Evaluation of serum markers for improved detection of autologous blood transfusions

Holly D. Cox,[1] Geoffrey D. Miller,[1,2] Auriella Lai,[1] Dan Cushman,[2] Tomas Ganz[3] and Daniel Eichner[1,2]

[1]Sports Medicine Research and Testing Laboratory, Salt Lake City, UT; [2]University of Utah School of Medicine - Division of Physical Medicine and Rehabilitation, Salt Lake City, UT and [3]Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Correspondence: hcox@smrtl.org
doi:10.3324/haematol.2018.190918

Supplemental Material

Table of Contents **Page Number**

Methods

Study Participants

Thirty-four healthy volunteers actively engaged in endurance sports, 20 males and 14 females, between the ages of 18 – 40 years were included in the study. Informed written consent was obtained from each participant. Approval for this study (US Clinical Trial NCT02684747) was granted by the University of Utah Institutional Review Board (IRB_00083533). All blood donation and transfusion-related procedures were closely monitored by on-site, trained medical staff using standard operating procedures defined by the Associated Regional and University Pathologists (ARUP) laboratories.

Study Outline

The randomized, single-blind, placebo concurrent, autologous transfusion study was performed as previously described.[11] In summary, 3 baseline blood samples were collected 2 weeks prior to blood withdrawal. At blood withdrawal, 1 unit of blood was collected in a bag containing citrate, phosphate, dextrose, and adenine (CPDA). Leukocytes were removed by filtration and the packed red cells were collected by centrifugation and stored at 4 °C for 21 days. After storage, 17 participants received blood transfusion and 17 participants received saline transfusion. Participants were randomly assigned to the blood or saline transfusion group and a curtain was used to hide the contents of the transfusion bags so that the participants were blinded to their group. Venous blood samples (K2EDTA) and serum samples (SST) were collected every day for the first week post-transfusion followed by additional collections at days 13, 20, 27 and 34. Complete blood counts were measured on the Sysmex XT 2000i automated hematology analyzer. Plasma and serum were separated by centrifugation at 1500 x g for 15 min and stored at -80 °C.

Serum Measurements

Ferritin, C-reactive protein (CRP), serum iron, and unsaturated iron binding capacity (UIBC) were measured on the Olympus AU400 (Beckman Coulter). IL-6 was measured using the Quantikine high sensitivity ELISA (R&D Systems, HS600B). Serum transferrin receptor (sTFR) was measured using the Quantikine IVD ELISA (R&D Systems). All assays were performed according to manufacturer instructions.

Hepcidin Quantification

Hepcidin was measured in serum using the mass spectrometry method described by Murphy *et. al*. with minor modifications.[12] Stable isotope-labeled hepcidin was used as the internal standard. Recombinant hepcidin and the internal standard were obtained from Peptides International (Louisville, KY).

Statistical Analysis

With a sample size of 34 participants, the study had a power of 0.92 to detect a 25% change in a variable assuming a population standard deviation of 30%, using a two-tailed t-test and a 5% significance level. A loss or drop-out of 10 participants would still provide a power of 0.80. The baseline values were calculated as the mean of 3 measurements collected over 2 weeks prior to blood withdrawal. All values are reported as the mean ± standard error (SEM). Data analysis was performed using Microsoft Excel with Data Analysis add-in. Statistical significance was determined using pairwise, two-tailed t test with Bonferroni correction for comparison of 12 groups. P values of ≤ 0.05 (Figure 1) or ≤ 0.01 (Supplemental Tables) were considered significant.

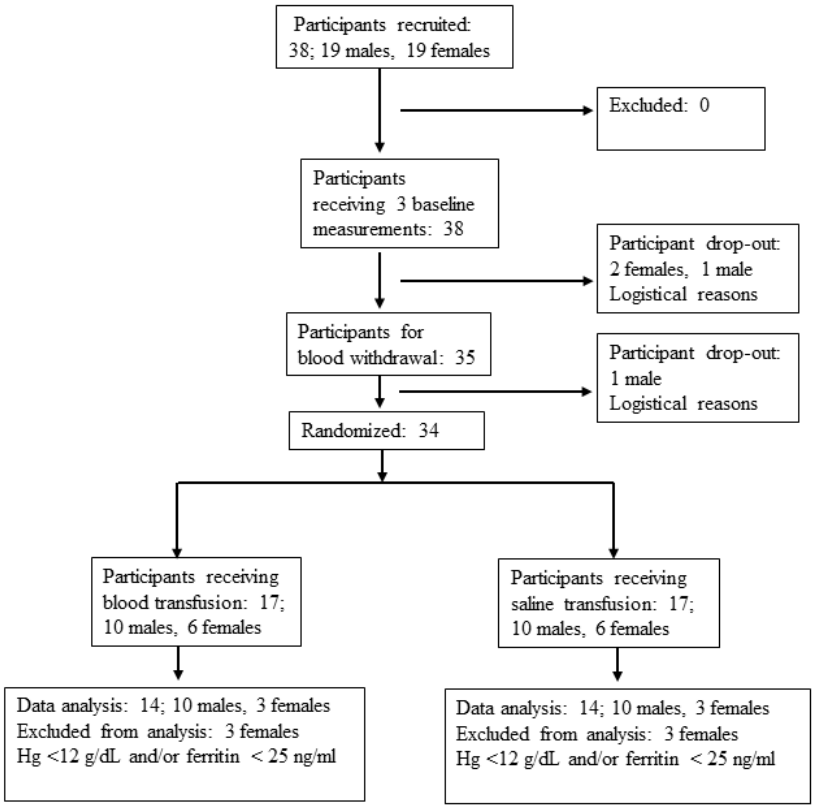
Participant Eligibility:

