A PHASE II, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY FOR HIGH-DOSE HYDROXOCOBALAMIN (VITAMIN B12A) FOR VASOPRESSOR REFRACTORY HYPOTENSION FOLLOWING CARDIOPULMONARY BYPASS

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A PHASE II, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY FOR HIGH-DOSE HYDROXOCOBALAMIN (VITAMIN B12A) FOR VASOPRESSOR REFRACTORY HYPOTENSION FOLLOWING CARDIOPULMONARY BYPASS

Study Product: *Hydroxocobalamin (Vitamin B12a), marketed as Cyanokit®*

Protocol Number: (IRBe) *17-011130*

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List of Abbreviations

LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
CFR	Code of Federal Regulations
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
IND	Investigational New Drug Application
IRB	Institutional Review Board
LOS	Length of Stay
PHI	Protected Health Information
PI	Principal Investigator
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure

Study Summary

Title	A phase II, randomized, double-blind, placebo-controlled study for High-dose Hydroxocobalamin (Vitamin B12a) for Vasopressor Refractory Hypotension Following Cardiopulmonary Bypass
Running Title	Vitamin B12a Vasoplegic Syndrome
Protocol Number	17-011130
Phase	Clinical study phase II
Methodology	Study design type: double blind; Randomized, placebo
Overall Study Duration	2 years
Subject Participation Duration	30 days
Single or Multi-Site	Single
Objectives	To demonstrate an improvement in arterial blood pressure and a decrease in vasopressor requirement with administration of B12a in patients with post-cardiotomy vasoplegic syndrome on high-dose vasopressors.
Number of Subjects	50
Diagnosis and Main Inclusion Criteria	Vasoplegic syndrome – inclusion criteria are patients with vasoplegic syndrome as defined below who are also on high-dose vasopressors.
Study Product, Dose, Route, Regimen	Hydroxocobalamin marketed as Cyanokit®, 5g, IV, Infused once over 15 min. Unblinded dose of Cyanokit® can be ordered within the first 4 hours at the discretion of the primary care team.
Duration of Administration	Infuse drug over 15 minute period.
Reference therapy	Placebo
Statistical Methodology	

1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with the applicable United States government regulations and Mayo Clinic research policies and procedures.

The primary aim of this study is to demonstrate an improvement in mean arterial blood pressure over the first 4 hours after administration of B12a as a 5g infusion. Secondary aims include evaluation for a decrease in vasopressor requirement in patients with post-cardiotomy vasoplegic syndrome on high-dose vasopressors after administration of B12a as a 5g infusion over the first 4 hour. Additional secondary aims include assessment of the effect of B12a on other important clinical endpoints and to potentially assist in power analysis and study design in larger future clinical trials. These endpoints include indices of perfusion such as lactate, and serum acid base balance, time to discharge from the ICU, multi-organ dysfunction, need for mechanical circulatory support, coagulopathy and transfusion requirements as well as mortality.

Significance:

VS is a common complication after cardiopulmonary bypass (CPB), occurring in up to 8-12% of cases[1-4]. It remains a clinical diagnosis with a significant burden of disease. One series reports a mortality of 24% and another at 25% in patients with prolonged catecholamine refractory VS[5, 6]. In addition prolonged intensive care unit (ICU) and hospital stays as well as the risks associated with increased catecholamine administration result in further increased morbidity. Calculating the economic costs associated with prolonged ICU stays has inherent limitations, however, 2015 unadjusted estimates of the cost of ICU stays were approximately $4500/day and as high as $10,794/day in mechanically ventilated patients[7, 8]. Although these costs decline with increasing days in the ICU they may be higher in the often more complex cardiac surgical population [9]. Therefore finding therapies that decrease the need for vasopressor support and ideally decrease ICU stays as well as mortality is of substantial significance.

Innovation:

Nitric oxide and it's downstream effects likely play a central role in the pathophysiology of VS[1]. Hydroxocobalamin (B12a) an inhibitor of nitric oxide and guanylate cyclase is emerging as potential therapeutic target[10, 11]. This has been demonstrated through its use in several case reports in both cardiac surgery and during liver transplantation[2, 12]. At our institution a series submitted for publication of 24 patients showed a significant improvement in blood pressure and decrease in vasopressor requirement after B12a administration in patients with VS after cardiac surgery.

1.1 Background

i) Introduction and Feasibility

Hypotension that fails to respond to the current armamentarium of vasopressors is a common complication after cardiopulmonary bypass (CPB), occurring in 8-12% of cases[1, 2, 4]. Commonly labeled "vasoplegic syndrome" (VS), although no universally accepted formal definition exists, it is generally defined as hypotension occurring as a result of low systemic vascular resistance (SVR) in the setting of normal to high cardiac output (CO), and euvolemia. Subsequent hypoperfusion can lead to end organ damage and in prolonged catecholamine refractory VS mortality approaches 25% [5, 6].

Routine treatment of vasodilatory hypotension after CPB has mirrored treatment of vasodilatory shock in other inflammatory contexts like sepsis and anaphylaxis. Hemodynamics are supported through both volume optimization as well vasopressors and inotropes. Catecholamines continue to be the most widely used agents with epinephrine, norepinephrine, phenylephrine and dobutamine being available choices. Vasopressin is also commonly used with the pathophysiological basis for its use in these patients being vasopressin deficiency which is believed to contribute to VS. Corticosteroids are another option in patients believed to be steroid deficient and the recent ATHOS-3 trial highlights the possibility of angiotensin II being a therapeutic agent in these patients.[13]

As described above, treatment for the most part has been directed at the physiological consequences of the syndrome with less emphasis on addressing the underlying pathology. The pathogenesis of VS is multifactorial and not fully understood and has been discussed in previous reviews [1, 6, 14]. Upregulation of inducible nitric oxide synthase (iNOS) is believed to play an important role and has been a target for therapeutic intervention through the use of methylene blue for more than 20 years, particularly in cases refractory to catecholamine administration[15]. Nitric oxide (NO) is involved in basal vasomotor tone through its activation of guanylyl cyclase and cGMP. In addition to its action in the regulation of small resistance arterioles and therefore systemic blood pressure it also plays a role in pulmonary vascular resistance (PVR) as well autoregulation of important vascular beds including in the heart, the brain and the kidneys [16]. Beyond its role as a vasodilator there is evidence suggesting NO contributes to a decrease in cellular responsiveness to catecholamines[16].

