High-Dose IV Hydroxocobalamin (Vitamin B12) in Septic Shock

A Double-Blind, Allocation-Concealed, Placebo-Controlled Single-Center Pilot Randomized Controlled Trial (The Intravenous Hydroxocobalamin in Septic Shock Trial)

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BACKGROUND: Elevated hydrogen sulfide (H_2S) contributes to vasodilatation and hypotension in septic shock, and traditional therapies do not target this pathophysiologic mechanism. High-dose IV hydroxocobalamin scavenges and prevents H_2S formation, which may restore vascular tone and may accentuate recovery. No experimental human studies have tested high-dose IV hydroxocobalamin in adults with septic shock.

RESEARCH QUESTION: In adults with septic shock, is comparing high-dose IV hydroxocobalamin with placebo feasible?

STUDY DESIGN AND METHODS: We conducted a phase 2 single-center, double-blind, allocation-concealed, placebo-controlled, parallel-group pilot randomized controlled trial comparing high-dose IV hydroxocobalamin with placebo in critically ill adults with septic shock. Patients meeting Sepsis 3 criteria were randomized 1:1 to receive a single 5-g dose of high-dose IV hydroxocobalamin or equivalent volume 0.9% saline solution as placebo. The primary outcome was study feasibility (enrollment rate, clinical and laboratory compliance rate, and contamination rate). Secondary outcomes included between-group differences in plasma H_2S concentrations and vasopressor dose before and after infusion.

RESULTS: Twenty patients were enrolled over 19 months, establishing an enrollment rate of 1.05 patients per month. Protocol adherence rates were 100% with zero contamination. In the high-dose IV hydroxocobalamin group, compared to placebo, there was a greater reduction in vasopressor dose between randomization and postinfusion (-36% vs 4%, $P < .001$) and randomization and 3-h postinfusion (-28% vs 10%, $P = .019$). In the high-dose IV hydroxocobalamin group, the plasma H_2S level was reduced over 45 mins by -0.80 ± 1.73 μM, as compared with -0.21 ± 0.64 μM in the placebo group ($P = .3$).

INTERPRETATION: This pilot trial established favorable feasibility metrics. Consistent with the proposed mechanism of benefit, high-dose IV hydroxocobalamin compared with placebo was associated with reduced vasopressor dose and H_2S levels at all time points and without serious adverse events. These data provide the first proof of concept for feasibility of delivering high-dose IV hydroxocobalamin in septic shock.

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KEY WORDS: critical care; feasibility; hydroxocobalamin; hydrogen sulfide; ICU; outcomes; sepsis; septic shock; vasodilatory shock; vitamin B12

ABBREVIATIONS: H_2S = hydrogen sulfide; IQR = interquartile range; MAP = mean arterial pressure; NO = nitric oxide; RCT = randomized controlled trial; SDB = sulfide dibimane

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Take-home Points

Study question: In adults with septic shock, is comparing high-dose (5-g) IV hydroxocobalamin with placebo feasible?

Results: Comparing high-dose IV hydroxocobalamin is feasible, and patients who received IV hydroxocobalamin, compared with those who received placebo, showed reduced vasopressor dose and plasma hydrogen sulfide levels.

Interpretation: Consistent with the proposed mechanism of benefit, these data provide the first proof of concept for delivering high-dose IV hydroxocobalamin as a targeted therapy for septic shock.

Septic shock is a form of vasodilatory shock that affects millions of people worldwide and kills more than 40% of the afflicted.[1] IV fluid resuscitation, timely and empiric broad-spectrum antibiotics, and supportive care are cornerstones in the management of septic shock. Currently, no guideline-recommend therapies directly target the gasotransmitter-related mechanisms that cause and potentiate septic shock.

Plasma concentrations of nitric oxide (NO) and hydrogen sulfide (H_2S) are elevated early in sepsis, contribute to vasodilation and circulatory dysfunction, and are associated with poor outcomes in septic shock.[2-4] High-dose (5 g) IV hydroxocobalamin (vitamin B12) scavenges and prevents NO and H_2S formation and has the potential to reduce capillary leak, to promote capillary membrane stabilization, and to accentuate recovery.[5-7] Preclinical studies found that high-dose IV hydroxocobalamin improved mean arterial pressure (MAP) and survival compared with placebo.[8,9] Although preclinical studies have demonstrated the potential benefit of high-dose IV hydroxocobalamin, no experimental human studies have evaluated its impact in septic shock.[10]

The purpose of this phase 2 single-center pilot randomized controlled trial (RCT) was to establish the feasibility and fidelity of comparing high-dose hydroxocobalamin with placebo in patients with septic shock. We hypothesized that this trial would be feasible based on an enrollment rate of at least 0.7 patients per month, protocol compliance rate of $\geq$ 90%, and contamination rate of $\leq$ 10%.