Healthy male and female participants between the ages of 18 – 40 who are actively engaged in endurance sports.

Exclusion Criteria:

Persons with chronic disease, pregnant women or women who plan to become pregnant during the study period. Persons with [Hg] > 16.7 or hematocrit< 35% or >55%. Persons excluded from blood donation using the criteria defined by the American Association of Blood Banks: http://www.fda.gov/downloads/BiologicsBloodVaccines/BloodBloodProducts/QuestionsaboutBlood/UCM272981.pdf

Athletes who plan to participate in an organized sporting event within 30 days following the transfusion phase of the study.



Supplemental Figure S1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram.

Supplemental Table S1. Response of Serum Markers to Blood Transfusion

Blood Transfusion									
Day	[Hb] (g/dL)	Ret%	OFF-score	Hepcidin (nM)	Ferritin (ng/ml)	sTFR (mg/L)	Iron (μg/dL)	TSAT (%)	IL-6 (pg/ml)
Base	14.78 ± 0.24	1.04 ± 0.08	87.28 ± 3.13	4.25 ± 0.95	107.45 ± 16.64	1.09 ± 0.06	117.83 ± 6.59	0.33 ± 0.02	0.85 ± 0.15
Post-out	13.69 ± 0.28	1.18 ± 0.09	73.88 ± 4.47	1.58 ± 0.23	91.09 ± 15.90	1.00 ± 0.07	119.51 ± 11.43	0.33 ± 0.03	1.67 ± 0.89
Pre-trans	14.18 ± 0.31	1.14 ± 0.10	78.59 ± 4.08	3.30 ± 0.96	65.36 ± 12.03	1.32 ± 0.07	104.79 ± 9.29	0.27 ± 0.02	1.10 ± 0.23
Day 1	15.11 ± 0.31	1.14 ± 0.09	86.67 ± 4.11	5.00 ± 0.93	75.59 ± 11.86	1.36 ± 0.08	113.15 ± 17.12	0.29 ± 0.04	0.88 ± 0.18
Day 2	15.29 ± 0.31	1.11 ± 0.09	90.23 ± 4.70	3.12 ± 1.02	76.23 ± 13.05	1.44 ± 0.12	108.06 ± 10.85	0.27 ± 0.03	0.65 ± 0.08
Day 3	15.14 ± 0.31	1.07 ± 0.08	89.86 ± 4.70	5.28 ± 1.95	73.55 ± 12.44	1.32 ± 0.06	129.02 ± 10.49	0.33 ± 0.03	1.61 ± 0.47
Day 4	14.93 ± 0.27	0.98 ± 0.07	90.46 ± 3.92	4.20 ± 0.72	67.68 ± 11.84	1.32 ± 0.06	137.36 ± 14.35	0.35 ± 0.03	2.64 ± 1.18
Day 5	15.05 ± 0.35	0.96 ± 0.07	92.18 ± 4.70	4.90 ± 1.31	74.32 ± 13.03	1.32 ± 0.06	142.21 ± 18.55	0.37 ± 0.05	0.88 ± 0.15
Day 6	14.98 ± 0.38	0.93 ± 0.07	92.42 ± 4.54	3.56 ± 0.59	79.79 ± 13.48	1.25 ± 0.07	115.83 ± 8.64	0.30 ± 0.02	0.65 ± 0.11
Day 13	14.91 ± 0.34	0.87 ± 0.07	93.77 ± 4.39	6.08 ± 0.93	85.59 ± 12.35	1.14 ± 0.07	135.32 ± 14.76	0.36 ± 0.04	1.03 ± 0.28
Day 20	14.74 ± 0.34	0.80 ± 0.06	94.17 ± 3.98	6.66 ± 1.35	89.21 ± 12.01	1.14 ± 0.06	105.77 ± 12.65	0.29 ± 0.04	1.95 ± 0.56
Day 27	14.76 ± 0.31	0.98 ± 0.08	88.91 ± 4.07	3.97 ± 0.90	82.11 ± 12.98	1.06 ± 0.05	118.98 ± 9.47	0.32 ± 0.03	0.72 ± 0.14
Day 34	14.75 ± 0.35	0.94 ± 0.05	89.56 ± 3.98	4.09 ± 0.71	85.73 ± 13.14	1.07 ± 0.06	125.25 ± 13.84	0.32 ± 0.04	0.64 ± 0.11