Given these factors it's possible that treatment at the level of the underlying pathological process, inhibition of iNOS and its downstream pathways, leads to a higher achievable target blood pressure as catecholamines become more effective on top of the underlying improvement in blood pressure achieved through NO removal alone. Additionally some of the adverse effects seen as catecholamine doses increase including arrhythmias, as well as malperfusion through important vascular beds and subsequent adverse physiological sequelae may be attenuated [17].

The concept of treating post-cardiotomy VS at the level of its pathological root led to the use of Methylene Blue (MB) in the 1990s. The first series of six patients was reported in 1996 and was followed with a number of case reports and series over the following decade [17-20].

MB was initially used as a rescue medication in cases of catecholamine refractory VS with hesitation about its use as first line therapy. Two randomized controlled trials then pointed to

benefit both with prophylactic use and as early therapy post-CPB prior to the development of catecholamine refractory VS. Ozal et al. showed benefit with use of MB as a preventative therapy in patients at high risk of VS[21] and Levin and colleagues reported benefit with the early use of MB in patients with VS post-CPB[5].

In spite of these benefits there are several reasons to be cautious when using MB as therapy in post-CPB VS. Firstly, given there is no clear definition of VS, it isn't clear which patients are truly vasoplegic and therefore may benefit from its use. On top of this a physiological diagnosis by no means guarantees the underlying process is NO and cGMP mediated and therefore a diagnosis of VS alone may not predict response definitively. In addition it remains unclear when MB should be administered in relation to the onset of VS. It has been proposed that there may be windows of opportunity for treatment of VS with the first 8 hours being possibly the ideal time to intervene [15, 22]. On top of this, a retrospective review published in 2013 showed an independent association between MB administration and mortality [23]. These uncertainties coupled with several potentially serious adverse effects have led several authors to conclude MB is better confined to use as a rescue medication and should be avoided in certain populations[23-25].
MB inhibits monoamine oxidase A (MAO-A) resulting in prolonged action of serotonin, norepinephrine and epinephrine. Although this can help augment blood pressure the inhibition of MAO-A can lead to serotonin syndrome when MB is combined with other agents that also increase serotonin, such as fentanyl and SSRIs[26]. Other unfavorable effects of MB include increasing pulmonary vascular resistance (PVR), decrease in mesenteric and renal blood flow, cardiac arrhythmias and worsening gas exchange[4, 21, 25]. In large doses it has also been associated with hemolytic anemia and hyperbilirubinemia [27].

1.2 Investigational Agent

Hydroxocobalamin marketed as Cyanokit®, 5g, IV, Infused once over 15 min. Unblinded dose of Cyanokit® can be ordered within the first 4 hours at the discretion of the primary care team. The drug comes in a 250ml glass vial with 5g of lyophilized hydroxocobalamin to be reconstituted in 200ml of Normal Saline, Dextrose or Lactated Ringers. It appears as a dark red solution after reconstitution.

1.3 Preclinical Data

ii) Preliminary Studies

Concerns over the host of adverse effects seen with the use of MB have led to a search for other NOS inhibitors. One such medication is hydroxocobalamin also known as vitamin B12a (B12a). B12a has been used in Europe for the treatment of cyanide toxicity for over 40 years with an excellent safety profile[28]. Hypertension is one of the possible effects of B12a likely through inhibition of both NOS and cGMP [2, 10]. Given its lack of MOA-A inhibition it does not result in serotonin syndrome. Several case reports have demonstrated its effectiveness in patients with vasoplegia with one suggesting it reduced the risks of vasospasm associated with high dose

catecholamine administration and another hypothesizing a synergistic effect when used with MB [2, 12, 29].

1.4 Clinical Data to Date

To date no prospective studies have looked at the use of B12a in this population. Currently it is FDA approved for use in cyanide toxicity with a more extensive body of literature for that indication[30].

At our institution a protocol was put in place for the use of B12a in patients with refractory VS after cardiac surgery. A retrospective review of the data sent for publication found a statistically significant increase in blood pressure and decrease in vasopressor requirement in 24 patients who received B12a in post-cardiotomy VS. This included both patients who had not received MB and patients who were B12a non-responders.

In addition a case series was recently published in the Canadian Journal of Anesthesiology also showing a favorable response in a subset of patients with post-cardiotomy VS receiving B12a [31].

1.5 Dose Rationale

The selected dose is the current FDA approved dose for use in cyanide toxicity. In our review of the data of patients with VS receiving B12a this has been associated with a significant change in blood pressure. The protocol requires the full dose of 5g to avoid the heterogeneity of intervention that might occur if partial doses were given. In order to avoid blood pressure overshoot, which has been described in this population, the protocol allows for titration of vasopressors while the B12a is infusing, to maintain the desired maximum blood pressure [31].

The drug is sold for intravenous administration and this is the most practical route given the patient population.

1.6 Risks and Benefits

The potential risks include mishandling of patient information, anaphylaxis and transient skin discoloration due to study drug administration, as yet unknown adverse effects of the study drug, the potential that placebo is inferior to the study drug and therefore patients receiving placebo are adversely affected through omission. Plasma discoloration has also been shown to interfere with intermittent hemodialysis in which case there is the potential for need for continuous renal replacement therapy (CRRT) use instead. CRRT has been used uneventfully for this reason in the past [32]. These risks are mostly low or transient in nature and for most people are outweighed by the benefits which include.

Direct benefit from receiving the study drug, increased vigilance in regards to hemodynamic monitoring, and a large potential scientific yield given the magnitude of this problem both in

terms of the frequency with which it occurs and the high rate of morbidity and mortality associated. Anecdotal findings and retrospective review of case series point to this being a promising therapeutic option however prospective trials like this one are needed to more strongly support its use. For patients that have no response to the administration of study drug or placebo standard of care will be followed. Patients in this scenario would not be at a disadvantage to what they would have otherwise received. Rescue with methylene blue will also be an option in circumstances where it is considered potentially useful.

