parameters was assembled and organized by Hierarchal Clustering Analysis. Metabolites with Pearson's correlations > 0 (red) are positively-correlated and those < 0 (blue) are negatively-correlated. **Top-Right:** Twenty-four (24) statistically-significant ($p < 0.05$) metabolite correlations with ΔNTBI grouped by metabolic pathway and showed with Pearson's correlation (R), coefficient of determination (R^2), and p-value. **Bottom-Right:** Representative graphs of most statistically-significant metabolite from each of the first four metabolic pathways vs ΔNTBI, shown with linear correlations and statistics. **Bottom-Left:** Representative graphs showing that ΔNTBI positively correlates with patient Δiron levels but does not correlate with the chronological age of the pRBCs nor with measured patient outcomes such as PICU mortality or length-of-stay (LOS).



Limitations

- Secondary analysis of previously collected samples with limited patient morbidity and mortality data
- All transfusates are sampled at the end of transfusion after pRBCs have been at room temperature in plastic with decreased blood volume (higher plastic bag surface/blood volume ratio)
- Small sample size with a large number of variables
- Absolute metabolite quantification not used for these samples
- Did not look at variables correlated with RBC quality (morphology score, O_2 off-loading, osmotic fragility, mechanical resistance)

Conclusions

- Patient ΔNTBI levels correlate with stored pRBC metabolites representative of:
  - Metabolic age of the stored donor pRBCs (markers of proteolysis, hypoxanthine, lactate)
  - Membrane remodeling of the stored donor pRBCs (acylcarnitines, unsaturated free fatty acids)
  - Oxidative stress in the stored donor pRBCs (glutathione homeostasis)
  - Possible donor gut microbiome metabolism (markers of indole and tryptophan metabolism)
- Markers of stored pRBC metabolic age seem to correlate better with patient ΔNTBI levels than the chronological age of the stored pRBCs

Implications

- Using markers of "metabolic age" may be a better determinant of a stored pRBC unit's functional age and may influence recipient physiology such as iron homeostasis and even possibly patient complications from pRBC transfusion
- Using markers of a stored pRBC unit's "metabolic age" may be able to predict increases in recipient NTBI levels post-transfusion which may have implications in recipient post-transfusion recovery and transfusion efficacy in critically-ill children
- The metabolic state of the donor and possibly the donor gut microbiome metabolism at the time of pRBC donation may affect the quality of stored pRBCs and their efficacy in transfused critically-ill children

Future Directions

- Correlate absolute quantification of these metabolites with ΔNTBI levels using available laboratory heavy-labeled standards and establish a threshold cut-off value for the most significant correlations
- Validate these correlations using another available dataset of patients
- Prospectively validate significant metabolites with ΔNTBI in a larger, multicenter study
  - Associate ΔNTBI with more clinically-relevant patient morbidity and mortality parameters (e.g. AKI, ventilator days, documented development of sepsis, PICU LOS, hospital LOS, in-hospital mortality, etc.)