Supplementary Online Content

Fowler III AA, Truwit JD, Hite RD, et al. Effect of vitamin C infusion on organ failure and biomarkers of inflammation and vascular injury in patients with sepsis and severe acute respiratory failure: the CITRIS-ALI randomized clinical trial. *JAMA*. doi:10.1001/jama.2019.11825

CITRIS-ALI Supplemental Material

This supplementary material has been provided by the authors to give readers additional information about their work.

Materials & Methods

1. **Vitamin C Preparation:** Vitamin C for Injection (Ascor®), is a colorless to pale yellow, preservative-free, hypertonic, sterile, and non-pyrogenic solution of ascorbic acid, containing no bacteriostatic or antimicrobial agent was supplied by McGuff Pharmaceuticals, Santa Ana, CA in amber-colored 50 ml vials, containing pH balanced L-Ascorbic Acid ($C_6H_8O_6$) for injection at a concentration of 500 mg/ml. McGuff Pharmaceuticals reviewed the manuscript prior to submission but had no role in the study design, study conduct, data analysis, or data interpretation.

2. **Subject Randomization/Vitamin C Preparation**: Subjects were randomized 1:1 to receive vitamin C or placebo, using randomization generated by the VCU Health Investigational Drug Service using the website: www.randomization.com. Study site Investigational pharmacies prepared study drug in 50 ml infusion bags containing dextrose 5% in Water (D5%W) under USP 797 standards and placed study drug in ICU refrigerators. Nursing administered study agent through a central or peripheral intravenous line. ICU nursing monitored subjects during and after study drug infusion. Subjects were monitored both by study coordinator and by site investigators. Investigators tracked and monitored any adverse event. All study drug doses were administered in the ICU. Record of study drug administration was recorded in specially designed case folders.

3. **Exclusion Criteria:** Patients were excluded from the CITRIS-ALI trial if any of the following 14 items were present. **1)** known allergy to vitamin C, **2)** inability to obtain informed consent, **3)** age less than 18 years, **4)** greater than 48 hours since meeting ARDS criteria, **5)** presence of diabetic ketoacidosis, **6)** surrogate or physician not committed to full support, **7)** pregnancy/breast-feeding, **8)** moribund patient not expected to survive 24 hours, **9)** home mechanical ventilation (via tracheostomy or noninvasive), **10**) home oxygen greater than 2 liters/minute, **11)** interstitial lung disease/diffuse alveolar hemorrhage, **12)** active kidney stone, **13)** non-English speaking, **14)** ward of the state.

4. **Plasma Vitamin C Quantification Method:** Plasma specimens were reduced with dithiothreitol (2mg/ml) followed by precipitation with 20% trichloroacetic acid then vortex-mixed and centrifuged. Supernate vitamin C levels were measured using high-pressure liquid chromatography (HPLC) with UV detection (via cGLP bioanalytical validation according to FDA guidelines on bioanalytical method validation). Chromatography was performed on a Zorbax SB-AQ, 4.6 x 150 mm, 5 µm column (Agilent Technologies, Santa Clara, CA), with a mobile phase using a gradient buffer (dipotassium phosphate), ion pairing reagent (tretrabutyl ammonium chloride), and acetonitrile at a flow rate 0.850 mL/min. Detection was at 265 nm and vitamin C levels quantified using peak area analysis and external standardization. Ascorbic acid standards (5 - 2,500 µM) were freshly prepared and treated in the same way as the test plasma samples. The limit of quantification was 5 µM.

5. **Luminex Assay Method:** Plasma biomarkers were analyzed using custom-designed Human Magnetic Luminex Screening Assays according to the manufacturer's instructions (R&D Systems, MN, USA). Levels of thrombomodulin (TM) and C-reactive protein (CRP) were measured using a Luminex LX200 instrument with xPONENT 3.1 software (Luminex Corp., Austin, TX). Biomarker concentrations were calculated from standard curves of Median Fluorescence Intensity (MFI) for each analyte by generating a five-parameter logistic (5-PL) curve-fit and multiplying by the dilution factor. Samples outside the standard range were further diluted and assayed again.