2 Study Objectives

Primary Objective

1. Change in Mean Arterial Pressure – To assess the efficacy during the first 4 hours after B12a administration on increasing mean arterial pressure in patients with vasoplegic syndrome on high-dose vasopressors as defined by a norepinephrine equivalent of 0.1mcg/kg/min.

Secondary Objective

1. Change in vasopressor infusion rates. (first 4 hours)
2. Change in Systolic Blood Pressure (first 4 hours)
3. Thirty day mortality
4. Hospital length of stay (LOS)
5. ICU LOS
6. Ventilator time (hrs)
7. Urine output
8. Total crystalloid and albumin administration (first 48 hrs)
9. End organ dysfunction during the first seven days will not be directly collected in the CRF but will be captured retrospectively in supportive documentation: (Stroke, AKI, Liver enzyme elevation, lactate, renal replacement therapy)
10. Maximum SOFA score (First 7 days)
11. New arrhythmias (First 7 days)
12. Need for Rescue with MB
13. Need for mechanical circulatory support
14. Change in INR, platelet count, fibrinogen
15. Need for transfusion of allogeneic packed red blood cells, plasma, platelets and fibrinogen.

3 Study Design

3.1 General Description

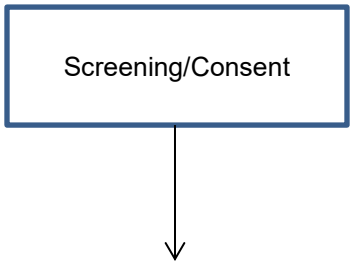
This will be a phase I/II, single-center, double-blind, placebo-controlled, randomized, intent-to-treat study among a placebo group and a group receiving B12a for clinically diagnosed VS after CPB. Subjects will be screened on the medical record and consented in the preoperative area or patient/family will be approached for consent if they are close to qualifying for the study after the surgical procedure. If consent is given they will be enrolled and monitored after cardiopulmonary bypass for cardiac surgery for inclusion criteria as defined in table 1. B12a will be administered as an infusion of 5g for the first dose and must be reconstituted to a 25mg/mL concentration. The initial 5g dose will be given over a period of 15 min. If there is a non-sustained response after the first dose a second dose may be administered within the first 4 hours at the discretion of the primary care team. This strategy is consistent with current FDA approved dosages. If there is no response, rescue with methylene blue will be at the discretion of the primary anesthesiologist or intensivist and will be measured as an outcome. Patients/Legal Authorized Representative (LAR) will be consented on the basis of 1 through 4 and randomized for inclusion based on 5 in the table below. Exclusion criteria are also included table 1. After randomization and drug administration initial vital signs and hemodynamic measurements will be taken. The electronic medical record will then be used to collect patient data retrospectively. Patients will receive 1 follow up phone call at 1 month after enrollment.

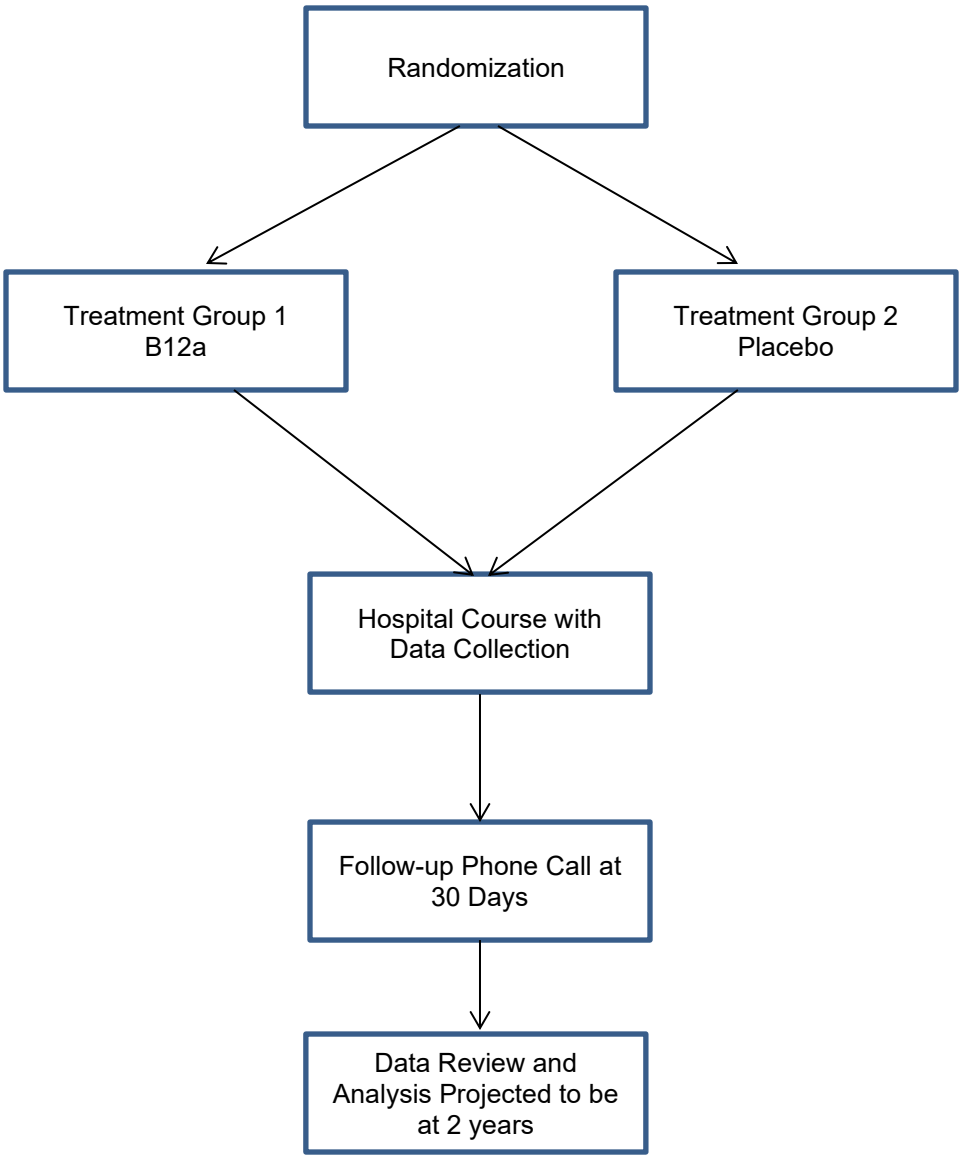
3.2 Number of Subjects

1000 to 2000 subjects will be enrolled in order to accrue a total of 50 subjects.

