Intravenous Magnesium for Sickle Cell Vasoocclusive Crisis (Magnesium in Crisis (MAGIC)) PECARN Protocol Number 025

Pediatric Emergency Care Applied Research Network
National Institute for Child Health and Human Development (NICHD)

Protocol Version 1.05
Version Date: September 7, 2012
Printing Date: September 7, 2012

This protocol is PECARN Protocol Number 025, and the lead PECARN investigator for this protocol is David Brousseau, M.D., Medical College of Wisconsin.

This protocol is partially funded by R01 HD062347 from the *Eunice Kennedy Shriver* National Institute for Child Health and Human Development (NICHD) and by R01 HL103427-01A1 from the National Heart, Lung and Blood Institute (NHLBI). The remainder of funding is from the Pediatric Emergency Care Applied Research Network infrastructure funding detailed below.

The PECARN Research Node Centers are the University of Michigan at Ann Arbor, Cincinnati Children's Hospital Medical Center, Columbia University, University of California at Davis Medical Center, Children's Hospital of Pittsburgh, and Children's National Medical Center, and are supported by Cooperative Agreements U03-MC00003, U03-MC22684, U03-MC00007, U03-MC00001, U03-MC22685, and U03-MC00006 from the Emergency Medical Services for Children (EMSC) Program, Maternal and Child Health Bureau, Health Resources and Services Administration.

This document was prepared by the PECARN Data Coordinating Center (DCC) located at the University of Utah School of Medicine, Salt Lake City, Utah. The document was written and typeset using LaTeX 2_ε. The DCC at the University of Utah is supported by Cooperative Agreement U03-MC00008 from the Emergency Medical Services for Children (EMSC) Program, Maternal and Child Health Bureau, Health Resources and Services Administration.

PROTOCOL TITLE:

Intravenous Magnesium for Sickle Cell Vasoocclusive Crisis

Short Title: Magnesium in Crisis (MAGIC)
PECARN Protocol Number: 025

Lead Investigator and Author:
David Brousseau, M.D.
Medical College of Wisconsin

Protocol Version: 1.05
Version Date: September 7, 2012

I confirm that I have read this protocol, I understand it, and I will conduct the study according to the protocol. I will also work consistently with the ethical principles that have their origin in the Declaration of Helsinki and will adhere to the Ethical and Regulatory Considerations as stated. I confirm that if I or any of my staff are members of the Institutional Review Board, we will abstain from voting on this protocol, its future renewals, and its future amendments.

Principal Investigator Name: _____

Principal Investigator Signature: _____

Date: _____

THIS PAGE IS INTENTIONALLY BLANK.

Contents

List of Tables

List of Figures

Abstract

Background: It is well known that children with sickle cell disease (SCD) are at risk for acute pain crises, however no one knows why a pain crisis starts. Once a pain crisis starts, there are no treatments that have been shown to shorten the length or severity of the crisis. The usual treatment for these pain crises, intravenous (IV) fluids and pain medicines using drugs like morphine or codeine, has changed little over the past three decades. Thus, it is necessary to identify additional medicines that can help treat acute pain crises. The purpose of this study is to determine the safety and efficacy of intravenous magnesium in shortening the duration of a pain crisis and to determine the health-related quality of life and short term outcomes of children treated with intravenous magnesium during an acute pain crisis.

Design and Methods: This is a multi–center, randomized, double–blind, placebo controlled trial. Consented, eligible patients will be enrolled in the study when the decision is made to admit for pain management. Patients will then be randomized to either the study drug group or the placebo group to receive a dose of medication (or placebo) every eight hours after the initial infusion for a total of six doses, or until discharge. Patients will be followed up by phone one week after discharge and will return for follow up within 3 months after discharge. Sites that participate in the Quality of Life portion of the study, will administer the PedsQL quality of life, PedsQL Multidimensional Fatigue Scale, and the PedsQL Sickle Cell Disease Module up to four times throughout the study: before the study drug is started, after the last dose is given, during the follow up phone call, and at the final follow-up visit.

Significance: Data from these studies will lead to a greater understanding of the pathophysiology of sickle cell crisis and provide additional insight into potential therapeutic responses invoked by magnesium therapy.

1 Study Summary

1.1 Hypothesis

The hypotheses of this multi–center, randomized, double–blind, placebo controlled trial are that

1. intravenous magnesium is an effective agent for the treatment of patients hospitalized for an acute sickle cell pain episode;

2. the addition of intravenous magnesium sulfate to standard inpatient therapy is safe for patients hospitalized with an acute sickle cell pain

episode;

3. the beneficial effect of intravenous magnesium is, in part, due to increased endothelial cell nitric oxide production and/or improvement of endothelial dysfunction.

4. children receiving magnesium therapy will have better health-related quality of life (HRQL) at completion of treatment and better short term outcomes after hospital discharge than those receiving placebo therapy;

5. the PedsQL Sickle Cell Disease module will be responsive to temporal changes in health status of the child

1.2 Specific Aims

This proposal has the following Specific Aims:

Specific Aim 1. To compare the hospital length of stay, in hours, for individuals admitted because of acute sickle cell pain when intravenous magnesium (versus placebo) is added to standard therapy.

Specific Aim 2. To determine the safety profile of intravenous (IV) magnesium sulfate in the treatment of acute painful episodes in individuals with sickle cell disease (SCD).

Specific Aim 3. To determine the effect of intravenous magnesium sulfate on the nitric oxide pathway, measures of hemolysis, and markers of endothelial injury and inflammation.

Specific Aim 4. To determine the effect of intravenous magnesium on the health-related quality of life and short term outcomes of children with sickle cell disease presenting with an acute pain episode.

Specific Aim 5. To determine the psychometric properties of the PedsQL Sickle Cell Disease-specific (HRQL) measure.

1.3 Study Endpoints

1.3.1 Primary Endpoint

The primary efficacy outcome measure is length of stay (LOS) in the hospital, recorded in hours from the time of the start of the first study drug administration until the time of discharge from the hospital, or twelve hours after the last intravenous opioid, whichever occurs first.

1.3.2 Secondary Endpoints

One secondary outcome measure is the number of morphine equivalents per kilogram of body weight used during the hospitalization. This will include both intravenous, other parenteral, and oral doses of opioid. Additionally, at some sites, the HRQL and short term outcomes of children participating in the trial will be determined. HRQL of the child will be measured using the PedsQL generic core scales, the PedsQL fatigue scales and the pilot PedsQL sickle cell disease specific measure. Short term outcomes will be measured by determining the days of school/daycare/work missed and days of pain for the patient and days of work/school missed for the caregiver (if applicable) from the time of discharge from the hospital.

1.3.3 Safety Endpoints

Safety endpoints of this study are:

1. hypotension (defined as >20% SBP reduction from the measurement taken immediately prior to infusion) associated with study drug infusion;
2. weakness associated with study drug infusion;
3. warm sensation associated with study drug infusion;
4. rehospitalization within 7 and 28 days of hospital discharge.

In addition to these specific endpoints, adverse events will be recorded from the time of randomization through hospital discharge, or day 7 of hospitalization, whichever occurs first.

1.3.4 Additional Analyses

To help understand the potential effects of magnesium sulfate on the nitric oxide pathway, hemolysis, endothelial injury and inflammation, measurements will include:

1. plasma nitrite and the potential for plasma to scavenge nitric oxide as measures of functionality of the nitric oxide pathway;
2. plasma levels of sVCAM-1, sP-selectin, IL-1β, IL-6, TNF-α, and IFN-γ as measures of endothelial injury and systemic response to inflammation;

3. cell free hemoglobin, LDH, hemoglobin, and reticulocyte count as measures of hemolysis.

1.4 Patient Eligibility

1.4.1 Inclusion Criteria

Patients will be eligible for enrollment if they meet **all** of the following inclusion criteria:

- age 4-21 years, inclusive; AND
- Hb SS or Hb Sβ° thalassemia disease; AND
- failed intravenous opioid pain management in the ED prior to the decision to admit the patient; AND
- decision to admit the patient to the inpatient unit for sickle cell pain crisis.

1.4.2 Exclusion Criteria

Patients will be ineligible for enrollment if **any** of the following exclusion criteria are met:

- patient received more than 12 hours of intravenous pain medication prior to enrollment; OR
- patient discharged from an inpatient unit within 72 hours of arrival in emergency department for current pain crisis; OR
- previous enrollment in this study (only one admission per child is eligible); OR
- history of allergy/intolerance to both intravenous morphine and hydromorphone; OR
- known other cause for current pain – (avascular necrosis, gall bladder disease, priapism, etc.); OR
- patient with greater than 10 admissions for pain crisis in the past year; OR
- patient maintained on daily opioids or chronic transfusions for chronic sickle cell pain; OR
- transfusion within the previous two months; OR
- known kidney or liver failure (elevation of LFTs would not warrant exclusion); OR

- known pulmonary hypertension; OR
- patient known to be pregnant; OR
- diagnosis of bacterial infection, fever $\geq 39.5^{\circ}$C, acute chest syndrome, hemodynamic instability or sepsis; OR
- current oral magnesium supplementation or current enrollment in another therapeutic study protocol; OR
- previously diagnosed clinical stroke; OR
- current or planned use of neuromuscular blocker, nifedipine, ritodrine, or terbutaline; OR
- allergy to magnesium sulfate.

Note: Current or past use of hydroxyurea is not an exclusion criteria for enrollment.

1.5 Inclusion of Women and Minorities

This study will only enroll subjects from 4 to 21 years of age, inclusive. Approximately 50% are anticipated to be female, and the study will primarily involve subjects of African–American descent since SCD disproportionately affects this group.

1.6 Anticipated Recruitment and Study Duration

Our target accrual will be approximately 208 subjects.

The participating PECARN clinical sites have access to over 400 sickle cell patients requiring hospital admission per year. Assuming a 50% enrollment rate of eligible subjects, the accrual of approximately 208 subjects is anticipated to be completed within 36 months.

2 Background and Significance

In the United States, there are over 18,000 hospitalizations and 75,000 hospitalization days annually for children suffering vasoocclusive pain episodes secondary to sickle cell disease (SCD).[1] The treatment for these acute painful events, intravenous fluids and analgesia with opioids, has changed little over the past three decades.

Recently, the study investigators have developed a novel approach for the initial management of acute pain episodes, intravenous magnesium. Intravenous magnesium, an effective and safe drug for many pediatric conditions including asthma, acts as a vasodilator through both endothelial dependent

and endothelial-independent mechanisms. The safety profile, ease of administration, and low cost of magnesium make its potential as a therapy for acute sickle cell crisis even more exciting. A preliminary study of 19 children with sickle cell crisis showed a significant decrease in length of stay (LOS) when magnesium was added to standard therapy; the median LOS with magnesium was 3.0 days, compared with 5.0 and 4.0 days for the two previous hospitalizations.[2] Nevertheless, this study was not randomized, not blinded, not placebo–controlled, and not adequately powered to assess safety, therefore necessitating the current study.

This is a multi–center, randomized, double–blind, placebo controlled trial to assess the efficacy and safety of the addition of IV magnesium to standard therapy for sickle cell pain episodes. In order to reduce pain and suffering, an effective intervention should be undertaken as early as possible during the clinical course of a painful episode. In addition to examining the efficacy and safety of magnesium, translational studies performed prior to initiation of magnesium, during magnesium therapy, and several months after the episode while in steady state will potentially elucidate the mechanism of action for magnesium sulfate and provide insights into the pathobiology of acute vasoocclusive pain episodes.