Study Design and Methods

Trial Design

We conducted a phase 2 single-center, double-blind, placebo-controlled, parallel-group pilot RCT comparing high-dose IV hydroxocobalamin with placebo in critically ill patients with septic shock. The institutional review board, committee 5, at the Medical College of Wisconsin approved the study protocol (Identifier: PR000032950). An independent data safety monitoring board oversaw the study for serious adverse events. This study was registered with www.clinicaltrials.gov (Identifier: NCT03783091). The Food and Drug Administration granted hydroxocobalamin an exemption from investigational new drug regulations (Identifier: 143695).

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Site and Study Participants

The pilot study was conducted in the medical and surgical ICUs at Froedtert and the Medical College of Wisconsin. The medical ICU is a closed 26-bed unit staffed by two teams, each led by an academic intensivist. The surgical ICU is a closed 20-bed unit staffed by one team led by an academic intensivist. We enrolled adults 18 years of age or older within 48 h of medical or surgical ICU admission and with a primary diagnosis of septic shock receiving norepinephrine infusion of 0.10 μg/kg/min for a minimum of 15 min (or equivalent dose phenylephrine, epinephrine, or dopamine).[11] Septic shock was defined by Sepsis 3 criteria, based on (1) hypotension despite IV fluid resuscitation of at least 30 mL/kg, (2) vasopressor use, (3) lactic acidosis (lactic acid $>$ 2 mM), or (4) a combination thereof. We excluded patients with known hypersensitivity to any component of the hydroxocobalamin formulation (risk outweighs benefit), pregnancy (potential teratogenic), those with a history of urinary calcium oxalate crystals (risk outweighs benefit), moribund patients (benefit not actualized) as deemed by the attending physician, and those with active hemolysis or bleeding requiring two or more hemoglobin measurements per day.

Screening, Consent, and Randomization

The study team screened all adults admitted to the medical and surgical ICUs who were receiving vasopressors to determine if they met septic shock criteria Monday through Friday between 8:00 AM and 5:00 PM. All patients or their legally authorized representative provided written informed consent. A randomized allocation concealment sequence was created using OnCore software (Advarra). The randomization scheme consisted of a sequence of five blocks such that each block contained six treatment arms (A, B, C, D, E,

and G). Three of the arms (letters) were assigned to the treatment and three of the arms were assigned to the placebo, and only the investigational pharmacy was aware of the arm assignments. Each block's arm sequence was assigned in random order using the OnCore randomization algorithm.

Masking

Study and treatment teams, patients, surrogate decision-makers, data collectors, and adjudicators were masked. The investigational pharmacy was aware of the study assignment to prepare the appropriate infusion (high-dose IV hydroxocobalamin or 0.9% placebo) for administration. The process to prepare and mask the intervention and placebo can be found in e-Appendix 1.

Data Collection

Baseline demographic, clinical, biochemical, and outcomes variables were captured and collected by a masked member of the study team using the electronic medical record and exported onto a secure web-based application (Research Electronic Data Capture).

Baseline demographic variables included age, sex, and race. Clinical variables included quantity of IV fluids in the preceding 24 h (in liters); time to first antibiotic (in hours); severity of illness scores, including the Sequential Organ Failure Assessment score[12] and Acute Physiology and Chronic Health Evaluation II score,[13] and Charlson Comorbidity Index[14]; norepinephrine (or equivalent, or both) dose (in micrograms per kilogram per min)[11] at the time of consent, at randomization, 30 min after infusion, and 3 h after infusion; and source of sepsis. Biochemical variables included serum lactic acid (millimole per liter), serum leukocyte count (10^3 per microliter), serum creatinine (milligrams per deciliter), and monobromobimane-reactive H_2S level (micromole per liter). Outcome variables collected include enrollment rate, study protocol compliance rate, study arm contamination rate, duration of norepinephrine (or equivalent) use (in days and hours), duration of mechanical ventilation (in days), duration of ICU stay (in days), ICU and hospital mortality (yes or no), and complications (yes or no).