3.3 Duration of Participation

Patients will be randomized and administered study drug if criteria are met within the first 24hrs after being removed from cardiopulmonary bypass. Once study drug is given, hemodynamic variables will be monitored the first 30 minutes, all adverse events will be monitored the first 12 hours and development of acute kidney injury (AKI) and stroke will be monitored for 72 hours. Data will be collected the first 7 days through the electronic medical record and at 30 days the patient will be called for follow up and assessment of 30 day mortality.





3.4 Primary Study Aims

Change in Mean Arterial Pressure over the first 4 hours post B12a administration

3.5 Secondary Study Aims

1. Change in vasopressor infusion rates. (first 4 hours)
2. Change in Systolic Blood Pressure (first 4 hours)
3. Thirty day mortality

4. Hospital length of stay (LOS)
5. ICU LOS
6. Ventilator time (hrs)
7. Urine output (Daily for 7 days)
8. Total crystalloid and albumin administration (amount given over first 48 hrs)
9. End organ dysfunction during the first seven days will not be directly collected in the CRF but will be captured retrospectively in supportive documentation: (Stroke, AKI (AKIN criteria), Liver enzyme elevation, lactate, renal replacement therapy all daily for first seven days)
10. Maximum SOFA score (daily for first 7 days)
11. New arrhythmias (daily for first 7 days)
12. Need for Rescue with MB
13. Need for mechanical circulatory support
14. Change in INR, platelet count, fibrinogen (daily for first 7 days)
15. Need for transfusion of allogeneic packed red blood cells, plasma, platelets and fibrinogen. (Units and L transfused over first 48 hrs)
16. Compare vitamin B12 assay and heavy metal quant in patients who receive study drug

3.6 Primary Safety Aims

Primary safety endpoints will include evaluation for development of anaphylaxis, blood pressure overshoot as defined by a systolic blood pressure above 140mmHg during the first 4 hours of administration, as well as the following.

1. Thirty day mortality
2. Hospital length of stay (LOS)
3. ICU LOS
4. Ventilator time in hours
5. End organ dysfunction during the 1st seven days will not be directly collected in the CRF but will be captured retrospectively in supportive documentation:
    i. Stroke (CT or MRI confirmed hemorrhagic or ischemic
    ii. AKI as defined by AKIN criteria
6. SOFA score
7. New arrhythmias
8. Need for mechanical circulatory support
9. Statistically significant change in INR, platelet count, fibrinogen
10. Need for transfusion of allogeneic packed red blood cells, plasma, platelets and fibrinogen.

3.7 Identification of Source Data

The following source data will be directly recorded on the Case Report Form (CRF):

- Vital signs (T0±30 min, T0 represents time of drug administration)
    - Cardiac index

  - MAP
  - SBP
  - Systolic Pulmonary Blood Pressure
  - Right Atrial Pressure

- Vasopressor infusion rates (T0±30 min, T0 represents time of drug administration)
  - Norepinephrine mcg/kg/min
  - Epinephrine mcg/kg/min
  - Vasopressin unit/min
  - Calcium mg/hour
  - Dopamine mcg/kg/min

The following source data will be directly collected in the Case Report Form (CRF), if we are not able to capture the data in the CRF due to limitations, it will be captured retrospectively in supportive documentation (study source documents, EMR):

- Laboratory tests will be taken as is standard in this patient population with no added tests specific for the study. Results and clinical interpretation with be via the EMR
  - Creatinine, ABG (FiO2, PaO2, PaCO2, pH, Bicarb, Base Deficit, methemoglobin percentage), Lactate, INR, PTT, AST, ALT, Bilirubin, Hemoglobin, platelets
- Vitamin B12 assay and heavy metal quant results 24 hours and 72 hours after drug administration
- Urine output at each 24 hours.
- Vital signs
- Cardiac index at 15 min intervals from administration time T45 through the first 4 hours if the participant has a continuous pulmonary artery catheter. Cardiac index 30 minutes after administration time, times two, followed by every hour for 4 hours if the patient has a non-continuous pulmonary artery catheter and based upon the availability of the technician/therapist.
- MAP at 15 min intervals from administration time T45 through the first 4 hours
- SBP at 15 min intervals from administration time T45 through the first 4 hours
- Systolic Pulmonary Blood Pressure at 15 min intervals from administration time T45 through the first 4 hours
- Right Atrial Pressure at 15 min intervals from administration time T45 through the first 4 hours
- Hospital length of stay (LOS) (Days)
- ICU LOS (Days)
- Ventilator time (Hours)
- End organ dysfunction during the first seven days will not be directly collected in the CRF but will be captured retrospectively in supportive documentation:
  - Stroke (CT or MRI confirmed hemorrhagic or ischemic
  - AKI as defined by AKIN criteria
- New arrhythmias as diagnosed by in the EMR
- Need for mechanical circulatory support (LVAD, ECMO, Intra-aortic Balloon Pump, Impella)

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

- Patients/LAR will be consented prior to going to the operating room or in the intensive care unit after surgery.
- The benefits and risks will be discussed.