Grey boxes = statistically significant difference from baseline (for post-out and pre-transfusion), or from pre-transfusion (for day 1 - 34) or $p \leq 0.01$, n = 10 males, 4 females. Significance was determined using a pairwise, two-tailed, t-test with Bonferroni correction for comparison of 12 groups. *Ret% data for a subset of the participants, n = 10, was previously reported.[1]

Supplemental Table S2. Response of Serum Markers to Saline Transfusion

Saline Transfusion									
Day	[Hb] (g/dL)	*Ret%	OFF-score	Hepcidin (nM)	Ferritin (ng/ml)	sTFR (mg/L)	Iron (µg/dL)	TSAT (%)	IL-6 (pg/ml)
Base	15.09 ± 0.26	1.11 ± 0.06	88.04 ± 3.23	3.91 ± 0.74	81.64 ± 7.81	1.11 ± 0.08	113.89 ± 9.29	0.38 ± 0.03	1.01 ± 0.11
Post-out	14.00 ± 0.28	1.21 ± 0.07	74.43 ± 3.08	1.57 ± 0.25	71.29 ± 6.85	1.01 ± 0.07	92.61 ± 9.31	0.33 ± 0.04	0.85 ± 0.12
Pre-trans	14.24 ± 0.27	1.28 ± 0.11	75.35 ± 3.41	2.00 ± 0.56	40.25 ± 4.85	1.30 ± 0.08	92.99 ± 9.19	0.33 ± 0.05	0.85 ± 0.12
Day 1	14.31 ± 0.22	1.30 ± 0.11	75.39 ± 3.01	1.69 ± 0.54	46.07 ± 5.63	1.28 ± 0.08	90.01 ± 7.25	0.32 ± 0.05	0.83 ± 0.10
Day 2	14.47 ± 0.23	1.34 ± 0.10	75.85 ± 3.57	1.25 ± 0.32	44.72 ± 5.09	1.34 ± 0.09	110.01 ± 9.16	0.37 ± 0.05	1.02 ± 0.14
Day 3	14.63 ± 0.24	1.32 ± 0.08	77.80 ± 2.87	2.37 ± 0.63	46.04 ± 5.31	1.43 ± 0.09	101.67 ± 9.96	0.33 ± 0.05	0.95 ± 0.16
Day 4	14.89 ± 0.26	1.25 ± 0.08	82.36 ± 3.60	2.06 ± 0.56	49.70 ± 5.87	1.40 ± 0.11	118.41 ± 14.90	0.38 ± 0.05	0.95 ± 0.18
Day 5	14.64 ± 0.28	1.28 ± 0.07	78.79 ± 3.67	1.45 ± 0.27	44.92 ± 4.65	1.39 ± 0.10	91.41 ± 16.32	0.26 ± 0.06	1.10 ± 0.23
Day 6	14.62 ± 0.19	1.30 ± 0.08	78.18 ± 2.42	1.32 ± 0.26	40.49 ± 4.29	1.42 ± 0.10	102.81 ± 11.38	0.35 ± 0.05	1.11 ± 0.14
Day 13	14.55 ± 0.28	1.17 ± 0.08	81.14 ± 3.87	2.56 ± 0.62	44.79 ± 5.78	1.29 ± 0.12	98.83 ± 14.98	0.33 ± 0.06	1.34 ± 0.30
Day 20	14.79 ± 0.26	1.08 ± 0.08	86.31 ± 3.57	1.96 ± 0.34	47.32 ± 4.56	1.40 ± 0.09	101.22 ± 12.91	0.33 ± 0.06	0.97 ± 0.20
Day 27	15.05 ± 0.30	1.04 ± 0.07	89.79 ± 2.96	2.76 ± 0.71	42.29 ± 3.74	1.29 ± 0.08	121.48 ± 13.78	0.40 ± 0.04	1.00 ± 0.14
Day 34	14.94 ± 0.25	1.03 ± 0.07	89.07 ± 2.82	1.72 ± 0.37	45.26 ± 4.34	1.32 ± 0.08	96.50 ± 9.66	0.32 ± 0.05	0.97 ± 0.13

Grey boxes = statistically significant difference from pre-transfusion (day 1 - day 34) or from baseline (post-out and pre-transfusion), $P \leq 0.01$, n = 10 males, 4 females. Significance was determined using a pairwise, two-tailed, t-test with Bonferroni correction for comparison of 12 groups. *Ret% data for a subset of the participants, n = 8, was previously reported. [1]

References

1. Cox HD, Miller GD, Lai A, Cushman D, Eichner D. Detection of autologous blood transfusions using a novel dried blood spot method. Drug Test Anal. 2017;9(11-12):1713-1720.