Study Interventions

In the intervention group, a single 5-g dose of high-dose IV hydroxocobalamin (200 mL) was infused over 15 min through a central venous catheter within 24 h of ICU admission. In the placebo group, an equivalent volume (200 mL) of 0.9% saline solution was infused over 15 min through a central venous catheter within 24 h of ICU admission. In both groups, best practices for management of sepsis and septic shock were used, including resuscitation with crystalloid of at least 30 mL/kg within the first 3 h of sepsis recognition, obtaining microbiologic cultures before starting antimicrobial therapy, initiating broad-spectrum antibiotics as soon as possible after recognition of sepsis, infectious source control as soon as medically and logistically practical after diagnosis, and use of low tidal volume (6 mL/kg predicted body weight) ventilation in patients with acute respiratory distress syndrome.[15] Norepinephrine was the first-choice vasopressor for all patients and was titrated to achieve a MAP of 65 mm Hg.[15] Epinephrine, phenylephrine, and vasopressin were initiated and titrated at the discretion of the care team.

Plasma Sulfide Measurements

Because high-dose IV hydroxocobalamin has been shown to reduce plasma H_2S concentrations and previous reports indicate increased H_2S concentrations are inversely proportional to BP and patient survival, we chose to evaluate the impact of high-dose IV hydroxocobalamin on plasma H_2S concentrations in patients with septic shock. In both groups, we measured monobromobimane-reactive H_2S at the time of randomization and 30 min after IV infusion of high-dose IV hydroxocobalamin. Full details of monobromobimane-reactive H_2S measurement can be found in e-Appendix 1.

Study Outcomes

Enrollment began in July 2019 with an initial primary outcome of a 15% reduction in vasopressor support. However, enrollment was paused in March 2020 because of the COVID-19 pandemic, and the primary outcome was changed to establish feasibility criteria for future trial design. Feasibility criteria included enrollment rate (per month), clinical and laboratory protocol compliance rate, and contamination rate. Contamination rate was defined as the number of times the intervention (hydroxocobalamin) was administered to a patient randomized to placebo and the number of times the placebo was administered to a patient randomized to the intervention. A convenience sample of 20 patients was chosen for the primary outcome of establishing enrollment rate (per month), compliance rate with clinical and laboratory protocols, and contamination rate.[16]

Secondary outcomes included change in monobromobimane-reactive H_2S levels between time of randomization and 30-min after infusion and norepinephrine (or equivalent) dose between time of randomization and 30 min and 3 h after infusion between groups and between survivors and nonsurvivors.

Tertiary outcomes of relevance for future clinical trials included the duration of norepinephrine (or equivalent) use, vasopressor-free days (number of days alive and free of vasopressors of 28 days), ICU-free days (number of days alive and out of the ICU of 28 days), ventilator-free days (number of days alive and free of mechanical ventilation of 28 days), ICU mortality, hospital mortality, and persistent organ dysfunction syndrome or death at 28 days.[17,18] Persistent organ dysfunction plus death at 28 days is defined as the persistence of organ dysfunction and is present when a critically ill patient is receiving a vasopressor, dialysis, mechanical ventilation, or a combination thereof at the outcome assessments time point.[17]

Safety Data

We monitored for serious adverse events of allergic reactions and renal failure with calcium oxalate crystalluria.[19] In addition, high-dose IV hydroxocobalamin has been reported (in vitro) to interfere with laboratory assays, particularly causing artificially increased hemoglobin, bilirubin, triglycerides, cholesterol, total protein, albumin, and alkaline phosphatase in $>$ 10% of cases on at least one analyzer.[20] We monitored for laboratory interference by asking the clinical care team to report laboratory test results that were out of range up to 48 h after infusion.

Statistical Methods

The original sample size estimation used the variability values from the Angiotensin II for the Treatment of Vasodilatory Shock 3 trial.[21] With 13 patients per group, the study would have 80% power to detect a 15% difference in the percent change of norepinephrine at 3 h between the two groups at a one-sided 5% significance level. The COVID-19 pandemic had a profound impact on study execution, as follows: (1) the pandemic halted all nonessential research activities in March 2020, (2) all clinical research staff thereafter were working remotely, (3) clinical workload increased to optimize pandemic preparedness and infection control activities, (4) the types of patients admitted to the ICUs changed substantially after the start of the COVID-19 pandemic, and (5) we did not receive an extension in funding. As per the CONSORT and SPIRIT Extension for RCTs Revised in Extenuating Circumstances guidelines, these extenuating circumstances required us to change the statistical plan (mitigation strategy) to minimize the effects of the circumstances on study

execution.[22] Accordingly, we changed the primary outcome to feasibility outcome as described above. The study was completed with a convenience sample of 20 representative patients from a single center to establish enrollment rate per month, clinical and laboratory protocol compliance rate, and contamination rate in the setting of multiple periods of surge in COVID-19 admissions during the pandemic. Based on a previous RCT conducted in a similar patient population at the same institution, feasibility was defined by an enrollment rate of at least 0.7 patients per month, protocol compliance rate of $\geq$ 90%, and contamination rate of $\leq$ 10%.[23]