Table 1

Inclusion

1. Any patient for whom we are able to obtain consent prior to their procedure or after their procedure in the intensive care unit.
2. Patient must have a PA catheter, or a CCO/Flo Trak for the procedure
3. Is presenting for a procedure in which CPB will be required
4. Is considered high risk for VS
    a. Patient considered to be at high risk for the development of VS will be identified per the guidelines developed by Levin et al[11]. This includes any patient:
        I. With pre-CPB vasopressor requirement(s)
        or
        II. On pre-operative ACE, or beta-blocker or calcium channel blocker therapy
        or
        III. With a euroSCORE>6
        or
        IV. Presenting for pericardiectomy, aneurysm surgery, LVAD surgery or heart transplant, CABG or valve surgery other than a single mitral valve repair.
5. Has no contraindications to arterial line or PA catheter placement
6. Patient must fall into the defined parameters below for the majority of measurements over a 30 minute period (ex: 3 out of 5 measurements or 6 of 10, etc).
    a. Develops high-dose vasopressors for 30 min or longer and refractory hypotension consistent with VS within 24 hours of coming off CPB.
    b. “high-dose vasopressor” will be defined as:
        I. Norepinephrine infusion≥0.1mcg/kg/min ***and/or***
        II. Dopamine infusion≥ 15mcg/kg/min
        and/or
        III. Epinephrine infusion≥0.1mcg/kg/min
        Or
    c. Norepinephrine equivalent infusion of ≥ 0.1mcg/kg/min (Table 1 Appendix)
    d. “Hypotension consistent with VS” will be defined as:
        I. MAP≤75 mmHg via arterial line monitor
7. Cardiac index>2.3L/min/m^2 via PA catheter monitor.
8. If at the time study drug arrives, the patient who previously met criteria has fallen out, they must once again meet requirements only one time before the drug is given.

4.2 Exclusion Criteria

- Age<18 years
- Known pregnancy or patients without a documented pregnancy test if not menopausal.
- Known prior anaphylactic or allergic reaction to B12a
- CKD stage 4 or worse (GFR $\leq$ 30 ml/min) before study drug administration
- ECMO (extracorporeal membrane oxygenation) or IABP (intra-aortic balloon pump)prior to study consent and post cardiopulmonary bypass
- Patients currently on cardiopulmonary bypass
- Circumstances for which the safety of the patient could be jeopardized by continued adherence to the study protocol

4.3 Subject Recruitment, Enrollment and Screening

Study coordinators will screen all adult (age > 18) patients who are scheduled to undergo coronary artery bypass graft surgery with or without valve repair/replacement, complex cardiac valve surgery, pericardial resection, and/or ascending aortic surgery at Mayo Clinic Rochester. Eligible patients will be approached by a study coordinator for consent before their elective surgical procedure or patient/LAR will be approached after the surgical procedure in the intensive care unit if the patient is close to qualifying for the study. After confirming the patient's inclusion and exclusion criteria, the study coordinator at the institution will obtain informed consent and assign a study ID number to the study participant. Screening logs will be maintained to allow generation of a CONSORT diagram.

4.4 Early Withdrawal of Subjects

- We expect early withdrawals to be rare given it is a single intervention.
- Patients will be free to withdraw consent at any time during the study period.
- Patients who withdraw will be included in intention to treat analysis and safety assessment and analysis.

4.4.1 When and How to Withdraw Subjects

.

- Patient/LAR will be able to withdraw consent at any time if the patient no longer wishes to participate.

5 Study Drug

5.1 Description

- The study drug, hydroxocobalamin is marketed as Cyanokit® and comes in a 200ml glass vial as a red powder which is reconstituted in 200ml of normal saline giving a dark red solution.

5.2 Treatment Regimen

- 5g, IV, over 15 min as a single dose. Can order unblinded dose of Cyanokit® within the first 4 hours at the discretion of the primary care team.

5.3 Method for Assigning Subjects to Treatment Groups

- Patients will be randomized by research pharmacy if they meet inclusion criteria.

5.4 Preparation and Administration of Study Drug

- Both the study drug and placebo will be randomized by the research pharmacy.

5.5 Subject Compliance Monitoring

- Patient non-compliance is not expected to occur as the patient will be intubated and ventilated and it is a single dose IV medication administered by OR and ICU staff.

5.6 Prior and Concomitant Therapy

5.7 Packaging

- A full one time dose will be administered and so no drug return is expected.
- Drug will be documented by the RN, CRNA or anesthesiologist of record.

5.7.1 Return or Destruction of Study Drug

- As per current Mayo practice.

6 Study Procedures

Patient randomized to B12a or placebo. Data will be measured prospectively for the first 30 minutes and then taken from the EMR after that. All tests besides vitamin B12 assay and heavy metal quant will be done as standard of care and data will only be collected if done as standard of care. Vitamin B12 assay and heavy metal quant will be collected at 24 hours (± 1 hour) and 72 hours (± 1 hour) after drug administration.

Study Activity	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day6	Day 7	30 Day F/U
Study Drug	X	X (window -1, -0)							
Informed consent	X	X							
History	X	X							
Concurrent meds	X	X							
Physical exam (Ht, Wt, VS)	X	X							
Patient monitoring for inclusion	X	X							
Randomization Res Pharm	X	X							
CBC w/diff, plts	X	X	X	X	X	X	X	X	
Serum chemistry [a]	X	X	X	X	X	X	X	X	
INR/PTT/Fib/Plt	X	X	X	X	X	X	X	X	
ABG/Lactate[b]	X	X	X	X	X	X	X	X	
Vitamin B12 Assay[c]		X	X	X	X				
Heavy Metal Quant[c]		X	X	X	X				
Hemodynamic monitoring									
Patient Call for follow up on adverse events and mortality									X

a: Albumin, alkaline phosphatase, total bilirubin, BUN, calcium, chloride, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [13], sodium, creatinine
b: PaO2, PaCO2, pH, bicarb, SpO2, lactate, base deficit
c: Vitamin B12 assay, heavy metal quant to be collected at 24 hour (± 1 hour) and 72 hour (± 1 hour) after study drug administration
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7 Statistical Plan

7.1 Sample Size Determination

Sample size

Power analysis using the results of our retrospective review revealed a study population of 50 patients randomized to either B12a or control would detect a mean arterial pressure (MAP) increase of 7.3mmHg with a power of 0.8 and a p value of 0.05. The expected event rate triggering randomization is between 10% and 20% based on both historical data from previous series and evaluation of the percentage of patients meeting inclusion criteria based on a random selection of patients after cardiac surgery at our institution [5, 14]. This will require consent of 1000 to 2000 patients in order to randomize 50 patients. Our institution performs approximately 3000 cardiac surgical cases requiring CPB per year meaning we would need to consent between 4% and 8% of our cardiac surgical volume over a 2 year study period.