2.1 General Background

SCD is caused by a genetic disorder of hemoglobin (hemoglobin βGlu6Val) that predisposes deoxyhemoglobin S to polymerize and form long crystals that distort and damage the red cell membrane.[3–5] The two major clinical manifestations of SCD are chronic hemolytic anemia and vasoocclusion, resulting in tissue ischemia and infarction. Factors that appear to contribute to vasoocclusive disease include:

1. the extent of HbS polymerization,
2. changes in the sickle red cell membrane including cytoskeletal rigidity and an enhanced adhesive phenotype,
3. localized disturbances in the vascular endothelium, leading to both a proadhesive phenotype and abnormal vasomotor tone, and
4. systemic effects, such as an augmented inflammatory response.

The vascular pathology of SCD is complex. Increased sickle RBC adhesion to the endothelium, sickling of hypoxic red cells, and hemolysis that leads to increased plasma free Hb are likely major initiating factors that lead to endothelial injury dysfunction and ultimately to vasoocclusion

(Figure 1).

Cell-free Hb impairs the bioavailability of nitric oxide (NO) and contributes to the generation of reactive oxygen species (ROS), oxidized lipids (e.g. isoprostanes) and oxidative endothelial injury. These pathologic events induce perturbations of endothelial derived vasoregulatory molecules resulting in deficits in endothelial-dependent vasodilation. The abnormal red cell interactions coupled with oxidative and inflammatory endothelial injury result in pathologic alterations in the vessel wall, including an inflamed and proadhesive endothelial cell phenotype. These events lead to secondary adhesive and thrombotic events, including red cell, leukocyte and platelet adhesion to the damaged vessel wall, and entrapment of sickled RBCs, precipitating acute vascular occlusion.

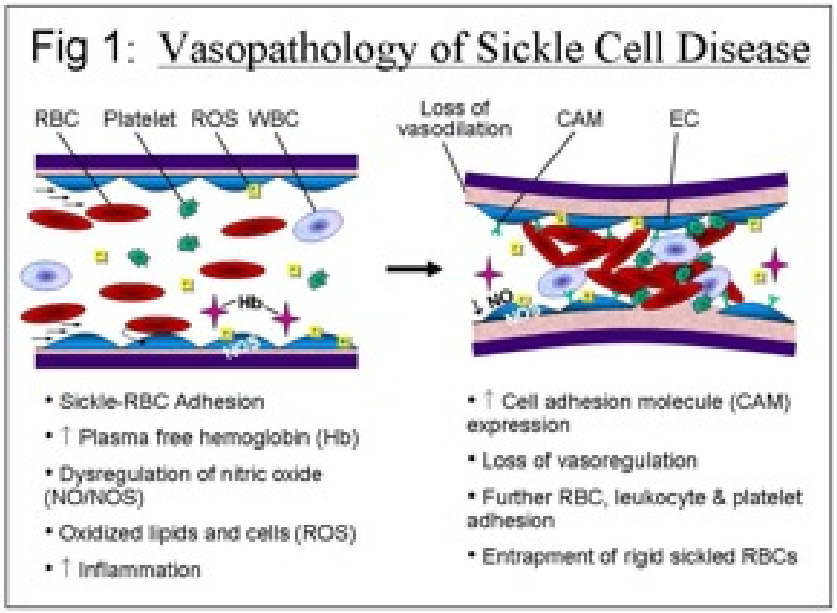

Figure 1: Complex vascular pathology in sickle cell disease.

2.2 Vasoocclusive Crisis and Hospital Admissions

Sickle cell anemia affects approximately one in 400 African American children. Much of the morbidity of the disease is due to recurrent vasoocclusive pain crises, which often result in hospitalizations, and adversely affect quality of life for children with sickle cell disease.[6, 7]

The average length of stay for a child hospitalized for a vasoocclusive crisis is over four days. It is estimated that there are approximately 18,000 admissions to the hospital nationwide each year for children as a result of these pain crises.[1] This results in over 75,000 days spent in the hospital each year by children due to the pain crises associated with sickle cell anemia. While improvement has been made in the chronic treatment of sickle cell disease with the addition of hydroxyurea therapy,[8–11] the treatment for acute pain crises has changed little over the past three decades.

2.3 Magnesium Therapy of Acute Pain

Magnesium is an NMDA (N–methyl–D–aspartate) receptor antagonist.[12] The NMDA receptor appears to modulate the response to painful stimuli. The NMDA receptor is modulated by Mg^{++} which blocks the ion channel in a voltage dependent manner from the outside. The ion channel opens by expelling Mg^{++}. The NMDA receptor appears to play an important role in both chronic and acute pain. In cancer patients with pain poorly responsive to strong opioids, a single magnesium infusion led to significant pain relief starting at 15 minutes, resulting in almost complete relief of pain by 45 minutes, and duration of pain relief lasting greater than four hours.[13] Infusions of magnesium have been studied in the setting of acute migraine headache. The rationale for magnesium therapy of migraine headache is that magnesium may affect both the NMDA receptor and vascular tone. Randomized placebo-controlled trials have shown significant relief of pain after magnesium infusion[14] especially if the migraine has an associated aura.[15] Other studies have shown modest benefit.[16] Thus, magnesium infusion may have a role in the management of a vascular pain syndrome.

2.3.1 Rationale for Vasoocclusive Crises

The long response time to clinical improvement (two weeks)[17] for oral magnesium raises the possibility of a more rapid response with the intravenous form, or suggests that the intravenous form is working via a different mechanism. The use of intravenous magnesium for people with sickle cell pain crisis is less well studied. In 1964, Hugh-Jones published a case series of five pediatric patients who showed significant, rapid improvement in pain with a combination of intravenous magnesium sulfate and an alkali.[18] There was no comparison group for this case series so an effect on length of stay could not be determined; however, these patients showed no untoward side effects at doses up to one gram of intravenous magnesium every six to eight hours.

This report together with the pilot trial performed in the lead investigator's center when combined with recent improved understanding of magnesium's effects on vasomotor tone underscore its potential as a therapeutic agent for acute sickle cell painful episodes.

Magnesium is a known vasodilator that can exert its effects through at least two independent mechanisms: a direct inhibition of calcium in the vascular smooth muscle wall and an endothelial dependent release of nitric oxide.[19–24] Magnesium can also modulate endothelial inflammation.[25–29] For example, magnesium deficiency is associated with increased levels of the inflammatory cytokines IL-1, IL-6 and TNF-α as well as endothelial expression of VCAM 1.[27–29] Furthermore, treatment of cultured endothelial cells with magnesium attenuates the inflammatory response to lipopolysaccharide (LPS).[26]

We hypothesize that magnesium imparts its beneficial effect through improvement of vascular dysfunction in SCD, either by modulation of vasodilation or by improvement of endothelial cell inflammation. Therefore, in Specific Aim 3 of this project, the mechanisms by which magnesium appears to have a beneficial effect on vasoocclusive crisis will be explored.

2.3.2 Safety in Children

Intravenous magnesium has been shown to be safe and effective in pediatric patients for a variety of other diagnoses, including several studies of pediatric asthma.[30–32] These studies used intravenous doses ranging from 25mg/kg to 75 mg/kg, with maximum doses of 2.0 to 2.5 grams, with no reported adverse side effects. Of note, these doses are still much lower than used in the obstetrical literature, where four grams is the standard bolus dose for pre-eclampsia, and continuous infusions of 2 grams/hour are used for up to 48 hours.[33]

2.3.3 Effect on Erythrocytes in SCD

Intracellular dehydration of sickle red blood cells likely contributes to vasoocclusive pain crises. Magnesium can also decrease erythrocyte potassium and water losses via the K/Cl cotransport system and thus improve sickle red cell dehydration.[17, 34] Twenty individuals with sickle cell disease treated chronically with oral magnesium pidolate showed increased cellular hydration as evidenced by decreased mean hemoglobin concentration (MCHC), and decreased distribution of red cell widths. Clinically, the subjects experienced a decreased number of painful days.[34] Although this study was

neither randomized, nor blinded, it suggests a potential role for oral magnesium in the chronic treatment of sickle cell disease. While the long-term treatment with oral magnesium improves red cell hydration status, it is unlikely that acute intravenous magnesium would affect erythrocyte parameters during the short duration of our preliminary study using intravenous magnesium beginning in the emergency department.[17, 34] In agreement, we found that the short duration of IV magnesium in our pilot study did not cause a significant change in sickle RBC hydration status (as measured by MCHC) or in vitro adhesion to thrombospondin.[2] Therefore, the beneficial effect of IV magnesium is more likely due to effects on the vasculature independent of cell hydration status, although an effect on hemolysis was not evaluated in the pilot study. In this study we will analyze both markers of hemolysis (e.g. plasma free hemoglobin and LDH) and production of nitric oxide (measurement of nitrite levels).

2.4 Impaired vasodilation in SCD

Vascular function is impaired in sickle cell disease.[35, 36] Patients with sickle cell disease have both increased circulating levels of vasoconstrictors, such as endothelin and thromboxane, and increased production and consumption of the vasodilator nitric oxide.[36] This imparts an inherent vascular instability in sickle cell disease, where acute perturbations in the bioavailability of nitric oxide likely contribute to acute vasoocclusive events in SCD. Studies of arterial forearm blood flow in individuals with SCD show increased endothelium-dependent vasodilation both at baseline and when stimulated with acetylcholine, which augments the endothelial release of NO, prostaglandins, and endothelium-derived hyperpolarizing factor.[37] The exaggerated vasodilation in SCD is due to both nitric oxide and nitric oxide-independent mechanisms.[36, 37] Nitric oxide plays an important role in maintaining systemic blood flow by promoting vasodilation as well as inhibiting platelet aggregation, leukocyte adhesion and endothelial adhesion molecule expression. There is a growing body of evidence that at baseline, individuals have a compensatory increase in nitric oxide production that is overwhelmed by scavenging of the nitric oxide by cell-free hemoglobin and increased reactive oxygen species in plasma lipids and the vascular wall.[36–38]

2.5 Inflammation and Endothelial Injury in SCD

SCD is considered a pro–inflammatory condition. The leukocyte count is elevated in SCD and correlates with a more severe clinical course, includ-

ing increased risk of stroke and early death.[39–42] Moreover, patients with SCD have chronically elevated acute phase proteins, which increase further during crisis.[43] Further evidence of endothelial inflammation and injury includes the finding of increased levels of circulating endothelial cells in patients with SCD, with higher levels of circulating endothelial cells at the time of vasoocclusive crises;[44] the circulating endothelial cells expressed an activated phenotype, including increased expression of the adhesive molecules VCAM-1, E-selectin, P-selectin and ICAM-1.[44] Human and murine SCD is associated with increased levels of the proinflammatory cytokines IL-1β, IL-6, TNF-α, and IFN-γ and that tend to further increase with acute vasoocclusive events.[45–48] Soluble VCAM-1 (sVCAM-1) is elevated in both human and murine SCD, consistent with significant endothelial cell injury associated with vasoocclusive events.[49, 50] The cell adhesion molecule P-selectin is expressed in both platelets and endothelial cells with increased endothelial expression of P-selectin reported in human and murine SCD.[51–55]

In a murine model of moderately severe SCD, Hebbel, Kaul and colleagues found that cycles of hypoxia and reoxygenation trigger an exaggerated inflammatory response with increased leukocyte number, rolling and adhesion, as well as increased production of reactive oxygen species by the vascular endothelium.[56, 57] To further support the claim that SCD is a "proinflammatory state" Holtzclaw recently found that SCD mice had increased mortality and an exaggerated inflammatory response to low dose LPS challenge compared to control mice.[48] This included elevated levels of TNF-α, IL-1β and sVCAM-1 in the serum and bronchoalveolar lavage fluid of SCD mice. Taken together, these data suggest that SCD is a proinflammatory state. In Specific Aim 3, the investigators will examine the levels of soluble VCAM-1, soluble P-selectin, and proinflammatory cytokines in subjects enrolled in this study during acute painful crises both before and after magnesium therapy as well as in the same individuals when they return to steady state.