All analyses were performed on an intention-to-treat basis using all randomized patients. Mean $\pm$ SD, median (interquartile range), and proportions (95% CI) were used to summarize demographic and clinical characteristics. Categorical variables were compared between groups using the Fisher exact test. Continuous variables were compared between groups using the Wilcoxon rank-sum test. The enrollment rate was estimated as the observed rate with Poisson distribution-based 95% CIs. The compliance and contamination probabilities were estimated as the observed proportions with exact 95% binomial CIs. Change in vasopressor dose was quantified as percent change compared with randomization and compared between groups using Wilcoxon rank-sum test. The same test was used to compare all continuous outcomes, such as changes in plasma H_2S levels, length of ICU stay, ICU-free days, ventilator-free days, and vasopressor-free days. The Fisher exact test was used for binary outcomes such as hospital mortality and complications. Aside from the key secondary outcomes, all other analyses were hypothesis generating, and therefore, no adjustments were made for multiple tests of significance. Interim analyses were not performed. Analyses were performed using R version 4.0.3 software (R Foundation for Statistical Computing).

Results

Recruitment and Baseline Patient Data

Study enrollment began on July 19, 2019, paused on March 16, 2020, because of the COVID-19 pandemic, resumed on November 11, 2020, and was completed on October 28, 2021. Over the 19 months of enrollment, 1,234 patients were screened, 70 were eligible, and 20 patients gave consent and were randomized. Ten patients were randomized to each study arm. All randomized patients were analyzed in accordance with their assigned arm and were followed up through hospital discharge. Figure 1 shows the process of patient screening, eligibility, randomization, and analysis by arm. Table 1 shows baseline patient characteristics, which were similar between groups.

Primary Outcome

Twenty patients were enrolled over 19 months, corresponding to an enrollment rate of 1.05 patients/mo (95% CI, 0.66-1.58 patients/mo). Twenty patients (100%; 95% CI, 83%-100%) completed the assigned