6. **Enrollment by trial site:** Virginia Commonwealth University Medical Center, 59, Cleveland Clinic Foundation, 45, Medical College of Wisconsin, 52, University of Kentucky, 11, Emory University, 3.

eTable 1. Vital Signs

Parameter	Time (h)	Vitamin C	Placebo
Heart rate mean (SD), bpm	0	94.6 (22.1)	101.0 (21.2)
	48	91.9 (20.1)	93.0 (22.7)
	96	92.3 (21.8)	93.5 (21.8)
	168	92.0 (19.6)	93.5 (15.2)
Temperature mean (SD), ℃	0	37.1 (1.1)	37.0 (1.1)
	48	37.0 (0.7)	37.0 (1.1)
	96	36.9 (0.9)	37.1 (0.7)
	168	37.0 (0.8)	37.1 (0.7)
Systolic blood pressure mean (SD), mm Hg	0	109 (15.5)	107 (18.2)
	48	117 (19.4)	116 (22.9)
	96	123 (19.8)	126 (24.2)
	168	121 (19.8)	123 (22.7)
Diastolic blood pressure mean (SD), mm Hg	0	57 (11.6)	57 (10.0)
	48	60 (10.9)	61 (11.7)
	96	64 (14.4)	65 (14.6)
	168	63 (12.8)	64 (14.0)

eTable 2. Adjusted All-Cause 28-Day Mortality

Patient Characteristic at Enrollment	HR	SE	Z-score	95% CI	P-value
Vitamin C Treatment vs. Placebo	.51	.16	-2.15	.28 – .94	.03
Age, each additional year	1.02	.01	2.01	1.00 – 1.04	.04
Female gender vs. Male	.91	.28	-.31	.50 – 1.65	.76
Race					
White	1 [Reference]				
Black	.39	.19	-1.93	.15 – 1.02	.05
Asian	–	–	–	–	–
American Indian	9.30	10.29	2.02	1.06 – 81.33	.04
Alaska Native	–	–	–	–	–
Pacific Islander	.42	.56	-.65	.03 – 5.57	.51
Patient Place of Residence					
Home independently	1 [Reference]				
Home with personal assistance	5.86	2.59	4.00	2.46 – 13.95	<0.001
Home with professional help	2.84	2.98	.99	.36 – 22.26	.32
Intermediate care or Rehabilitation facility	–	–	–	–	–
Skilled Nursing Facility	7.48	4.86	3.09	2.09 – 26.75	<0.001
Other	.86	.89	-.15	.11 – 6.57	.88
Sepsis Source					
Urinary	1 [Reference]				
Thorax	.95	.79	-.06	.19 – 4.80	.95
Abdomen	1.29	1.11	.29	.24 – 7.02	.77
Skin	–	–	–	–	–

Sinuses	–	–	–	–	–
Central Venous Catheters	–	–	–	–	–
Central Venous System	–	–	–	–	–
Unknown	1.06	1.18	.05	.12 – 9.37	.96
Other	.57	.73	-.44	.04 – 7.14	.66
mSOFA, each incremental unit	1.19	.07	2.79	1.05 – 1.34	.01
Use of Dialysis at enrollment	3.31	1.17	3.38	1.65 – 6.62	.001
ICU Admission Source					
Emergency Department	1 [Reference]				
Operating Room	.00	.00	.00	–	1.00
Ward, or Stepdown Unit	1.35	.61	.65	.55 – 3.29	.51
Other Special Care Unit	3.56	2.10	2.15	1.12 – 11.30	.03
Other Hospital	1.85	.63	1.81	.95 – 3.61	.07
Direct Admit	–	–	–	–	–

Multiple Cox proportional hazards model with adjusted Hazard Ratios (HRs) for all-cause 28-day mortality, adjusted for characteristics at the time of enrollment.

Abbreviations: CI: Confidence Interval; ICU: Intensive Care Unit; mSOFA: Modified Sequential Organ Failure Assessment score; SE: Standard Error.

eTable 3a. Mixed Effects Modeling: Ignoring Any Random Effects:

Covariance Parameter Estimates

	Estimate	Std.Err	Z-value	p-value
SP(POW)	0.9920	0.0009	1112.00	<0.0001
Residual	13.3248	1.0349	12.88	<0.0001

Fixed Effects Tests

	Numerator D.F.	Denominator D.F.	F-Value	p-value
Drug	1	179	0.01	0.9390
Hour	3	420	43.67	<0.0001
Drug*Hour	3	420	3.52	0.0152