2.6 Importance of Evaluating Health-related Quality of Life in Children with Sickle Cell Disease

It is increasingly being recognized that patient reported outcomes (PROs) are important outcomes to measure effectiveness of proposed therapies. A PRO is a "measurement of any aspect of a patient's health status that comes directly from the patient without the interpretation of the patient's responses by a physician or anyone else".[58, 59] Utilizing PRO as a secondary outcome in an interventional trial can aid in understanding the efficacy of

the intervention and provide insight into aspects of a patient's well-being that have historically been ignored. The proposed project seeks to determine the (HRQL) and short term outcomes (post hospital discharge: missed school/daycare/work and days of pain for the patient and missed work/school for the caregiver) of children presenting with an acute sickle cell pain episode who are randomized in the parent trial to receive treatment with intravenous magnesium sulfate or placebo.

In addition, the laboratory at the lead site has recently finished the development of the PedsQL Sickle Cell Disease module. Thus, we propose to utilize the longitudinal and randomized nature of this clinical trial to determine the responsiveness and minimally important difference (MID) of this measure to further define its psychometric properties.

2.7 Significance of Study

In summary, the data from other studies using magnesium for acute pain, and its safety record in the pediatric population, as well as its low cost, make it a potentially beneficial addition to the treatment of acute sickle cell painful events. This study will directly measure the effect of intravenous magnesium on the length of stay for children hospitalized with sickle cell vasoocclusive pain events and evaluate the safety of magnesium infusions in children with sickle cell disease, with the potential for direct and immediate benefit. The results could therefore change the standard of care for children with acute vasoocclusive crisis. Furthermore, the information gained from this study will increase our understanding of the pathophysiology of vasoocclusive crisis, leading to improved therapeutic approaches to the treatment of sickle cell crisis.

3 Preliminary Studies

3.1 Safety of IV magnesium in children with SCD

The safety of IV magnesium in children with sickle cell disease was evaluated in the pilot study.[2] Only one child, in the higher dose group, experienced any side effects. The child had an asymptomatic decrease in blood pressure to the low normal range after receiving IV morphine and IV magnesium within 15 minutes. She received a normal saline bolus and her blood pressure returned to her pre-medication level. She received no more doses of magnesium. All serum magnesium levels were within the normal range at initiation of magnesium therapy, and the highest magnesium level (obtained one hour

after infusion) for any child in the study was 2.9 mg/dL (normal 1.9 – 3.0 mg/dL), well below both the therapeutic (4–7 mg/dL) and toxic levels (greater than 7 mg/dL) in the obstetrical literature.[60] There was also no increase in the readmission rate for the children discharged to home after the admission in which they received magnesium.

3.2 Safety of IV magnesium in children with asthma

The principal investigator and author of this protocol, Dr. Brousseau, has previously investigated the efficacy and safety of intravenous magnesium sulfate in pediatric asthma patients. In a randomized, double blind trial, intravenous magnesium (dose of 40mg/kg; max 2 grams) was shown to increase peak flow rates and decrease hospitalizations in children with moderate to severe asthma. This benefit was accomplished with no reported side effects.[31]

The combined safety record of magnesium from the previous work in children and the results from our pilot study support the safety of a randomized trial of intravenous magnesium for sickle cell pain crisis.

3.3 Effect of IV magnesium on hospital length of stay in children with SCD

Investigators from the Wisconsin Sickle Cell Center at the Medical College of Wisconsin, completed a pilot study investigating the efficacy and safety of the addition of intravenous (IV) magnesium to standard therapy for nineteen children admitted to the hospital for sickle cell pain crisis.[2] The initial twelve children received 40 mg/kg (max 1.5 grams) of IV magnesium sulfate every eight hours for three doses. There was a significant decrease in the length of stay for those children when compared to their two most recent hospitalizations meeting the same admission criteria (median length of stay: 3.0 days with magnesium compared to 4.5 days and 4.0 days for their previous two admissions). In addition to the decrease in length of stay, there was a statistically significant 33% decrease in morphine equivalents used as well, suggesting that magnesium also decreased severity of pain. The pilot study was neither randomized, nor placebo–controlled, and therefore only provides preliminary evidence about the potential efficacy of the therapy. The current protocol will test for true efficacy and to further evaluate safety.

4 Study Design

This trial will be analyzed as an intention-to-treat study. Per-protocol analyses will also be performed for safety and laboratory related endpoints.

4.1 Hypothesis

The hypotheses of this multi–center, randomized, double–blind, placebo controlled trial are that

1. intravenous magnesium is an effective agent for the treatment of patients hospitalized for an acute sickle cell pain episode;
2. the addition of intravenous magnesium sulfate to standard inpatient therapy is safe for patients hospitalized with an acute sickle cell pain episode;
3. the beneficial effect of intravenous magnesium is, in part, due to increased endothelial cell nitric oxide production and/or improvement of endothelial dysfunction.
4. children receiving magnesium therapy will have better health-related quality of life (HRQL) at completion of treatment and better short term outcomes after hospital discharge than those receiving placebo therapy;
5. the PedsQL Sickle Cell Disease module will be responsive to temporal changes in health status of the child

4.2 Specific Aims

This proposal has the following Specific Aims:

Specific Aim 1. To compare the hospital length of stay, in hours, for individuals admitted because of acute sickle cell pain when intravenous magnesium (versus placebo) is added to standard therapy.

Specific Aim 2. To determine the safety profile of intravenous (IV) magnesium sulfate in the treatment of acute painful episodes in individuals with sickle cell disease (SCD).

Specific Aim 3. To determine the effect of intravenous magnesium sulfate on the nitric oxide pathway, measures of hemolysis, and markers of endothelial injury and inflammation.

Specific Aim 4. To determine the effect of intravenous magnesium on the health-related quality of life and short term outcomes of children with sickle cell disease presenting with an acute pain episode.

Specific Aim 5. To determine the psychometric properties of the PedsQL Sickle Cell Disease-specific (HRQL) measure.

4.3 Study Endpoints

4.3.1 Primary Endpoint

The primary efficacy outcome measure is length of stay (LOS) in the hospital, recorded in hours from the time of the start of the first study drug administration until the time of discharge from the hospital, or twelve hours after the last intravenous opioid, whichever occurs first.

4.3.2 Secondary Endpoints

One secondary outcome measure is the number of morphine equivalents per kilogram of body weight used during the hospitalization. This will include both intravenous, other parenteral, and oral doses of opioid. Additionally, at some sites, the HRQL and short term outcomes of children participating in the trial will be determined. HRQL of the child will be measured using the PedsQL generic core scales, the PedsQL fatigue scales and the pilot PedsQL sickle cell disease specific measure. Short term outcomes will be measured by determining the days of school/daycare/work missed and days of pain for the patient and days of work/school missed for the caregiver (if applicable) from the time of discharge from the hospital.

4.3.3 Safety Endpoints

Safety endpoints of this study are:

1. hypotension (defined as >20% SBP reduction from the measurement taken immediately prior to infusion) associated with study drug infusion;
2. weakness associated with study drug infusion;
3. warm sensation associated with study drug infusion;
4. rehospitalization within 7 and 28 days of hospital discharge.

In addition to these specific endpoints, adverse events will be recorded from the time of randomization through hospital discharge, or day 7 of hospitalization, whichever occurs first.

4.3.4 Additional Analyses

To help understand the potential effects of magnesium sulfate on the nitric oxide pathway, hemolysis, endothelial injury and inflammation, measurements will include:

1. plasma nitrite and the potential for plasma to scavenge nitric oxide as measures of functionality of the nitric oxide pathway;
2. plasma levels of sVCAM-1, sP-selectin, IL-1β, IL-6, TNF-α, and IFN-γ as measures of endothelial injury and systemic response to inflammation;
3. cell free hemoglobin, LDH, hemoglobin, and reticulocyte count as measures of hemolysis.

5 Study Activities

The study workflow is summarized in Figure 2.

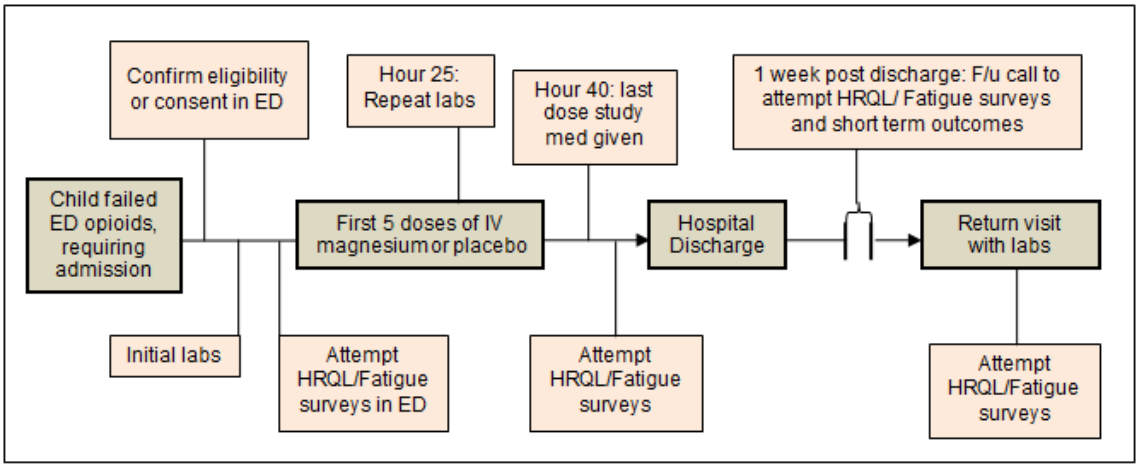

Figure 2: Summary of study workflow.

5.1 Screening and Enrollment

Patients with SCD will be screened in the emergency department and informed consent will be obtained as described in Section 9.2 on page 46. Permission or consent, and child assent, may also be obtained in advance of an emergency visit, during a hematology clinic visit or in the hospital. In this instance, verification of eligibility will be performed at the time of subject identification in the emergency department.

In order to complete the HRQL surveys, children with a history of severe cognitive impairment should not complete the child report of HRQL due to their presumed inability to comprehend the surveys. However, the parents of these children will be able to participate and will complete the parent-proxy HRQL forms and assessment of short term outcomes.

5.2 Randomization

The randomization will be accomplished through the use of an interactive voice randomization service via telephone. Equal allocation randomization will be stratified by age (4-11 years and 12-21 years), clinical center, and hydroxyurea use (yes/no) in randomly varying block sizes. Randomization tables will be provided by the CDMCC to each clinical center pharmacy. Randomization tables will be maintained in the hospital research pharmacy. After the randomization number is obtained, it will be transcribed into the study prescription, and the research pharmacist will fill the prescription according to the assigned study arm.

5.3 HRQL/Fatique Survey

At participating sites, the HRQL surveys marked only with a unique identifier will be attempted in the emergency department prior to the start of the first infusion(Time 0).