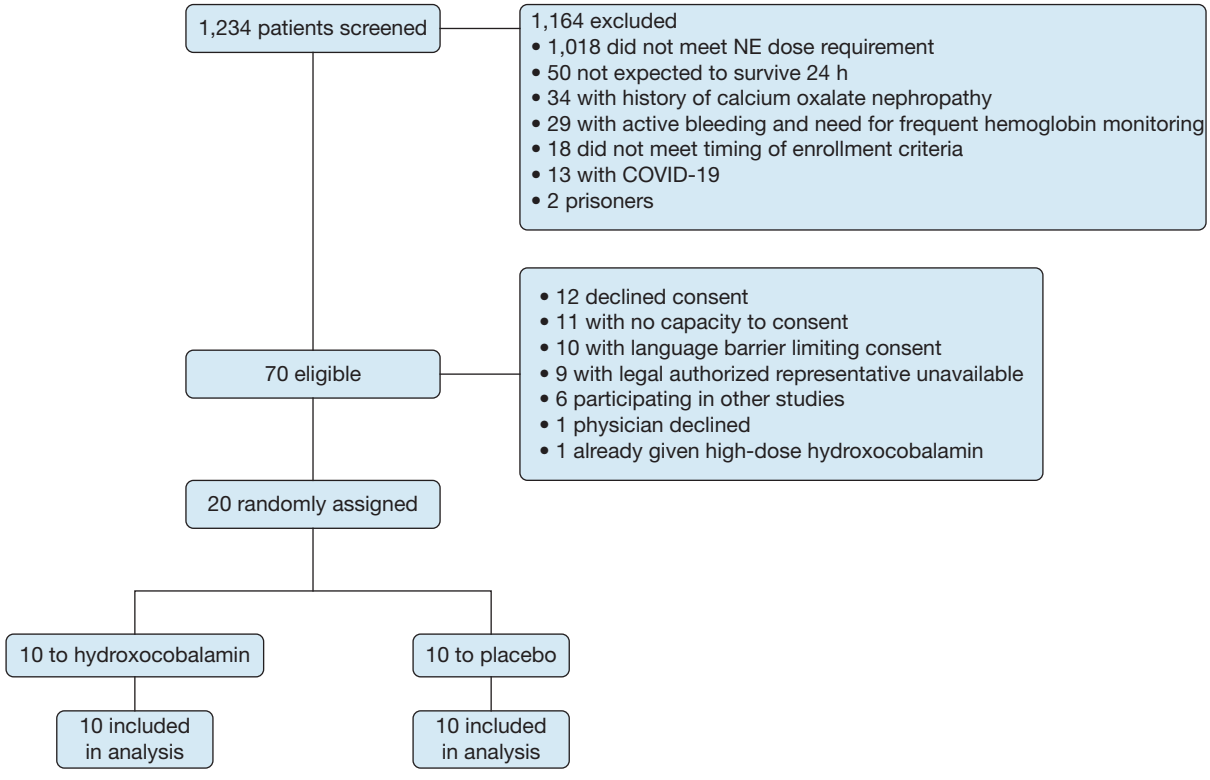

Figure 1 – *Consolidated Standards of Reporting Trials diagram showing the process of patient screening, eligibility, randomization, and analysis by arm.*

intervention and laboratory protocols. The contamination rate was zero (95% CI, 0%-16%), and no patient was lost to follow-up.

Secondary Outcomes

The high-dose IV hydroxocobalamin group, compared with the placebo group, showed a greater reduction in vasopressor dose between randomization and 30 min after infusion (–36% [interquartile range (IQR), –48% to –31%] vs 4% [IQR, –5% to 13%]; $P < .001$) and randomization and 3 h after infusion (–28% [IQR, –67% to –12%] vs 10% [IQR, –14% to 49%]; $P = .019$) (Table 2). Figure 2 shows change in vasopressor dose between groups.

For the entire cohort, the baseline monobromobimane-reactive H_2S level was 5.88 ± 2.69 μM. In the high-dose IV hydroxocobalamin and placebo groups, the baseline monobromobimane-reactive H_2S levels were 6.25 ± 3.49 μM and 5.52 ± 1.89 μM, respectively. In the high-dose IV hydroxocobalamin group, the monobromobimane-reactive

TABLE 1] Baseline Patient Characteristics

Characteristic	Hydroxocobalamin (n = 10)	Placebo (n = 10)
Age, y	64 (58-74)	57 (51-72)
Female sex	5 (50)	5 (50)
Race		
White	8 (80)	8 (80)
Black	2 (20)	2 (20)
Type of patient		
Medical ICU	9 (90)	8 (80)
Surgical ICU	1 (10)	2 (20)
Heart failure	2 (20)	3 (30)
COPD	1 (10)	2 (20)
Cancer	2 (20)	1 (10)
Diabetes	5 (50)	4 (40)
Hypertension	8 (80)	5 (50)
Charlson comorbidity index score	4 (2-6)	4 (2-5)
Mechanical ventilation	9 (90)	8 (80)
Total IV fluid in previous 24 h, L	2.50 (2.0-3.38)	3.0 (1.50-3.75)
Time to first antibiotic, h	2.47 (1.09-4.27)	3.82 (2.41-4.33)
Norepinephrine alone	3 (30)	2 (2)
Norepinephrine and vasopressin	0 (0)	1 (10)
Norepinephrine and vasopressin and epinephrine	7 (70)	7 (70)
Total norepinephrine equivalent dose at T0, μm/kg/min	0.29 (0.20-0.36)	0.34 (0.24-0.51)
Inotropic agent	4 (40)	3 (30)
Serum lactic acid, mM	4.5 (3.2-7.2)	3.6 (2.4-8.0)
APACHE II score	28 (26-35)	25 (20-37)
SOFA score	14 (10.2-14)	14 (8.2-14.8)
Source of sepsis		
Abdominal	0 (0)	3 (30)
Bloodstream	1 (10)	4 (40)
CNS	1 (10)	0 (0)
Other	2 (20)	1 (1)
Pulmonary	7 (70)	3 (30)
Skin	1 (10)	1 (1)
Stress dose steroids	8 (80)	9 (90)

Data are presented as No. (%) or median (interquartile range). APACHE II = Acute Physiologic and Chronic Health Evaluation; SOFA = sequential organ failure assessment; T0 = at randomization.

H_2S level was reduced over 45 min (15-min infusion, 30-min observation) by −0.80 ± 1.73 μM compared with −0.21 ± 0.64 μM in the placebo group ($P = .3$). Among survivors, the monobromobimane-reactive H_2S level was reduced by −1.29 ± 1.96 μM in the high-dose IV hydroxocobalamin group compared with −0.12 ± 0.82 μM in the placebo group ($P = .24$). Figure 3 shows change in monobromobimane-reactive H_2S level between groups.