Problems and Limitations

Several potential limitations exist with this study. Firstly there is still no universally accepted definition of VS in the literature. Therefore physiological variables chosen for inclusion will by necessity be arbitrary. We chose variables based on definitions used in previous reviews. Furthermore, previous work suggests NOS inhibitors may be most effective early in the disease process and may be less effective after prolonged refractory VS [5, 22]. Given this we decided intervention with the study drug should occur while vasoplegia is refractory but before intervention would be considered as a rescue and therefore infusion of norepinephrine equivalents of 0.1mcg/kg/min^2 for 30 min was considered appropriate.

7.2 Statistical Methods

Descriptive Statistics

The primary outcome is change in MAP from baseline over the first 4 hours postoperatively at 15 min increments post randomization. Randomized groups will be compared using analysis of covariance (ANCOVA), adjusting for baseline MAP prior to randomization. A p-value <0.05 is considered statistically significant.

Secondary outcomes will be compared by treatment arms without adjustment for multiple comparisons. Change in rates of vasopressor and inotropic infusions will be compared by

ANCOVA where the outcome is defined as the infusion rate at 30 minute intervals for the first 4 hours post B12a administration and models adjust for the baseline value. Change in systolic blood pressure will be analyzed similarly. Length of stay and ventilator time outcomes will be compared by Wilcoxon rank-sum tests; mortality and binary outcomes will be compared between randomized arms with a Pearson chi-square or Fisher's exact test when appropriate.
As a tertiary aim and exploratory analysis, longitudinally collected data such as daily SOFA score will be modeled in a repeated measures analysis. Treatment groups will be compared by assessing whether B12 treatment affects the slope of or change in SOFA scores over time.

Handling of Missing Data

Missing data is expected to be rare as these outcomes are routinely collected in the electronic medical record. The primary analysis will consider complete cases. Two sensitivity analyses may be performed, including multiple imputation of missing data under the missing at random mechanism and an analysis under the not missing at random mechanism. For the latter, methods may include shared parameter models or worst-case imputation when the cause of the missing data is early mortality

Primary Hypothesis:

The use of an intravenous infusion of vitamin B12a in patients with post-cardiotomy vasoplegic syndrome receiving high dose vasopressors will result in a statistically significant increase in mean arterial blood pressure compared to placebo over the first 4 hours after administration.

Secondary Hypothesis:

The use of an intravenous infusion of vitamin B12a in patients with post-cardiotomy vasoplegic syndrome will have a statistically significant decrease in vasopressor requirement over the first 4 hours after administration.

7.3 Subject Population(s) for Analysis

- All-randomized population: Any subject randomized into the study, regardless of whether they received study drug. Inclusion and exclusion criteria are provided in table 1.
- All-treated population: Any subject randomized into the study that received at least one dose of study drug
- All-completed population: Only subjects who completed ALL study related procedures and follow-up will be included

8 Safety and Adverse Events

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)
Any unanticipated problem or adverse event that meets the following three criteria:

- Serious: Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**
- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected.
- Related: A problem or event is "related" if it is possibly related to the research procedures.

Adverse Event
An untoward or undesirable experience associated with the use of a medical product (i.e. drug, device, biologic) in a patient or research subject.

Patients undergoing cardiac surgery with CPB sustain a multitude of adverse events as a result of their medical condition and the exposure to surgical procedure and CPB. Therefore, in order to make the safety evaluation of vitamin B12a meaningful, only selected adverse events that are medically important and/or may have a causal relationship to vitamin B12a will be collected.

Serious Adverse Event
Adverse events are classified as serious or non-serious. Serious problems/events can be well defined and include;

- death
- life threatening adverse experience
- hospitalization
- inpatient, new, or prolonged; disability/incapacity
- persistent or significant disability or incapacity
- birth defect/anomaly

and/or per protocol may be problems/events that in the opinion of the sponsor-investigator may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

All adverse events that do not meet any of the criteria for serious, should be regarded as **non-serious adverse events**.

Adverse Event Reporting Period

We will monitor for all adverse events over the first 12 hours after the administration of study drug and monitor for acute kidney injury (AKI) and stroke for 72 hours after drug administration. Patients will continue to be monitored per standard ICU protocol after this point.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event will also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the sponsor-investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization aside from the anticipated post-surgical hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should not be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.1 Recording of Adverse Events

Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event section of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results should recorded in the source document.

All adverse events occurring during the study reporting period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period must be followed up, to determine the final outcome. Any serious adverse event that occurs during the Adverse Event Reporting Period and is considered to be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

8.2 Reporting of Serious Adverse Events and Unanticipated Problems

When an adverse event has been identified, the study team will take appropriated action necessary to protect the study participant and then complete the Study Adverse Event Worksheet and log. The sponsor-investigator will evaluate the event and determine the necessary follow-up and reporting required.

8.2.1 Sponsor-Investigator reporting: notifying the Mayo IRB

Monitoring activities will be performed by the PI and study team. The investigators will allocate adequate time for monitoring activities. The Investigators will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit. A data safety monitoring board is not required by this institution.

The sponsor-investigator will report to the Mayo IRB any UPIRTSOs and Non-UPIRTSOs according to the Mayo IRB Policy and Procedures and it will include the following:
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- Subject's name:
- Medical record number:
- Disease/histology (if applicable):
- The date the adverse event occurred:
- Description of the adverse event:
- Relationship of the adverse event to the research (drug, procedure, or intervention*):
- If the adverse event was expected:
- The severity of the adverse event: (use a table to define severity scale 1-5**)
- If any intervention was necessary:

- Resolution: (was the incident resolved spontaneously, or after discontinuing treatment)
- Date of Resolution:

The sponsor-investigator will review all adverse event reports to determine if specific reports need to be made to the IRB and FDA. The sponsor-investigator will sign and date the adverse event report when it is reviewed. For this protocol, only directly related SAEs/UPIRTSOs will be reported to the IRB.