After the last dose of IV magnesium or placebo is administered in the hospital, the HRQL surveys will be attempted again (Time 1). The parent, if not present at the bedside at this time, may be called to complete the questionnaires over the phone. To capture the greatest effect of the study medication, the HRQL surveys should be completed within 1 to 1 $\frac{1}{2}$ days of administration of the last dose. In the unlikely event the patient is discharged to home prior to hour 48, the assessment for Time 1 should occur at the time of discharge. Our earlier work demonstrated that the average length of stay for children hospitalized for an acute painful event is 4 days, thus we expect few discharges prior to 48 hours.[1]

Eight to ten days post-discharge the HRQL surveys will be attempted again by child and caregiver via telephone interview at each clinical site (Time 2). Short term functional outcomes will also be assessed at this follow-up phone call. Alternatively, we will use mail to send and receive surveys for those families who choose to complete the survey by hand. The final assessment of HRQL will be attempted at the post discharge clinic visit at each site (Time 3). Pain level and change in pain, using self report measured with standard pain scales, will be also be assessed at Time 2 and Time 3.

5.4 Study Drug Administration

The subject will be randomized to receive 40 mg/kg (max 2.4 grams) of IV magnesium sulfate infused at a concentration of 40 mg/ml (1 ml/kg, max 60 ml), or the equivalent volume (1 ml/kg, max 60 ml) of normal saline placebo. The infusion will be administered over 20 minutes.

The first dose will ideally be given in the emergency department; however, a window will be allowed for enrollment following arrival on the inpatient floor (assuming this is still within the 12 hour IV opioid window in the exclusion criteria).

Every eight hours after the initial infusion for a total of six doses (or until discharge if it occurs before all six doses are given), the patient will receive a repeat dose of study drug.

Subjects will be monitored with a continuous monitor and pulse oximeter during the first infusion of study drug. The blood pressure, heart rate, and saturation will be recorded prior to beginning the infusion, and every 10 minutes, with the last measurement at least 30 minutes after completing the infusion.

For subsequent infusions, the blood pressure, heart rate, and saturation will be recorded prior to beginning the infusion, and every 20 minutes, with the last measurement at least 20 minutes after completion of the infusion.

The infusion of intravenous magnesium has been associated with a warm sensation, and hypotension has been reported with large bolus doses. Should any subject report a warm sensation on infusion of the study drug, the infusion will be slowed to half the previous rate. If the infusion is slowed because of a sensation of warmth, the blood pressure, heart rate, and saturation measurements will continue until at least 30 minutes after completing the infusion.

Should the subject experience a greater than 20% drop in systolic blood pressure from the pre-infusion measurement (defined as the blood pressure

reading obtained just prior to the start of the current study drug infusion), the infusion of the study drug will be stopped, a 10 cc/kg bolus of normal saline administered, and no subsequent doses of the study drug will be administered. Blood pressure monitoring will continue until the subject is no longer hypotensive.

5.5 Blood Sampling

Baseline blood samples will be obtained and repeated one hour after initiation of the fourth dose of study drug. Complete blood count, reticulocyte counts, serum LDH, BUN, creatinine, and calcium will be measured in the clinical site's clinical laboratory. For biomarkers analyzed for Specific Aim 3, up to 5.5 ml of whole blood will be collected and processed (Section 5.6) at the clinical site. Subjects will have similar blood sampling at the followup visit within three months of discharge.

5.6 Biomarker Blood Processing

For biomarker measurement, up to 5.5 ml of whole blood will be collected into 3.2% citrate (1:10 dilution, two x 3mL light blue top tubes) and the platelet poor plasma immediately isolated by centrifugation at 1500 g for 15 minutes, and the plasma aliquoted into 3 - 5 tubes ($\approx$600μL each) and immediately frozen at -60°C to -85°C as per a routine coagulation reference samples. An effort will be made to collect the research blue top citrated sample after the CBC tube (if obtained) to minimize hemolysis of the sample due to venipuncture. Collected plasma samples will be stored at -60°C to -85°C until use or shipment on dry ice to the central laboratory. This same protocol has previously been used to collect, store and ship samples of frozen citrated plasma samples to the central lab.[61]

5.7 Post-Discharge Followup

The family will be contacted approximately eight to ten days after discharge to determine whether the patient has required readmission to the hospital during the first seven days following discharge. The major reason for this followup telephone call is to maintain contact with the subject and confirm that they know that they should return within three months or as scheduled, after discharge for the final study evaluation. In addition, during this phone call, the HRQL surveys will be attempted and short term outcomes will be assessed at participating sites. At the follow-up visit, rehospitalizations will

also be queried, in addition to laboratory sampling. At participating sites, patients and parents will also attempt HRQL surveys at the follow-up visit.

Both all–cause rehospitalizations and sickle–cell related hospitalizations will be recorded. Medical records for all rehospitalized children will be reviewed to verify dates and diagnoses. If the subject was rehospitalized at a non-study hospital, then a release of information form will be requested so that the data can be obtained.

5.8 Summary of Study Activities

The study activities are summarized in Table 1 on the facing page. Infusions of study drug are administered at 0, 8, 16, 24, 32, and 40 hours of the study, and are not included in the Table.

Table 1: Summary of study activities

	ED Evaluation	*Baseline*	*Time 25 hrs*	*Time 40-52 hours*	*Next Scheduled*	*Daily*	*8 to 10 days*	*1-3 month*
Decision to admit patient	√							
History of present illness	√	X				√		
Physical examination	√				√	√		√
CBC	√				√	√		√
Reticulocyte count	√				√	√		√
Electrolytes, BUN, Cr		X	X					
Plasma free Hb		X	X		X			X
Plasma MetHb		X	X		X			X
Plasma nitrite		X	X		X			X
Calcium		X	X					
Serum magnesium		X						
LDH		X	X		X			X
Phone followup/Short term outcomes & pain							X	
Daily data collection						X		
Concomitant med review						X		
Adverse event review						X		
Complete HRQL surveys		X		X			X	X

√ denotes activities usually done as part of routine clinical care.
X denotes study activities that may not be part of routine clinical care.

6 Non-Study Hospital Therapy

6.1 Emergency Department (ED) Management

Patient initial evaluation, diagnostic work–up and pain management are at the discretion of the treating ED physician, in conjunction with the primary care physician or hematologist. The patient is only eligible for the study if he/she fails intravenous opioid pain management in the ED prior to the decision to admit for inpatient care.

The ED choice of opioid, and dosing is left to the treating ED physician using protocols established for pain management of sickle cell pain crisis at each participating institution. All of these treatment protocols involve the use of intravenous morphine unless contraindicated, in which case hydromorphone is the drug of choice. The use of intravenous non–steroidal anti–inflammatory drugs (NSAIDs) and laboratory testing is also at the discretion of the treating physician, although most institutions routinely perform a complete blood count and reticulocyte count as part of this initial evaluation. The use of NSAIDs during the ED course or during hospitalization will be documented, but is not a criterion for exclusion from the study. As the child is not eligible for enrollment until after having failed ED treatment, standardization of ED treatment is not part of this study.

6.2 Inpatient Management

Subjects will receive standard therapy for sickle cell acute painful episodes. The initial opioid treatment at each institution will be structured to aid in comparison of the effects of magnesium on pain and length of stay while still allowing institutional flexibility. Each child greater than or equal to 7 years of age will be started on 0.05 to 0.1 mg/kg/hour of intravenous morphine administered by patient–controlled–analgesia (PCA). Children younger than 7 years of age, who are frequently unable to operate a PCA, will receive intermittent bolus or continuous infusion of morphine at a similar dose per hour. The reason for any deviation from this protocol (for reasons of past history of allergy to morphine, or any other reason) will be documented on the "Outside of Protocol Specifications" log (Section 7.2.12 on page 37), but will not result in exclusion from the study. Other sickle cell specific medications (e.g. intravenous non–steroidal anti–inflammatory drugs) will be administered at the discretion of the treating physician, following treatment protocols specific to each institution. The dose and frequency of all other pain medications administered will also be recorded on the study flow sheet. Chronic medications unrelated to sickle cell disease, including anti–

epileptics and asthma controller medication, will not be affected by study participation.

7 Data Collection and Management

7.1 Clinical Investigator Responsibilities

Study data will be recorded on paper work forms, which will be retained at the clinical site in accordance with Section 14.3 on page 59. Data will then be entered into the electronic data capture (EDC) system provided by the CDMCC at the University of Utah School of Medicine.

The clinical investigator at each participating site is responsible for all aspects of study implementation, including administration of study drug, collection of accurate study data, and correct entry of the data into the EDC. These tasks may be specifically delegated to other individuals at the clinical site, but the clinical investigator is responsible to supervise all aspects of the study, and is responsible to assure that all staff involved in this study are adequately trained to perform the delegated tasks.

7.2 Phases of Data Collection

7.2.1 Screening, Enrollment, Consent, and Randomization Data

Screening. When a potentially eligible patient is in the emergency department, the clinical investigator or delegated study staff will screen the patient for eligibility. For patients who do not meet inclusion criteria, have previously been enrolled in the study, been identified as having a permanent exclusion criteria, or have refused on a prior visit no data will be entered into the study database. For patients who meet all inclusion criteria, data that will be recorded include yes/no answers to each of the exclusion criteria. If the patient is ineligible at this point, no further data will be recorded and the patient will not be approached.

Consent. If the patient has met the inclusion and exclusion criteria, the clinical investigator or delegated study staff will approach the patient and/or family to explain the study and obtain informed consent to participate. Data that will be recorded include whether the patient was approached, and whether informed consent was given. These will be yes/no questions. If the patient and/or family were not approached, the reason for not offering participation in the study will be recorded.

Enrollment and Randomization. For subjects consented to participate in the study, the date and time of signing the informed consent documents will be recorded. The date and time of randomization will also be recorded, as well as the resulting randomization number.

If a subject has agreed to participate in the study, but randomization does not occur, the reason for this will be recorded.

7.2.2 Pre-treatment Data

History of present illness. Pertinent review of systems findings will be recorded, and this historical information will be used to determine whether subsequent events are unchanged from baseline. Onset of pain will also be recorded. History of acute chest syndrome and asthma will be recorded.

In addition, the blood pressure obtained just prior to the start of each infusion will be recorded. The determination of hypotension is based on a 20% decrease below the value obtained prior to the start of the infusion. A table is provided in section 15 that demonstrates a 20% drop in systolic blood pressures for five point pre-infusion blood pressure categories.

Baseline physical examination. Pertinent physical findings, including vital sign measurements, will be recorded, and these findings will be used to determine whether subsequent events are unchanged from baseline. Additional data include the weight (kg) of the patient.

Baseline laboratory measurements. CBC and reticulocyte counts will be obtained and measured in the hospital clinical laboratory. For this study, the baseline hemoglobin, hematocrit, white blood cell total count, platelet total count, and reticulocyte count will be recorded.

Serum sodium, potassium, chloride, bicarbonate, BUN, creatinine, calcium, magnesium, and LDH will be measured and recorded.

In addition, biomarker samples will be obtained at this time, and will be processed as described in Section 5.6 on page 27.

Previous medications. All medications received by the subject in the 12 hours prior to ED arrival, as well as medications received after ED arrival but prior to first study drug infusion, will be identified and recorded. For opioid medications, the dose received will also be recorded.

Whether the patient is receiving hydroxyurea will be recorded as a yes/no question.

HRQL/Fatigue survey (at participating sites). The PedsQL will be self-completed by children ages 8-21 years and by parents of children ages 4 - 18 years. For children ages 5-7 years, the PedsQL will be completed with the aid of research personnel who will read aloud a script to the child as directed in the instructions by the developer. A faces response format is used to help the young child understand how to answer.