Exploratory Tertiary Outcomes

In this pilot study, no statistically significant differences were found in hospital mortality, ICU mortality, ICU-free days, or vasopressor-free days (Table 3).

Harm

No serious adverse events (new acute kidney injury secondary to oxalate nephropathy or unexpected death) or reports of color interference with laboratory assays occurred.

Discussion

We conducted, to our knowledge, the first human RCT comparing high-dose IV hydroxocobalamin (vitamin B12) with placebo in critically ill patients with septic shock. Our pilot feasibility trial established favorable metrics for enrollment rate, protocol compliance rate, and contamination rate. In addition, high-dose IV hydroxocobalamin, compared with placebo, was associated with reduced norepinephrine dose at all a priori defined time points and a clear trend toward reduced monobromobimane-reactive H_2S levels consistent with the proposed mechanism of effect. No serious adverse events occurred. These data provide the first proof of concept for feasibility in delivering high-dose IV hydroxocobalamin in a randomized controlled trial for septic shock targeting the H_2S-related mechanism of vasodilatory shock.

Despite the COVID-19 pandemic, including three surges in COVID-19 critical care patient admissions leading to reduced study team capacity for screening and enrollment, we screened > 1,200 patients receiving norepinephrine and we established a single-center enrollment rate of > 1 patient/mo. Before the pandemic, between July 2019 and February 2020, we enrolled 10 patients over a period of 8 months, with an enrollment rate of 1.25 patients/mo. In determining the overall enrollment rate in 94 contemporary RCTs in critically ill patients, Krutsinger et al[24] reported a mean enrollment rate of 0.83 patients/mo/site (95% CI, 0.57-1.21 patients/mo/site). In an analysis of 23 RCTs, Nalamalapu et al[25] reported an enrollment rate of 0.90 patients/site/mo (95% CI, 0.50-1.79 patients/site/mo) and concluded a recruitment rate of approximately 1 patients/site/mo in a multicenter RCT is a reasonable expectation. Our accrual was limited to weekdays in the daytime, strongly supporting higher accrual rates with dedicated research staffing. Although the study design shifted from clinical outcome to feasibility (changing N = 26 to N = 20), the pharmacologic effect was sufficient to be definite statistically despite the small loss in power. Our enrollment rate before the pandemic of 1.25 patients/mo and overall enrollment rate of 1.05 patients/mo lend credibility to the feasibility of conducting both a phase 2 single-center safety and efficacy trial and a phase 3 multicenter efficacy trial.

Clinically, septic shock is recognized by hypotension requiring vasopressor support and lactic acidosis. Multiple preclinical studies have demonstrated that high-dose IV hydroxocobalamin improves MAP and survival in septic shock.[26,27] In an RCT in a swine model

TABLE 2] Summary of Vasopressor Dose and Change in Dose at T0, T1, T2, and T3

Characteristic	Hydroxocobalamin (n = 10)	Placebo (n = 10)	*P* Value
Total norepinephrine dose, μg/kg/min			
T0[a]	0.29 (0.20-0.36)	0.34 (0.24-0.51)	.4
T1	0.25 (0.20-0.38)	0.31 (0.20-0.54)	.7
T2	0.14 (0.10-0.21)	0.30 (0.20-0.72)	.01
T3	0.13 (0.10-0.21)	0.26 (0.17-0.90)	.06
% Change			
T1 to T2	−36 (−48 to −31)	4 (−5 to 13)	< .001
T1 to T3	−28 (−67 to −12)	10 (−14 to 49)	.01

Data are presented as median (interquartile range), unless otherwise indicated. T0 = at randomization; T1 = 1 min before start of hydroxocobalamin or placebo infusion; T2 = 30 min after infusion; T3 = 3 h after infusion.
[a]Includes dose of norepinephrine and norepinephrine equivalent when other vasopressors were used, as per Goradia et al[11].