*** Relationship Index Example**

The relationship of an AE to the Investigational Drug is a clinical decision by the sponsor-investigator (PI) based on all available information at the time of the completion of the CRF and is graded as follows:

1. Not related: a reaction for which sufficient information exists to indicate that the etiology is unrelated to the study drug; the subject did not receive the study medication or the temporal sequence of the AE onset relative to administration of the study medication is not reasonable or the event is c1early related to other factors such as the subject's clinical state, therapeutic intervention or concomitant therapy.

2. Unlikely: a clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable and in which other drugs, chemicals, or underlying disease provide plausible explanations.

3. Possible: a clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug but which could also be explained by concurrent disease or other drugs or chemicals; information on drug withdrawals may be lacking are unclear.

4. Probable: a clinical event including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals and which follows a clinically reasonable response on withdrawal (de-challenge): re-challenge information is not required to fulfi1 this definition.

5. Definite: a reaction that follows a reasonable temporal sequence from administration of the drug, or in which the drug level has been established in body fluids or tissues, that follows a known or expected response pattern to the suspected drug, and that is confirmed by improvement on stopping or reducing the dosage of the drug, and reappearance of the reaction on repeated exposure (re-challenge).

** Severity Index Example
The maximum intensity of an AE during a day should be graded according to the definitions below and recorded in details as indicated on the CRF. If the intensity of an AE changes over a number of days, then separate entries should be made having distinct onset dates.

1. Mild: AEs are usually transient, requiring no special treatment, and do not interfere with patient’s daily activities.

2. Moderate: AEs typically introduce a low level of inconvenience or concern to the patient and may interfere with daily activities, but are usually ameliorated by simple therapeutic measures.

3. Severe: AEs interrupt a patient's usual daily activity and traditionally require systemic drug therapy or other treatment.

8.3 Unmasking/Unblinding Procedures

Masking and blinding will be carried out by the research pharmacy with unmasking to be performed at study completion through the research pharmacy and statistician.

8.4 Stopping Rules

In the case of anaphylaxis drug infusion will be stopped. In the event of blood pressure overshoot the Vasopressors will be titrated to stay under the upper limit blood pressure goal of the treatment team. Urine and skin discoloration is an expected side effect, there will be no change in protocol or management if this occurs.

The study drug has been used safely over a prolonged period with very few reported adverse events. We do not expect any other related adverse events.

8.5 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 "Study Monitoring, Auditing, and Inspecting"). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.5.1 Internal Data and Safety Monitoring Board

8.5.2 Independent Data and Safety Monitoring Board

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why

- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

Data Management

Data will be stored in a secure server accessed only by the PI and other study personnel as designated by the PI

Data Processing

Data will be collected by the study coordinator and will be entered into REDCap data base which is located on a secure drive and accessed only by the PI and other study personnel as designated by the PI.
Retrospective data captured in spreadsheet and kept in secure server

Data Security and Confidentiality

As above

Data Clarification Process

9.4 Records Retention

The sponsor-investigator will maintain records and essential documents related to the conduct of the study. These will include subject case histories and regulatory documents.

The sponsor-investigator will retain the specified records and reports for;

1. Up to 2 years after the marketing application is approved for the drug; or, if a marketing application is not submitted or approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified. OR
2. As outlined in the Mayo Clinic Research Policy Manual –"Retention of and Access to Research Data Policy" http://mayocontent.mayo.edu/research-policy/MSS_669717 whichever is longer

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, and government regulatory agencies, of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

11 Ethical Considerations

This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the sponsor-investigator before commencement of this study.

All subjects/LAR for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the Approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject/LAR, and the individual obtaining the informed consent.

12 Study Finances

12.1 Funding Source

We are hopeful of receiving a grant through the Society of Cardiovascular Anesthesiologists as well as study drug through Pfizer.

12.2 Conflict of Interest

No conflict of interest declared by study personnel

13 Publication Plan

The study will be linked to ClinicalTrials.gov prior to subject recruitment and enrollment, as well as posting of results to ClinicalTrials.gov within 12 months of final data collection for the primary outcome.

Link to protocol registration site for ClinicalTrials.gov: https://register.clinicaltrials.gov/

14 Protocol Addendum

Although B12 has an excellent safety profile, to date no study has evaluated serum levels of B12 and cobalt after dosing in this patient population. Recently, a reviewer on a grant application recommended adding a couple labs to the research study since the vitamin b12 contains the heavy metal cobalt. A decision was made by the investigator and the study team to collect labs up to 16 patients who receive study drug. The labs that will be collected are the vitamin b12 assay and heavy metal quant at 24 hours and 72 hours post drug administration. The study will be paying for these lab tests so the patient should not see any billing for them.
Obtaining these lab results will help to develop a pharmacokinetic profile in patients receiving the drug and it would help us understand the effects of Vitamin B12 in this study population.

15 References

1. Fischer, G.W. and M.A. Levin, *Vasoplegia during cardiac surgery: current concepts and management.* Semin Thorac Cardiovasc Surg, 2010. **22**(2): p. 140-4.
2. Roderique, J.D., et al., *The use of high-dose hydroxocobalamin for vasoplegic syndrome.* Ann Thorac Surg, 2014. **97**(5): p. 1785-6.
3. Oz, M., et al., *Cellular and molecular actions of Methylene Blue in the nervous system.* Med Res Rev, 2011. **31**(1): p. 93-117.
4. Lenglet, S., F. Mach, and F. Montecucco, *Methylene blue: potential use of an antique molecule in vasoplegic syndrome during cardiac surgery.* Expert Rev Cardiovasc Ther, 2011. **9**(12): p. 1519-25.
5. Levin, R.L., et al., *Methylene blue reduces mortality and morbidity in vasoplegic patients after cardiac surgery.* Ann Thorac Surg, 2004. **77**(2): p. 496-9.