Additional child demographic and disease variables will be obtained using a standard demographic form. A history of frequency of prior vaso-occlusive painful episodes requiring hospitalization in the prior three years will also be recorded.

7.2.3 Study Drug Administration Data

All study drug events will have data collected as described in the following paragraphs. There will be a lead in question for each administration that verifies if it occurred. This is because a subject might be discharged from the hospital prior to completing the study, or a previous administration may have been associated with adverse events that preclude further drug administration.

Study Drug. The date and time of beginning the infusion, the total dose of study drug in ml volume, and the date and time of completing the infusion will be recorded.

Vital sign monitoring. The heart rate, blood pressure and pulse oximeter saturation will be measured (prior to instituting the infusion), and at intervals during the infusion. For the first infusion, this interval is every 10 minutes until 30 minutes after completion of the infusion. For subsequent infusions, the interval is every 20 minutes until 20 minutes after completion of the infusion. If the infusion requires slowing, the same intervals will be used, but the number of measurements will be greater.

The heart rate, blood pressure, and saturation measurements will be recorded with the date and time of each measurement.

7.2.4 Daily Data Collection

Study day zero is defined as the day on which the subject was enrolled into the study. Study day zero begins at the time of enrollment and ends at 23:59. All subsequent study days are calendar days. The last study day

ends when the subject is discharged from the hospital, or 12 hours after the last intravenous opioid administration (whichever occurs first).

Study drug. For each administration of study drug, data will include the time of starting and ending the infusion, vital signs, and the occurrence of hypotension or other events associated with the infusion.

Opioid administration. On a daily basis, the clinical investigator or delegated study staff will review the patient record to identify all opioid administration that occurred that day, including oral and parenteral administration. For each opioid administered, the name of the drug and total amount received will be recorded. On the last study day, the time of the last intravenous opioid administration will be recorded.

Opioid doses will be recorded in the actual dose of the drug administered. The calculation of morphine equivalents will be done at the CDMCC.

Review for adverse events. On a daily basis, the clinical investigator or delegated study staff will review the patient record and (if necessary) ask the clinical providers about adverse events that have occurred during that day. If adverse events occur, it is expected that the event will be recorded on the adverse event log (Section 7.2.11 on page 37).

Medication side effects. On a daily basis, the clinical site investigator or delegated research staff will document any symptoms the subject reported.

Concomitant medications. On a daily basis, the clinical investigator or delegated study staff will review the patient record for all non-study drug administrations that have occurred during that day. These medications will be recorded on the concomitant medications log (Section 7.2.10 on page 36).

Pertinent clinical events. There will be specific questions (yes/no) concerning whether the subject has signs and symptoms of acute chest syndrome, priapism, stroke, or other major organ events relating to vasoocclusion.

Transfusions. All blood transfusions will be recorded, including the date and time of beginning and ending the infusion, the type of blood product, and the volume infused.

Laboratory measurements. On a daily basis, CBC and reticulocyte counts may be obtained and measured in the hospital clinical laboratory. For this study, the hemoglobin, hematocrit, white blood cell total count, platelet total count, and reticulocyte count will be recorded. If these hematologic parameters are measured more frequently than daily, all available measurements will be recorded.

Serum sodium, potassium, chloride, bicarbonate, BUN, creatinine, calcium, and LDH will be measured and recorded at baseline and following the fourth study drug infusion (see Section 7.2.5). All additional available measurements of these laboratory parameters will be recorded. Serum magnesium will be obtained at baseline (and will be entered into the database by the clinical investigative staff.)

7.2.5 Following Fourth Administration

Laboratory measurements. One hour after initiation of the fourth study drug infusion, serum sodium, potassium, chloride, bicarbonate, BUN, creatinine, calcium, and LDH will be measured and recorded. In addition, biomarker samples will be obtained at this time, and will be processed as described in Section 5.6 on page 27.

Blood will be obtained at the next scheduled blood draw following the draw occurring one hour after the fourth administration to measure LDH, and for biomarker analyses.

7.2.6 Maintaining Magnesium Blinding during Study

The baseline serum magnesium value will be recorded by the clinical investigative team, and will be available to the clinical providers.

In the event of suspected clinical magnesium toxicity, rather than attempting to unblind themselves by obtaining a magnesium level, the clinical providers should assume that the subject is receiving active drug (magnesium) and take appropriate steps to manage the toxicity. The clinical team and investigators should not attempt to unblind themselves, and if suspicion of magnesium toxicity is high, they should discontinue study drug on the assumption that the drug is active.

7.2.7 Hospital Discharge or 12 Hours After Last Opioid

Prior to hospital discharge, the subject will be asked his or her perception of whether they received active drug (magnesium) or not. This information will help to assess adequacy of blinding of the patient. In addition, details of the

hospital discharge or the timing of the last intravenous opioid administration will be obtained, such as the date and time.

At participating sites, the HRQL/fatigue survey will be attempted again within 10-12 hours of completion of the last dose of IV magnesium or placebo.

7.2.8 Telephone Followup (8 to 10 Days)

The families of all subjects will be called 8-10 days following discharge to determine rehospitalization rates or unscheduled care events within the 7 days following discharge from the hospital. In addition to this phone call, inpatient and ED records from the enrolling institution will be reviewed to ensure no additional/unreported visits were missed. Data that will be recorded include the dates and times of all attempted followup efforts, and for the successful followup, the occurrence of rehospitalization or unscheduled care. For each rehospitalization or unscheduled care event, the date will be recorded, as well as the location of care and a brief description of the event requiring care.

At participating sites, during the follow-up call, the HRQL/Fatigue surveys and short term outcomes surveys will be attempted at each clinical site.

7.2.9 Followup within Three Months

At the followup visit, rehospitalizations and unscheduled care events will be determined, as described for the telephone followup. Blood will be obtained for measurement of hemoglobin, hematocrit, white blood cell total count, platelet total count, reticulocyte count, LDH, and the values will be recorded. Biomarker samples will also be obtained at this time, and will be processed as described in Section 5.6 on page 27. Additionally, and at participating sites, the final assessment of HRQL will be attempted at this visit.

7.2.10 Concomitant Medications Log

All concomitant medications that are received between the time of randomization and the time of the primary endpoint (hospital discharge or 12 hours after the last intravenous opioid administration) will be recorded. Data will include the name of medication, date, dose, whether the medication was administered because of an adverse event, and if so, description of the adverse event.

7.2.11 Adverse Events Log

All adverse events that occur between the time of randomization and hospital discharge, or day 7 of hospitalization, whichever occurred first, will be recorded. Data will include the name of the event, the start and stop dates of the event, outcome and intensity of the event, actions taken to treat the event, actions taken with respect to study drug, relationship to study intervention, expectedness, and seriousness. If the event is serious, a serious adverse event record will be provided to the CDMCC.

Details about the data components for adverse event reporting are available in Section 10.2 on page 50.

7.2.12 Outside of Protocol Specifications Log

All protocol deviations (violations) will be recorded on the log. The data that will be recorded include the date the event was first identified, the date that the event actually occurred, which phase of study was involved, and a text description of the event.

7.3 Data Management

7.3.1 Electronic Data Capture (EDC) System

All data from this study will be entered into an electronic data capture (EDC) system used by the CDMCC. This system provides secure user access via the Internet, and maintains an audit log for all study events and data.

7.3.2 Data Monitoring

Throughout the study, clinical data managers at the CDMCC monitor incoming data to identify obvious errors or inconsistencies in the data that are entered. The CDMCC staff will attempt to identify areas of confusion and provide additional education to clinical site staff for data inconsistencies. Site monitoring (Section 12 on page 55) will also be used to assure high quality study data.

7.3.3 Data Query Management

The CDMCC uses its own query management tool to notify sites of data inconsistencies, and study staff at each site will use this tool to maintain communication with the CDMCC for resolution of these queries. The CDMCC will provide interim reports of the numbers of outstanding data queries, and

the time to resolution of queries, stratified by site. These reports are used to help keep all sites up to date with data entry and resolution of data queries.

7.3.4 Database Lock

After the last subject accrual and followup visit, the database will not be able to be locked until all data queries have been resolved. Quantitative data will be examined statistically, and previously unverified unusual values (such as laboratory findings) will be verified prior to locking the database. When all such verifications have been completed, the database will be locked prior to undertaking the final data analyses for the study.

7.3.5 Preparation of Public Release Database

A public release database will be prepared by the CDMCC in accordance with NIH requirements, within three years of accrual of the last subject into the trial. This database will only contain reliable data elements, will exclude all potentially identifying subject information, and will include sufficient documentation to enable it to be useful to non-study investigators. The public release database will only be available to investigators who fill out a data use agreement and have IRB approval from their own institution.

8 Data and Laboratory Analyses

8.1 Primary Endpoint Analyses

The primary efficacy outcome measure is length of stay (LOS) in the hospital, recorded in hours from the time of the start of the first study drug administration until the time of discharge from the hospital, or twelve hours after the last intravenous opioid, whichever occurs first.

Twelve hours after the last intravenous opioid is the only previously published marker for the ability to control pain without opioids in SCD. Using this alternate primary outcome allows adjustment for hospitalizations that are prolonged for reasons not related to pain medication use, in particular, transportation difficulties and continued hospitalization related to social situations.

Median LOS and interquartile ranges will be reported. A van Elteren test will be used to compare the distribution of the length of stay for subjects in each treatment arm. Although we believe that death is a rare event in this population, it is possible for subjects to die before the time of discharge.

For the purpose of the primary analysis, these subjects will be counted as having the worst possible ranking of the primary outcome.

In exploratory analyses, potential covariates to be considered include age, gender, history of asthma, history of acute chest syndrome, number of days of crisis prior to presentation, pain medication used prior to ED visit and in the ED prior to enrollment, and the hemoglobin at presentation and baseline for the individual patient. Clinical center will also be used as a covariate to account for differences in management between sites.

8.2 Secondary Endpoints Analyses

One secondary outcome measure is the number of morphine equivalents per kilogram of body weight used during the hospitalization. This will include both intravenous, other parenteral, and oral doses of opioid. Additionally, at some sites, the HRQL and short term outcomes of children participating in the trial will be determined. HRQL of the child will be measured using the PedsQL generic core scales, the PedsQL fatigue scales and the pilot PedsQL sickle cell disease specific measure. Short term outcomes will be measured by determining the days of school/daycare/work missed and days of pain for the patient and days of work/school missed for the caregiver (if applicable) from the time of discharge from the hospital.

The total amount of opioid pain medication used during the hospitalization (including the emergency department) will be recorded. The number of morphine equivalents for intravenous (including all parenteral) and oral opioids per kg of body weight used during the hospitalization will be assessed. The amount of medication used will be logged daily.

For children who receive drugs other than morphine, morphine equivalents will be calculated using the previously published conversions[62] shown in Table 2 on the next page. Based on the pilot study, a decrease of approximately 33% is anticipated as the effect size.

A van Elteren test will be used to compare the total number of morphine equivalents per kg of body weight between the two groups.

In exploratory analyses, potential covariates to be considered include age, gender, history of asthma, history of acute chest syndrome, number of days of crisis prior to presentation, pain medication used prior to ED visit and in the ED prior to enrollment, and the hemoglobin at presentation and baseline for the individual patient. Clinical center will also be used as a covariate to account for differences in management between sites.

Table 2: Conversion constants for calculating morphine equivalents.