Post-Hoc Difference Scores Tests From Model

	Estimate	Std.Err	t-Value	d.f.	p-value	95% CI
Placebo-Vit-C – 0 Hours	0.5159	0.5649	0.91	332	0.3618	(-0.5954, 1.6273)
Placebo-Vit-C – 48 Hours	0.5745	0.5781	0.99	351	0.3211	(-0.5626, 1.7115)
Placebo-Vit-C – 96 Hours	**0.3667**	**0.5907**	**0.62**	**359**	**0.5351**	**(-0.7950, 1.5284)**
Placebo-Vit-C – 168 Hours	-1.5991	0.6725	-2.38	419	0.0179	(-2.9210, -0.2772)

eTable 3b: Mixed Effects Modeling: Inclusion of Random Effect for Site:

Covariance Parameter Estimates

	Numerator D.F.	Denominator D.F.	F-Value	p-value
Drug	1	163	0.00	0.9990
Hour	3	291	58.32	<0.0001
Drug*Hour	3	291	3.74	0.0115

Fixed Effect Tests

	Numerator D.F.	Denominator D.F.	F-Value	p-value
Drug	1	163	0.00	0.9990
Hour	3	291	58.32	<0.0001
Drug*Hour	3	291	3.74	0.0115

Post Hoc Difference Score Tests From Model

	Estimate	StdErr	t-Value	d.f.	p-value	95% CI
Placebo-Vit-C – 0 Hours	0.5190	0.5654	0.92	327	0.3593	(-0.5932, 1.6312)
Placebo-Vit-C – 48 Hours	0.5771	0.5784	1.00	347	0.3191	(-0.5606, 1.7147)
Placebo-Vit-C – 96 Hours	0.3691	0.5911	0.62	355	0.5327	(-0.7934, 1.5316)
Placebo-Vit-C – 168 Hours	-1.5980	0.6731	-2.37	415	0.0180	(-2.9210, -0.2749)

eTable 3c: Mixed Effects Modeling: Inclusion of Random Effects for Subject Nested Within Site

Covariance Parameter Estimates

	Estimate	StdErr	Z-value	p-value
Site(Subject)	6.3945	1.3638	4.67	<0.0001
SP(POW)	0.9799	0.0053	183.20	<0.0001
Residual	7.0263	1.0056	6.99	<0.0001

Fixed Effect Tests

	Numerator D.F.	Denominator D.F.	F-Value	p-value
Drug	1	177	0.01	0.9430
Hour	3	420	43.69	<0.0001
Drug*Hour	3	420	3.52	0.0152

Post Hoc Difference Score Tests From Model

	Estimate	StdErr	t-Value	d.f.	p-value	95% CI
Placebo-Vit-C – 0 Hours	0.5159	0.5670	0.91	297	0.3636	(-0.5999, 1.6317)
Placebo-Vit-C – 48 Hours	0.5705	0.5811	0.98	316	0.3270	(-0.5728, 1.7137)
Placebo-Vit-C – 96 Hours	0.3715	0.5915	0.63	326	0.5304	(-0.7922, 1.5352)
Placebo-Vit-C – 168 Hours	-1.4602	0.6627	-2.20	394	0.0281	(-2.7631, -0.1573)

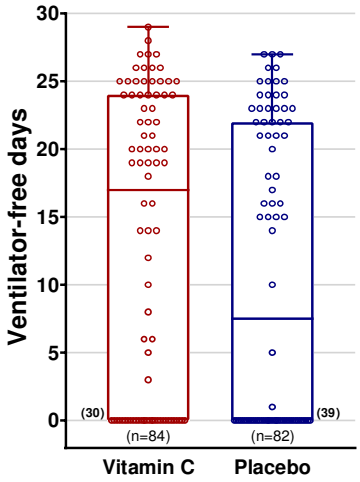
eTable 4. Missing Data Elements for Each Group Across Time