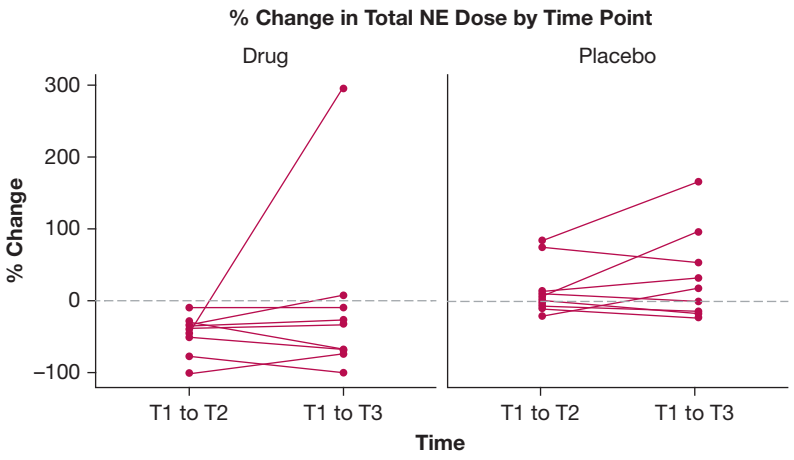

Figure 2 – *Graph showing between-group differences (percent change) in vasopressor dose between T1 and T2 and between T1 and T3. T1 = randomization; T2 = 30 min after infusion; T3 = 3 h after infusion. NE = norepinephrine.*

of septic shock, Bebarta et al[8] found pigs who received high-dose IV hydroxocobalamin, as compared with saline, showed higher MAP and lower serum inflammatory markers. In humans, case series have reported near immediate and sustained improvement in MAP in critically ill patients with vasodilatory shock who received high-dose IV hydroxocobalamin.[20] In our study, patients with septic shock who received high-dose IV hydroxocobalamin, compared with those who received placebo, showed a greater percentage change in vasopressor dose at randomization and 30 min after infusion (–36% vs 4%; $P \leq .001$) and randomization to 3 h after infusion (–28% vs 10%; $P = .019$). The total duration of norepinephrine (43 h vs 60 h; $P = .4$) and difference in vasopressor-free days (18 days vs 22 days; $P = .81$) did not meet statistical significance, but the differences may be clinically significant. Our trial found no differences in ICU mortality, hospital mortality, ICU-free days, or ventilator-free days; however, the trial was not powered for a difference in these outcomes, and a larger trial is be needed to evaluate for such differences.

At the circulatory and cellular level, septic shock is hallmarked by NO and H_2S-mediated vasodilation.[20,28] Hydrogen sulfide has been implicated in directly increasing vascular permeability and activating endothelial NO synthase to increase NO production.[20] Higher total plasma levels of H_2S, compared with lower levels, in early septic shock are correlated inversely with BP and are associated with higher mortality.[29-33] Because high-dose IV hydroxocobalamin binds free sulfide, we measured plasma monobromobimane-reactive H_2S levels. The baseline monobromobimane-reactive H_2S level for the entire cohort was

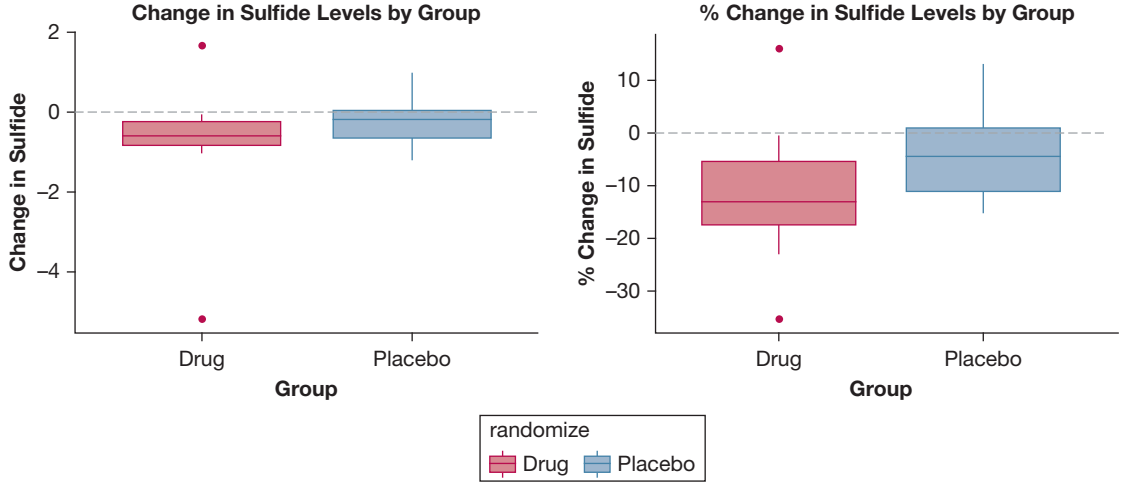

Figure 3 – *Box-and-whisker plots showing between-group differences (absolute difference and percent change) in plasma hydrogen sulfide levels before and after infusion (of intervention and placebo).*