Drug	Equivalent Dose
IV morphine	10 mg
IV hydromorphone	1.4 mg
PO morphine	30 mg
PO hydromorphone	7.5 mg
PO codeine	200 mg
PO oxycodone	25 mg

8.3 Safety Endpoints Analyses

Safety endpoints of this study are:

1. hypotension (defined as >20% SBP reduction from the measurement taken immediately prior to infusion) associated with study drug infusion;

2. weakness associated with study drug infusion;

3. warm sensation associated with study drug infusion;

4. rehospitalization within 7 and 28 days of hospital discharge.

In addition to these specific endpoints, adverse events will be recorded from the time of randomization through hospital discharge, or day 7 of hospitalization, whichever occurs first.

The proportions of subjects who experience hypotension, weakness, a warm sensation during infusion, or all-cause rehospitalization within 7 or 28 days, will be compared between study arms.

Adverse events (including serious) will be tabulated by study arm for DSMB reporting and for final analysis of the safety of magnesium in this setting. Adverse events will be coded using the MedDRA vocabulary.

8.4 Quality of Life Analysis

At participating sites, HRQL scores between children in the two arms of the trial will be compared using a t-test or Wilcoxon rank-sum test as appropriate at times 1, 2 and 3. We expect differences between groups at times 1 and

2 and similar scores at the follow-up visit at time 3. A Holm's adjustment for multiple testing will be applied.[63]

We will also perform a longitudinal analysis of HRQL scores over time. A linear mixed-effects, subject-specific model will be used. Treatment group and treatment-time interactions will be included as fixed effects. Both linear and quadratic terms for time will be considered. We will include random effects corresponding to each subject.

Missed days of work, missed days of school, and number of days post -discharge pain will also be compared using t-tests or Wilcoxon rank-sum tests.

We will determine the psychometric properties of the HRQL by calculating responsiveness and the minimally important difference (MID). To determine responsiveness we will calculate effect size as the mean change in the PedsQL sickle cell disease HRQL scores at baseline (Time 0) compared to scores at the follow-up visit (Time 3) divided by the standard deviation at baseline (Time 0).

MID will be calculated using three methods:

- Distribution method: The standard error of the measurement will be calculated as $\sigma\sqrt{1-r_{xx}}$ where σ is the standard deviation of the baseline HRQL score and r is the reliability (Cronbach's alpha) of the measure.

- Anchor method 1: We will use the pain score to anchor the HRQL score (i.e. a change in pain score of more than 1 point on a 10 point scale from time 0 to time 2 to time 3 will be compared to the change in HRQL to determine the MID for the HRQL measure).

- Anchor method 2: The patient's self-reported score on the global pain assessment at Time 2 will be compared to the change in HRQL from Time 0 to Time 2. Patients are then classified into one of seven global assessment change groups. Mean changes in HRQL score for each group will be calculated to determine the threshold for the MID.

8.5 Power and Sample Size Considerations

The sample size requirements for this trial reflect the power analysis for the primary endpoint, length of stay (LOS) in hours. A sample size of 91 subjects per group is needed to detect a 20–hour difference in length of stay, with $\alpha = 0.05$ and 80% power. The sample size calculation assumes a standard deviation of 2.0 days for LOS, based on our pilot study.[1]

Eligible subjects include both patients who have and have not received hydroxyurea. To account for up to 5% noncompliance (subjects who are randomized but get no study drug), the sample size should be inflated to 101 subjects per group. An additional 2% increase to account for interim monitoring boundaries gives 104 per group, or 208 total.

Significant subject drop–out is not anticipated in this study, since the primary and secondary outcomes are measurable entirely within the hospitalization. In addition, most the patients eligible for this study have established relationships with their clinical providers at the participating sites.

For the analysis of HRQL, some centers will collect the HRQL data, but not all are required to participate for the study aims related to HRQL to be complete.

8.6 Interim Analyses and Stopping Rules

Interim monitoring for superiority of one treatment approach over the other will clearly be appropriate in this study. Symmetric monitoring boundaries are appropriate as one cannot rule out a detrimental effect from active drug.

Numerous clinical trials have found early treatment differences that diminished or even reversed as more subjects were enrolled. In a multicenter clinical trial, it is not unusual for early recruitment to be confined to a subset of centers that receive early IRB approval or have a smoother run-in phase; the experience at these centers may differ from others. Also, a "learning curve" in delivering the study therapies, at some or all centers, is not inconceivable. Because of these issues, we have selected monitoring boundaries that are conservative at the early looks at the data; we believe that O'Brien-Fleming-type boundaries,[64] implemented using the Lan-deMets flexible alpha spending function approach,[65] are appropriate for this study setting.

As this is an expensive study to conduct, early stopping for futility (low chance that a treatment effect is found if the trial continues) is a consideration. A conditional power approach, wherein the chance of the study finding a treatment effect (given the data accrued thus far in the study) under various assumed true scenarios is assessed, may be appropriate for the DSMB to address futility issues if this becomes necessary.[66] This approach, which requires careful consideration of what treatment effect scenarios are realistic given the study data themselves, encourages dialogue and discussion among DSMB members. However, early termination of a clinical trial for futility may greatly reduce the value of the trial, and the investigators of this study do not anticipate that early termination for futility is likely.

The projected accrual period in this study is three years. We assume

that the DSMB will meet prior to study launch, and then after the accrual of approximately 40 subjects in each arm, and after the accrual of approximately 80 subjects in each arm. The final analyses will be the third look, following 36 months of subject accrual. While flexible alpha spending will be used, we assume that there will be two meetings at which the DSMB will perform interim analysis, and that 33%, and 66% of study data (technically, of total statistical information for the primary outcome) are available at the respective meetings. Thus, there would be two interim analyses, with an additional final analysis of the study data if the study is not terminated early.

8.7 Laboratory Analyses

To help understand the potential effects of magnesium sulfate on the nitric oxide pathway, hemolysis, endothelial injury and inflammation, measurements will include:

1. plasma nitrite and the potential for plasma to scavenge nitric oxide as measures of functionality of the nitric oxide pathway;

2. plasma levels of sVCAM-1, sP-selectin, IL-1β, IL-6, TNF-α, and IFN-γ as measures of endothelial injury and systemic response to inflammation;

3. cell free hemoglobin, LDH, hemoglobin, and reticulocyte count as measures of hemolysis.

8.7.1 Analytical Techniques

Plasma Nitrite. As nitric oxide is a soluble gas with a half-life of a few seconds, we will measure the stable end product of nitric oxide, plasma nitrite, which closely correlates with nitric oxide synthase production of nitric oxide.[67, 68] Plasma will be deproteinated by centrifugation (4°C, 30 min, 15,000 x g) through a 10 KD micron Centricon filter, pre-washed with nitrite/nitrate-free buffer. The eluate will be injected in to the reaction chamber of a Sievers chemiluminescence NO analyzer at room temperature, which contains potassium iodide (50 mg) in 5 ml glacial acetic acid. This solution specifically reduces nitrite to NO, which is purged from solution by helium and detected after reaction with ozone. This method can detect as little as 1 pmol of nitrite. The presence of nitrite will be confirmed by pretreatment of an aliquot of the sample with acidic sulfanilamide (10 mM).

This compound selectively destroys nitrite and will confirm that the signals we observe are due to the presence of nitrite.

Cell free hemoglobin and the potential for plasma to scavenge nitric oxide will be measured by Electron paramagnetic resonance (EPR) spectroscopy. Electron paramagnetic resonance studies will be performed on a Bruker Elexys X-band EPR system equipped with a liquid helium cryostat and a liquid nitrogen-based variable temperature unit. Using this equipment we can obtain EPR spectra at a temperature range from approximately 3.5 K to room temperature. EPR measurement of metHb will be performed at 5 K. Plasma samples will be stored at -60°C to -85°C before EPR spectra are taken as outlined above.

MetHb levels will be determined directly by EPR with reference to a standard curve generated using authentic metHb. Total (metHb +oxyHb) hemoglobin levels will be determined after treatment of plasmas with the nitric oxide donor compound PRIOLI/NO (50 μM). This compound rapidly releases 2 moles of nitric oxide per mole PROLIN/NO and will convert all the oxyHb to the metHb form.

Analysis of SCD plasma for markers of inflammation and endothelial injury. Human SCD is associated with multiple markers of inflammation and endothelial injury, including elevated leukocyte and platelet counts, acute phase proteins, cytokines, soluble adhesion molecules and activated circulating endothelial cells.[43, 44, 49, 69, 70] We will measure the cytokines IL-1β, IL-6, TNF-α, and IFN-γ with the human-specific Bio-Plex cytokine assay kit (Bio-Rad Laboratories). To determine plasma levels of the human-specific soluble VCAM-1 and soluble P-selectin, we will use ELISA kits DVC00 and BBE6 from R&D Systems Inc. (Minneapolis, MN).

Interpretation of data. These measures will determine the level of total cell-free hemoglobin as well as the potential for plasma to scavenge nitric oxide. It has been established that the presence of oxyHb in plasma will limit that ability of endogenous and exogenous nitric oxide to promote vasorelaxation[71] and so a decrease in these parameters should correlate with an increased bioavailability of endogenous nitric oxide. These measurements, in combination with plasma nitrite measurements, will give an insight into the effect of magnesium on the functionality of the nitric oxide pathway. The cell-free hemoglobin is a sensitive marker of hemolysis and will measure

changes in levels during acute crises before and after magnesium therapy as well as compared to steady state. The plasma levels of sVCAM-1, sP-selectin, IL-1β, IL-6, TNFα and IFN-γ will be a gauge of endothelial injury and the systemic response to inflammation during an acute vasoocclusive event with and without magnesium therapy as well as compared to levels at steady state within the same individual. All quantitative assays will be performed in duplicate or triplicate.

Anticipated results. We predict that nitrite levels will be low and cell-free hemoglobin elevated at the time of presentation to the ED during acute painful vasoocclusive crisis (pretherapy and placebo-treated samples) compared to steady state levels. We also predict that the proinflammatory markers (TNF-α, IFN-γ and IL-6), measures endothelial injury (sVCAM-1 and sP-selectin), will be elevated at the time of presentation to the ED during the acute painful vasoocclusive event (pretherapy and placebo-treated samples) and that the levels will decrease, but not normalize when the study subjects return for their follow up visit at steady state. If magnesium acutely increases nitric oxide production, then nitrite levels should increase in the group of patients receiving magnesium therapy. If magnesium improves vascular inflammation, then we predict that magnesium treatment will decrease the level of the proinflammatory cytokines and measures of endothelial injury (sVCAM-1 and sP-selectin). Of note, sP-selectin is also a marker of platelet activation and data will be interpreted in this light.

8.7.2 Statistical Analyses

To determine the effect of magnesium therapy, we will use the difference in measurements taken before and after magnesium/placebo therapy (during vasoocclusive crisis) for each individual. The two groups will be compared using the Wilcoxon rank-sum test. To study differences between subjects at the onset of an acute crisis compared to during steady state when well, we will compare the initial measurement with that of the follow-up visit using the Wilcoxon signed-rank test. In addition, we will report summary data such as the mean, median, 95% confidence interval, and interquartile range for each analyte. For subgroup comparisons, such as comparing groups with and without concomitant hydroxyurea therapy, we will use ANOVA.