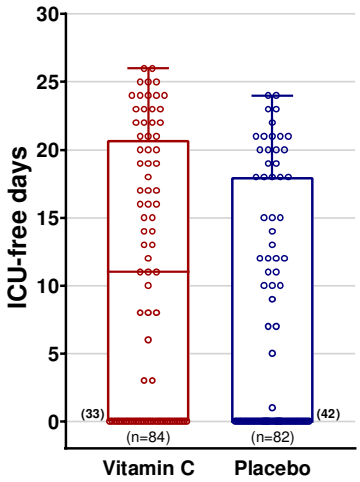
		Vitamin C (n=84)				Placebo (n=83)			
		0h	**48h**	**96h**	**168h**	**0h**	**48h**	**96h**	**168h**
Cumulative Died		0	2	4	14	1	12	19	25
Cumulative Graduated ICU		0	2	9	11	0	0	1	2
SOFA	Missing	10	21	30	51	5	21	29	47
	Mean (SD)	10.7 (3.7)	10.1 (5.0)	9.0 (4.9)	8.5 (4.7)	11.4 (3.6)	9.9 (4.4)	7.6 (4.3)	5.7 (3.8)
PaO_2/FiO_2	Missing	4	15	17	34	1	15	27	35
	Mean (SD)	2.7 (0.9)	2.0 (1.2)	1.9 (1.3)	1.6 (1.3)	2.5 (1.0)	1.9 (1.2)	1.7 (1.2)	1.1 (1.3)
GCS	Missing	0	4	9	28	0	10	17	30
	Mean (SD)	2.8 (1.0)	2.5 (1.2)	2.2 (1.4)	2.1 (1.3)	2.9 (0.8)	2.7 (1.0)	2.2 (1.2)	1.6 (1.4)
Cardiovascular	Missing	0	2	5	22	0	10	17	28
	Mean (SD)	2.4 (1.7)	2.0 (1.8)	1.2 (1.7)	1.1 (1.6)	2.7 (1.6)	1.9 (1.8)	1.0 (1.6)	0.5 (1.0)
Liver	Missing	6	10	21	39	4	16	20	41
	Mean (SD)	0.8 (1.2)	0.9 (1.3)	0.9 (1.3)	0.7 (1.3)	0.9 (1.1)	1.0 (1.2)	0.8 (1.2)	0.7 (1.2)
Platelets	Missing	0	2	7	21	0	10	18	28
	Mean (SD)	1.0 (1.2)	1.1 (1.3)	1.1 (1.3)	0.8 (1.1)	1.0 (1.3)	1.3 (1.3)	1.1 (1.3)	0.5 (1.0)
Creatinine	Missing	0	2	6	20	0	10	18	28
	Mean (SD)	1.0 (1.1)	1.0 (1.1)	0.8 (1.1)	0.8 (1.2)	1.2 (1.2)	0.9 (1.0)	0.7 (0.9)	0.9 (1.0)

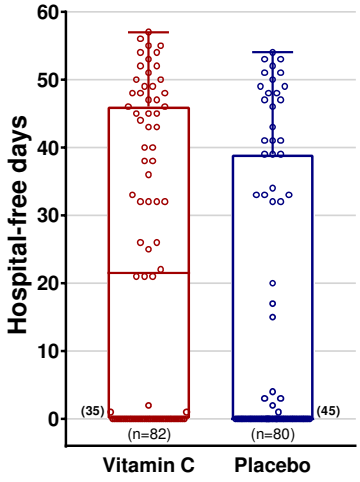
Abbreviations. SOFA: Sequential Organ Failure Assessment score, PaO_2/FiO_2: Ratio of arterial oxygen partial pressure to fractional inspired oxygen, GCS: Glasgow Coma Scale, CV: Cardiovascular, Cum: Cumulative, Grad: Graduated from the ICU (discharged alive), n Miss: Number of missing data elements, SD: Standard Deviation.

eTable 5: Power estimates for a variety of sample sizes, alpha=0.05 significance level, for multiple primary endpoints using the Holm-Bonferroni Correction

	Empirical Power					
	75/Group	**80/Group**	**85/Group**	**90/Group**	**95/Group**	**100/Group**
3 Co-Primary Endpoints: SOFA, CRP, TM	77%	77%	80%	80%	82%	83%







eFigure 1: Ventilator Free Days, ICU Free Days, Hospital Free Days Median values, the top and bottom of the boxes indicate the IQR with whiskers extending to the minimum and maximum. The circles indicate individual values as points superimposed on the graph. The small number at the bottom of the box indicates the number of zero values. The median value for the Placebo group in ICU free days and hospital free days equals 0, therefore the median lines in the graphs are not easily visible. Median ventilator free days to day 28 in Vitamin C-infused patients were not significantly different than Placebo patients (Vitamin C, median IQR, 17 (0-24) versus Placebo, median IQR, 7.5 (0-22), p=.11). Median ICU free days to day 28 in Vitamin C patients were significantly greater than Placebo patients (Vitamin C, median IQR, 11 (0-21) versus Placebo, median IQR, 0 (0-18), p=.03). Median hospital free days to day 60 in Vitamin C patients were significantly greater than Placebo patients (Vitamin C, median IQR, 22 (0-46) versus Placebo, median IQR, 0 (0-39), p<0.05).