TABLE 3] Secondary Outcomes

Outcome	Hydroxocobalamin (n = 10)	Placebo (n = 10)	*P* Value
Hospital mortality	4 (40	4 (40)	> .99
ICU mortality	3 (30)	4 (40)	> .99
Ventilator-free days	12 (0-25)	12 (0-18)	.97
ICU-free days	10 (0-21)	6 (0-17)	.63
New renal replacement therapy	3 (30)	4 (40)	> .99
Vasopressor-free days	18 (0-26)	22 (0-26)	.81
Duration of norepinephrine in survivors, n = 6, h	57 (21-375)	66 (18-192)	.4
PODS at 28 d	5 (50)	4 (40)	> .99
Serious adverse events[a]	0 (0)	0 (0)	NA
Reported laboratory interference	0 (0)	0 (0)	NA

Data are presented as No. (%) or median (interquartile range), unless otherwise indicated. NA = not applicable; PODS = persistent organ dysfunction syndrome.
[a]Serious adverse events include unexpected death and new acute kidney injury related to calcium oxalate nephropathy after randomization.

5.88 ± 2.69 μM, which is 4.5-fold higher than that observed in healthy humans (1.3 ± 1.5 μM).[34] In our study, patients with septic shock who received high-dose IV hydroxocobalamin, compared with those who received placebo, showed a numerically greater reduction in monobromobimane-reactive H_2S between randomization and 30 min after infusion (–13% [95% CI, –17% to –6%] vs –3% [95% CI, –11% to 1%]; $P = .11$). Similarly, among survivors, patients who received high-dose IV hydroxocobalamin, compared with those who received placebo, showed a numerically greater reduction in the monobromobimane-reactive H_2S level (–12% [95% CI, –21% to –6%] vs –2% [95% CI, –11% to 10%]; $P = .13$). Previous studies examining the role of plasma H_2S levels found that the total level correlates with norepinephrine dose and severity of illness.[3,33] However, no study before ours evaluated the impact of high-dose IV hydroxocobalamin on free H_2S levels in septic shock. Although we identified a numerically greater reduction in monobromobimane-reactive H_2S level between groups, a meaningful clinically important difference is unclear, and further studies are needed to confirm our exploratory findings.

The strengths of our randomized study include concealed allocation; masking to patients, clinicians, and members of the study team; comparison of an intervention art with a placebo-controlled arm; intention to treat with zero loss of follow-up; and incorporation of a biologically plausible metric for mechanism. Study limitations include a small cohort from a single center, which limits the ability to detect differences in clinical outcomes and to conduct meaningful subgroup analyses to identify groups of patients with septic shock who may benefit from high-dose IV hydroxocobalamin. Second, we excluded 1,164 of 1,234 screened patients (receiving norepinephrine) and did not record the cause of shock for the excluded patients. Despite these limitations, we identified 70 eligible patients and achieved, in the setting of a pandemic and limited research staff, a priori defined feasibility metrics and an enrollment rate (> 1 patient/mo) commensurate with most sepsis trials.[24] Third, despite taking measures to conceal the two interventions, patients receiving high-dose IV hydroxocobalamin can demonstrate chromaturia, which may be visible to patients and health-care providers. However, all study team members were quarantined from the bedside to remain masked to the intervention, and vasopressors were titrated to a MAP of > 65 mm Hg. Fourth, we did not assess concomitant cardiac dysfunction, and it is unclear how high-dose IV hydroxocobalamin impacts the subset of patients with septic shock who demonstrate cardiac dysfunction compared with those without cardiac dysfunction. However, as in other micronutrient trials,[35-37] we included patients in septic shock using current (Sepsis 3) criteria, and future trials could establish subgroups based on the presence or absence of cardiac dysfunction. Fifth, patients receiving high-dose IV hydroxocobalamin received antibiotic 1 h earlier than the placebo group. Finally, we did not randomize patients to different high-dose IV hydroxocobalamin doses or infusion times. High-dose IV hydroxocobalamin has a half-life of 26 to 31 h and was dosed at a concentration 100-fold higher than accumulated H_2S concentrations measured in

plasma. Lower doses may suffice, but require further study. However, the primary outcome of the study was to establish feasibility metrics, including enrollment rate, to inform future phase 2 and 3 efficacy study design, which could include testing different doses and infusion times.

Interpretation

This phase 2 double-blind, placebo-controlled single-center pilot RCT evaluating high-dose IV hydroxocobalamin in septic shock established both feasibility and hypothesis-generating outcomes data to inform phase 2 efficacy trial design.

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Additional information: The e-Appendix and e-Figures are available online under "Supplemental Data."

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