Previous work has shown differences in nitric oxide metabolite levels between those with sickle cell disease in acute crisis (mean 14.2 nmol/ml; s.d. 1.2) and those in steady state (mean 21.4 nmol/ml; s.d. 5.5).[49] Even conservatively assuming adequate blood samples on only half of the population,

45 in each group, we would still have greater than 80% power to detect a difference should the magnesium group return to baseline and the placebo group only return halfway to baseline. There is little previous work on the other laboratory values during a sickle cell crisis; the analyses are therefore somewhat exploratory. In reported results, we will focus on three main outcomes for the laboratory assessment: analysis on plasma nitrite (a marker of NO production), free hemoglobin (a marker of hemolysis), and VCAM-1 (a marker of endothelial injury). To adjust for the three outcomes, we will use a correction to $p < 0.05/3$ or $p < 0.016$ when assessing significance.

9 Human Subjects Protection

9.1 Institutional Review Board Approval

This protocol, the parental permission, subject consent, and child assent forms, if applicable, must be reviewed and approved by each Clinical Center's IRB before the study begins at that Clinical Center. In addition, the CDMCC must have documentation of current IRB approval at all times during the study. The CDMCC must also have a copy of the parental permission, informed consent, and child assent forms that were approved by the IRB for each Clinical Center before enrollment will be permitted at the Clinical Center. HIPAA authorization should be incorporated into the permission/consent process.

In the absence of documentation of on–going IRB approval in its possession, the CDMCC will not permit access to the randomization service or the electronic data capture (EDC) system by the clinical investigator or authorized staff from the clinical site. Lapses of IRB approval at any participating clinical site will be reported by the CDMCC staff to the study sponsor (Dr. Brousseau), the NICHD program officer, the PECARN Steering Committee, and the DSMB (at its scheduled meeting).

9.2 Recruitment and Informed Consent

Parents or legal guardians of children with symptoms of vasoocclusive crisis from SCD will be approached to provide permission for their child's participation in the study. Adult patients will be approached to provide informed consent for their own participation. The emergency medicine and hematology faculty and staff at each participating center will be informed about the study, and potential subjects may be pre-consented in the clinic or hospital. If the latter has occurred, eligibility will be verified in the emergency

department prior to enrollment in the study.

Parental or guardian permission, adult patient consent, and child assent will be obtained prior to initiation of study activities. Documentation of permission, consent, and assent will be maintained at the study site. The determination of whether a specific child must provide assent will be made according to the local Institutional Review Board (IRB) policies on this matter.

Financial compensation may be provided for the followup visit within three months following hospital discharge. This compensation must be approved by the Clinical Center's Institutional Review Board.

9.2.1 Parental Permission

For subjects under 18 years of age, this protocol requires that parents or other legally empowered guardians sign a parental permission form. The parent or legal guardian will be informed about the objectives of the study and the potential risks.

9.2.2 Child Assent

Subjects who are eligible for this study are in acute pain from the vasoocclusive crisis. This will often impair the ability of children to be able to provide assent for participation in the study. For this reason, waiver of assent will be requested from the IRB for children during the hospital phase of the study. When symptoms from the vasoocclusive crisis have resolved (prior to discharge or at the follow up visit), children who are capable of giving assent and who are alert and competent, will be asked, following an age-appropriate discussion of risks and benefits, to give assent to study participation. Assent will be waived if the child is too young, has a severely reduced mental age, decreased level of consciousness, psychological problems, or other legitimate reasons as judged by the Institutional Review Board at each Clinical Center.

9.2.3 Subject Consent

Subjects who are 18 years of age or older, will be informed about the objectives of the study, its potential risks and benefits, and required to sign an informed consent form in order to participate in the study. Subjects that are under 18 years of age at the time of enrollment who are 18 years of age at the time of the follow up visit will be required to sign an informed consent form at the time of the follow up visit.

9.3 Study Risks and Benefits

The risks to subjects in this protocol are reasonable in relation to the potential benefits to the participants and future patients with SCD.

9.3.1 Potential Risks

The clinical risks to subjects are those of receiving intravenous magnesium, a warm sensation on infusion, potential decrease in blood pressure, muscle weakness, flushing, dizziness, headache, constipation, and ileus. There may be other unknown risks of magnesium therapy in children with SCD although there were none encountered in the pilot trial. All subjects will still receive inpatient analgesia and supportive care for sickle pain crisis as is done at each study site.

Loss of confidentiality is always a risk in a study, but safeguards are in place to protect against this.

The amount of blood drawn for analysis of laboratory endpoints will pose minimal risk as the total volume will not exceed 3 cc/kg. In addition, there are risks of sickle cell disease that are not related to the study drug, including stroke, priapism, acute chest syndrome, and rehospitalization.

9.3.2 Potential Benefits to Participants

The study may result in decreased pain and decreased length of hospitalization for the subjects involved. Furthermore, the information gained from the analysis of nitric oxide may lead to further understanding of the physiology and treatment of sickle cell pain crisis. This could lead to new therapeutic options for future patients. Finally, this pediatric study may benefit adult patients in the future, as positive results from this study would be used to plan a future trial in adult patients.

9.3.3 Minimizing Risks of Participation

Multiple steps will be taken to minimize the risks of participation in this study. Clinical risks will be minimized by close monitoring of vital signs during study drug administration, and this protocol has precise instructions for slowing the infusion rate in the event of observed side effects (Section 5.4 on page 26). All of the participating clinical centers are tertiary pediatric hospitals with highly trained pediatric staff who can treat potential complications from magnesium infusion and are expert in the management of patients with sickle cell disease.

Loss of confidentiality is mitigated by the use of the PECARN CDMCC which has a highly secure IT infrastructure. Data security is described in Section 13 on page 57.

9.4 Discontinuation of Study Drug

If at any time prior to the final infusion of study drug the subject becomes hemodynamically unstable, septic, or has a stroke, no further study drug will be administered. Discontinuation of study drug may also occur if the clinical providers suspect clinical toxicity from magnesium. Discontinuation of study drug is not a withdrawal from study.

9.5 Withdrawal from Study

Subjects may completely withdraw from participation in this study at any time. If this occurs, the subjects will be followed for adverse events until hospital discharge or hospitalization day 7, whichever occurred first. These adverse events will be recorded.

Subjects may withdraw from receiving further study drug infusions but permit continued collection of study data.

Subjects may be withdrawn from receiving further study drug infusions by the clinical team caring for them because of contraindications or significant adverse events on a previous administration. While no additional study drug infusions will be administered, these subjects are *not* considered withdrawn from the study, and all study data will be collected and recorded.

10 Data and Safety Monitoring Plan

10.1 Data Safety Monitoring Board (DSMB)

This study will have a Data Safety Monitoring Board (DSMB) appointed in accordance with instructions from the NICHD program officer. The DSMB will have a charter, will approve the protocol prior to implementation, and will review interim analyses for safety and efficacy.

The purpose of the DSMB is to advise the Federal funding agency (NICHD), the study Principal Investigator (Dr. Brousseau), and the PECARN Steering Committee regarding the continuing safety of study subjects and the continuing validity and scientific merit of the study. The DSMB is responsible for monitoring accrual of study subjects, adherence to the study protocol, assessments of data quality, performance of individual clinical sites,

review of serious adverse events and other subject safety issues, and review of formal interim statistical analyses of treatment efficacy (Section 8.6 on page 42). The CDMCC will send reports relating to these topics to DSMB members prior to each DSMB meeting. It is anticipated that the DSMB will meet annually during patient accrual into the study, although the DSMB will have the discretion to alter meeting timing and frequency.

The Data Coordinating Center will staff DSMB meetings. The production and approval of DSMB minutes will be done in accordance with requirements of the NICHD. Each DSMB meeting will have a summary that will be provided to each participating clinical site for submission to the local Institutional Review Board (IRB).

10.2 Adverse Event Reporting

10.2.1 Definitions, Relatedness, Severity and Expectedness

Definition: An adverse event (AE) is any untoward medical occurrence experienced by a subject. An event constitutes a disease, a set of related signs or symptoms, or a single sign or symptom.

On each study day, the Clinical Center investigators will evaluate adverse events. Adverse events not previously documented in the study will be recorded on the adverse event record form. The nature of each experience, date and time (where appropriate) of onset, outcome, course, and relationship to treatment should be established.

Relatedness: The suspected relationship between study interventions and any adverse event will be determined by the Clinical Center investigator using the following criteria. *Relatedness may **not** be assessed by a research coordinator, and must be assessed by an investigator.*

Not Related: The event is clearly related to other factors, such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

Possibly Related: The event follows compatible temporal sequence from the time of beginning the assigned study intervention, but could have been produced by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

Probably Related: The event follows a reasonable temporal sequence from the time of beginning the assigned study intervention, and cannot be

reasonably explained by other factors such as the subject's clinical state, therapeutic interventions, or concomitant drugs administered to the subject.

Seriousness: The severity of clinical adverse events and laboratory abnormalities will be recorded by the Clinical Center investigator and categorized. A serious adverse event (SAE) is an adverse event that:

- results in death; or
- is life-threatening (the patient was, in the view of the Clinical Center investigator, in immediate danger of death from the event as it occurred); or
- requires inpatient hospitalization or prolongs and existing hospitalization; or
- results in persistent or significant disability or incapacity; or
- results in congenital anomaly/birth defect; or
- any other event that, based upon appropriate medical judgement, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

Expectedness of the Event: All adverse events, including serious adverse events, will be evaluated as to whether their occurrence was expected or unexpected. An adverse event is considered expected if it is known to be associated with sickle cell anemia, other underlying medical conditions of the subject, is directly related to study outcome, or is otherwise mentioned in the protocol, informed consent, or other study documents. Expected complications of sickle cell anemia include development of acute chest syndrome, decreases in hemoglobin requiring transfusion, priapism, stroke, or other major organ damage secondary to vasoocclusion. Other expected adverse events are reactions known to occur with magnesium administration, specifically hypotension, weakness, or a feeling of warmth at the site of infusion.

Treatment or Action Taken: For each adverse event, the Clinical Center will record whether an intervention was required:

- Intervention: Surgery or procedure
- Other Treatment: Medication initiation, change, or discontinuation
- None: No action taken

Finally, the Clinical Center will record the clinical outcome of each adverse event as follows:

- Death
- Recovered and the patient returned to baseline status
- Recovered with permanent sequelae
- Symptoms continue

10.2.2 Time Period for Adverse Events

For purposes of this study, adverse events occur following randomization through hospital discharge or day 7 of hospitalization, whichever occurs first. Specifically, events that occur following parental permission to participate in the study, but prior to actual randomization, will be not be reported as adverse events. These should be recorded as baseline conditions. Events that occur following discharge from the hospital will not be reported as adverse events. Serious adverse events will be followed until resolution or hospital discharge, whichever is earlier.

10.2.3 Data Collection Procedures for Adverse Events

After patient randomization, all adverse events (including serious adverse events), whether anticipated or unanticipated, will be recorded according to the date of first occurrence, severity, and their duration, as well as any treatment prescribed. Any medical condition present at the time of randomization, recorded in the patient's baseline history at study entry, which remains unchanged or improves, will not be recorded as an adverse event at subsequent evaluations. However, worsening of a medical condition that was present at the time of randomization will be considered a new adverse event and reported.

Abnormal laboratory values that are clinically significant will be recorded as adverse events and the Clinical Center investigator will assess the severity and relationship to the study. Laboratory values that are abnormal at the time of randomization and that do not worsen will not be recorded as adverse events.

Adverse events will be coded using the MedDRA coding vocabulary. Coding will be done centrally at the CDMCC because this requires specific training.