6. Gomes, W.J., et al., *Vasoplegic syndrome after open heart surgery.* J Cardiovasc Surg (Torino), 1998. **39**(5): p. 619-23.
7. Dasta, J.F., et al., *Daily cost of an intensive care unit day: the contribution of mechanical ventilation.* Crit Care Med, 2005. **33**(6): p. 1266-71.
8. Halpern, N.A. and S.M. Pastores, *Critical Care Medicine Beds, Use, Occupancy, and Costs in the United States: A Methodological Review.* Crit Care Med, 2015. **43**(11): p. 2452-9.
9. Gershengorn, H.B., A. Garland, and M.N. Gong, *Patterns of Daily Costs Differ for Medical and Surgical Intensive Care Unit Patients.* Ann Am Thorac Soc, 2015. **12**(12): p. 1831-6.
10. Gerth, K., et al., *Nitric oxide scavenging by hydroxocobalamin may account for its hemodynamic profile.* Clin Toxicol (Phila), 2006. **44 Suppl 1**: p. 29-36.
11. Weinberg, J.B., et al., *Inhibition of nitric oxide synthase by cobalamins and cobinamides.* Free Radic Biol Med, 2009. **46**(12): p. 1626-32.
12. Woehlck, H.J., et al., *Hydroxocobalamin for Vasoplegic Syndrome in Liver Transplantation: Restoration of Blood Pressure Without Vasospasm.* A A Case Rep, 2016. **7**(12): p. 247-250.
13. Khanna, A., et al., *Angiotensin II for the Treatment of Vasodilatory Shock.* N Engl J Med, 2017. **377**(5): p. 419-430.
14. Omar, S., A. Zedan, and K. Nugent, *Cardiac vasoplegia syndrome: pathophysiology, risk factors and treatment.* Am J Med Sci, 2015. **349**(1): p. 80-8.
15. Evora, P.R., et al., *Twenty years of vasoplegic syndrome treatment in heart surgery. Methylene blue revised.* Rev Bras Cir Cardiovasc, 2015. **30**(1): p. 84-92.
16. Loscalzo, J. and G. Welch, *Nitric oxide and its role in the cardiovascular system.* Prog Cardiovasc Dis, 1995. **38**(2): p. 87-104.
17. Kofidis, T., et al., *Reversal of severe vasoplegia with single-dose methylene blue after heart transplantation.* J Thorac Cardiovasc Surg, 2001. **122**(4): p. 823-4.
18. Evora, P.R., P.J. Ribeiro, and J.C. de Andrade, *Methylene blue administration in SIRS after cardiac operations.* Ann Thorac Surg, 1997. **63**(4): p. 1212-3.
19. Leyh, R.G., et al., *Methylene blue: the drug of choice for catecholamine-refractory vasoplegia after cardiopulmonary bypass?* J Thorac Cardiovasc Surg, 2003. **125**(6): p. 1426-31.
20. Evora, P.R. and R.L. Levin, *Methylene blue as drug of choice for catecholamine-refractory vasoplegia after cardiopulmonary bypass.* J Thorac Cardiovasc Surg, 2004. **127**(3): p. 895-6; author reply 896.
21. Ozal, E., et al., *Preoperative methylene blue administration in patients at high risk for vasoplegic syndrome during cardiac surgery.* Ann Thorac Surg, 2005. **79**(5): p. 1615-9.
22. Fernandes, D., et al., *Nitric oxide-dependent reduction in soluble guanylate cyclase functionality accounts for early lipopolysaccharide-induced changes in vascular reactivity.* Mol Pharmacol, 2006. **69**(3): p. 983-90.
23. Weiner, M.M., et al., *Methylene blue is associated with poor outcomes in vasoplegic shock.* J Cardiothorac Vasc Anesth, 2013. **27**(6): p. 1233-8.
24. Evora, P.R. and A.J. Rodrigues, *Methylene blue revised.* J Thorac Cardiovasc Surg, 2006. **131**(1): p. 250-1; author reply 251.
25. Andritsos, M.J., *Con: methylene blue should not be used routinely for vasoplegia perioperatively.* J Cardiothorac Vasc Anesth, 2011. **25**(4): p. 739-43.
26. Ng, B.K. and A.J. Cameron, *The role of methylene blue in serotonin syndrome: a systematic review.* Psychosomatics, 2010. **51**(3): p. 194-200.
27. Sills, M.R. and W.H. Zinkham, *Methylene blue-induced Heinz body hemolytic anemia.* Arch Pediatr Adolesc Med, 1994. **148**(3): p. 306-10.
28. DesLauriers, C.A., A.M. Burda, and M. Wahl, *Hydroxocobalamin as a cyanide antidote.* Am J Ther, 2006. **13**(2): p. 161-5.
29. Cai, Y., et al., *The use of intravenous hydroxocobalamin as a rescue in methylene blue-resistant vasoplegic syndrome in cardiac surgery.* Ann Card Anaesth, 2017. **20**(4): p. 462-464.
30. Petrikovics, I., et al., *Past, present and future of cyanide antagonism research: From the early remedies to the current therapies.* World J Methodol, 2015. **5**(2): p. 88-100.
31. Shah, P.R., et al., *Hydroxocobalamin for the treatment of cardiac surgery-associated vasoplegia: a case series.* Can J Anaesth, 2017.
32. Cheungpasitporn, W., et al., *High-dose hydroxocobalamin for vasoplegic syndrome causing false blood leak alarm.* Clin Kidney J, 2017. **10**(3): p. 357-362.

16 Attachments

Appendix

Table 1. (from ATHOS 3 trial) Angiotensin II for the Treatment of Vasodilatory Shock N Engl J Med 2017; 377:419-430

Drug	Dose	Norepinephrine equivalent
Epinephrine	0.1 mcg/kg/min	0.1 mcg/kg/min
Norepinephrine	0.1 mcg/kg/min	0.1 mcg/kg/min
Dopamine	15 mcg/kg/min	0.1 mcg/kg/min
Vasopressin	0.04U/min	0.05 mcg/kg/min

The conversion scale was developed based on the cardiovascular Sequential Organ Failure Assessment score[a] and the medical literature[b].[5,6] Vasopressin equivalence to norepinephrine was developed with the use of the Vasopressin and Septic Shock Trial data set (by JAR).[7]