10.2.4 Monitoring Serious Adverse Events

The Principal Investigator of the CDMCC (Dr. Dean) will act as the medical monitor for this study. If Dr. Dean is unavailable, a qualified physician will be designated to fulfill this function. Clinical Center investigators and/or research coordinators will report serious adverse events to the CDMCC within 24 hours. A detailed completed report will be required to be sent to the CDMCC within 3 working days of the event, and the medical monitor will assess all serious adverse events reported from Clinical Centers. For each of these serious adverse events, the Clinical Center will provide sufficient medical history and clinical details for a safety assessment to be made with regard to continuation of the trial. The medical monitor will sign each SAE report after review. All SAE reports will be retained at the CDMCC, and all SAE reports will be available for review by DSMB members and NICHD staff via the eRoom^{TM} facility.

In the unlikely event that the medical monitor believes an unexpected and study-related SAE warrants emergent cessation of enrollment in the trial, NICHD staff and the DSMB chairperson will be immediately consulted. If these individuals concur with the judgment of the medical monitor, or if the NICHD staff and the DSMB chairperson cannot be reached expeditiously, the CDMCC will notify the study investigator (Dr. Brousseau) and all Clinical Center investigators to cease enrollment in the trial. Resumption of enrollment will not occur without consent of the NICHD staff after discussion with the DSMB.

10.2.5 Reporting Procedures

Assuring patient safety is an essential component of this protocol. Each participating Clinical Center investigator has primary responsibility for the safety of the individual subjects under his or her care. All adverse events will be evaluated by the Clinical Center investigator, and will be classified as noted in Section 10.2.1. All adverse events occurring after study randomization through hospital discharge or day 7 of hospitalization, whichever occurs first, will be recorded and entered into the electronic data entry system provided by the CDMCC.

The Clinical Center investigator will report all *serious, unexpected, and study-related* adverse events to the CDMCC within 24 hours. A detailed completed report will be required to be sent to the CDMCC within 3 working days of the event. After receipt of the complete report, the CDMCC will report such *serious, unexpected, and study-related* adverse events to the

NICHD Program Official or Project Officer in an expedited manner (within 24 hours). In accordance with local IRB requirements, the Clinical Center investigator may be required to report such events to the IRB in addition to notifying the CDMCC. In the event that the medical monitor believes that such an event warrants emergent suspension of enrollment in the trial, and NICHD staff cannot be reached expeditiously, the CDMCC will notify the study investigator (Dr. Brousseau) and all Clinical Center investigators to cease enrollment in the trial. Resumption of enrollment will not occur without consent of the NICHD staff after discussion with the DSMB.

Unanticipated problems (UP) are defined as incidents, experiences, or outcomes that are unexpected, related to participation in the MAGIC study, and suggest that the research places subjects at a greater risk of harm than was previously known or recognized. The Clinical Center investigator will report unanticipated problems to the CDMCC within 24 hours. A detailed completed report will be required to be sent to the CDMCC within 3 working days of the event. After receipt of the complete report, the CDMCC will report these unanticipated problems to the NICHD Program Official or Project Officer in an expedited manner (within 24 hours). In accordance with local IRB requirements, the Clinical Center investigator may be required to report such unanticipated problems to the IRB in addition to notifying the CDMCC. In the event that the medical monitor believes that such an event warrants emergent suspension of enrollment in the trial, and NICHD staff cannot be reached expeditiously, the CDMCC will notify the study investigator (Dr. Brousseau) and all Clinical Center investigators to cease enrollment in the trial. Resumption of enrollment will not occur without consent of the NICHD staff after discussion with the DSMB.

After notification of the NICHD Program Official or Project Officer, and the DSMB chairperson, of *serious, unexpected, and study-related* adverse events or unanticipated problems (UP), decisions will be made whether to continue the study without change, and whether to convene the entire DSMB for an emergent meeting. If a decision is made to suspend enrollment in the trial, this will be reported to the study investigator (Dr. Brousseau) and all clinical investigators, who will be instructed to report this to their local IRB.

The DSMB will review all adverse events (not necessarily serious, unexpected, and study-related) during scheduled DSMB meetings. The CDMCC will prepare a Summary Report of Adverse Events for the DSMB meetings, classified with the MedDRA coding system.

10.2.6 Follow-up of Serious, Unexpected and Related Adverse Events

All serious, unexpected and related adverse events, that are unresolved at the time of the patient's termination from the study or discharge from the hospital, will be followed by the Clinical Center investigators until the events are:

- Resolved, subject is lost to follow-up, the adverse event is otherwise explained or has stabilized; OR
- 3 months has passed from the time of randomization.

Adverse experiences that begin after discharge from the hospital will not be reported as study adverse events.

11 Study Training

A formal training program for investigators and research staff (including research pharmacists) will be held prior to the start of enrollment. The training program will cover regulatory topics including applicable device regulations and good clinical practice. The training will also provide in depth explanations regarding study procedures, clinical care, adverse event reporting, data entry procedures, quality assurance, site monitoring and the informed consent process. A manual of operations will be provided to each Clinical Center investigator prior to the start of enrollment. The manual will detail specific information about the study procedures, regulatory information, safety reporting, and other necessary information. Updates and revisions to the manual will be made available electronically. The CDMCC, in collaboration with the study investigator (Dr. Brousseau), will be the main contact for study questions.

The Clinical Center investigators will attend two study meetings annually throughout the course of this study to discuss study issues and instruct Clinical Center investigators. Each Clinical Center investigator should instruct physicians at their home institutions about the study, and serve as local advocates for the study and answer questions as they arise.

12 Study Site Monitoring

The investigators recognize the importance of ensuring data of excellent quality. Site monitoring is critical to this process. Site monitoring has

been a very effective tool for maintaining data quality in previous PECARN studies, and we will utilize this process to ensure excellent quality data in the proposed study. Our site monitoring plan is designed to identify problems with sites and methods for handling problems that arise.

Site monitors must be provided with full access to study materials and the medical records for study subjects. If the medical records are in electronic form, the clinical investigator or an authorized individual must provide any assistance necessary to facilitate the site monitor's review of data in the electronic medical record.

12.1 Site Monitoring Plan

A supplemental study-specific monitoring plan, separate from the protocol, will be completed which outlines specific criteria for monitoring. This plan will include the number of planned site visits, criteria for focused visits, or additional visits, a plan for chart review, and a follow up plan for non-compliant sites. The monitoring plan also describes the type of monitoring that will take place (e.g. sample of all subjects within a site; key data or all data), the schedule of when these activities are to take place, how they are reported, and a time frame to resolve any issues found. Remote site monitoring data elements and schedule will be determined by the CDMCC.

12.2 Clinical Site Monitoring

Site monitoring visits are conducted during the study to review patient entry, data quality, and patient safety and to assure regulatory compliance. The ongoing site monitoring visits will include an on-site meeting of the monitor, the Clinical Center investigator and his/her staff. A site monitor will visit each study site during the study period and review compliance with the study methodology and adherence to Good Clinical Practice guidelines. The site monitor will provide each site with a written report and sites will be required to follow up on any deficiencies.

It is anticipated that the study monitoring visits for this protocol will consist of a site initiation visit (prior to patient enrollment), interim visits, and a close out visit. The site initiation may take place as a group training of site investigators and research assistants.

Interim visits will take place depending on grant budget, site enrollment, and compliance issues identified. The first interim visit will take place when at least three subjects have been enrolled at a specific Clinical Center Subsequent interim visit frequency will be determined by the results of the

first visit and financial resources available for conducting site monitor visits. During interim visits, review of regulatory compliance and documentation, 100% review of consent documentation is anticipated, along with statistically controlled sampling for source verification.

Close out visits will take place after the last subject is enrolled at the site. The close out visit agenda would include resolution of outstanding queries and a review of adverse events, regulatory documentation, and archiving plan. The close out visit may need to be accomplished remotely, depending on available financial resources at the time of study completion.

12.3 Remote Monitoring

The CDMCC will supplement on-site monitoring with remote monitoring activities. Remote monitoring involves detailed review of the data entered by the Clinical Center and telephone consultations with the Clinical Center investigator and/or research coordinator to review safety and data quality. This requires uploading de-identified copies of specific parts of the medical record to the CDMCC staff, who review those materials against the data recorded in the electronic data capture system.

12.4 Pharmacy Monitoring

The research pharmacy at each participating clinical center will be responsible for prescribing the correct study drug related to the randomization number. The pharmacy must maintain adequate records of all dispensed study drug. Each pharmacy will be site monitored by an individual separate from site monitors assigned for clinical site monitoring. In addition, the pharmacy may be requested to send copies of these documents to the CDMCC for remote monitoring.

13 Data Security

The data coordinating center (CDMCC) at the University of Utah has a dedicated, locked server room within its offices, and the building has 24 hour on-site security guards. The CDMCC has a state-of-the-art computer infrastructure and coordinates its network infrastructure and security with the Health Sciences Campus (HSC) information systems at the University of Utah. This provides the CDMCC with effective firewall hardware, automatic network intrusion detection, and the expertise of dedicated security

experts working at the University. Network equipment includes three high-speed switches. User authentication is centralized with two Windows 2003 domain servers. Communication over public networks is encrypted with virtual point-to-point sessions using secure socket layer (SSL) or virtual private network (VPN) technologies, both of which provide at least 128 bit encryption. The electronic data capture (EDC) software and other information systems that will be used by investigators and staff in this protocol use the SSL protocol to transmit data securely over the Internet.

Direct access to CDMCC machines is only available while physically located inside the CDMCC offices, or via a VPN client. All network traffic is monitored for intrusion attempts, security scans are regularly run against our servers, and our IT staff are notified of intrusion alerts. Security is maintained with Windows 2003 user/group domain-level security. Users are required to change their passwords every 90 days, and workstations time out after 10 minutes of inactivity. All files are protected at group and user levels; database security is handled in a similar manner with group level access to databases, tables, and views in Microsoft SQL Server.

The investigators and staff of the data coordinating center are fully committed to the security and confidentiality of data collected for this study. All personnel at the CDMCC have signed confidentiality agreements concerning all data encountered in the CDMCC. Violation of these agreements may result in termination from employment at the University of Utah. In addition, all personnel involved with CDMCC data systems have received Human Subjects Protection and HIPAA education.

14 Regulatory Issues

14.1 Food and Drug Administration (FDA)

This trial is being conducted under an Investigational New Drug application approved by the Food and Drug Administration (IND #78,057).

14.2 Health Insurance Portability and Accountability Act

Data elements collected in this study include patient identifying information, such as the date of birth and date of admission. Prior to statistical analyses, dates will be used to calculate patient age at the time of the study events. The final data sets (used for study analyses and archived at the end of the study) will be de–identified, and will exclude these specific dates.

Data elements for race, ethnicity, and gender are also being collected. These demographic data are required for Federal reporting purposes to delineate subject accrual by race, ethnicity, and gender.

Each Clinical Center will be required to obtain informed consent from a legal guardian of eligible patients before the patient is enrolled in the study. In most instances, HIPAA authorization will be obtained with the same documents. For purposes of the CDMCC handling potential protected health information (PHI) and producing the de–identified research data sets that will be used for analyses, all study sites have been offered a Business Associate Agreement with the University of Utah. Copies of executed Business Associate Agreements are maintained at the CDMCC.

14.3 Record Retention

For federally funded studies subject to the Common Rule, records relating to the research conducted shall be retained for at least 3 years after completion of the research. Completion of the research for this protocol should be anticipated to include planned primary and secondary analyses, as well as subsequent derivative analyses. Completion of the research also entails completion of all publications relating to the research. All records shall be accessible for inspection and copying by authorized representatives of the CDMCC, funding agency, or regulatory authorities at reasonable times and in a reasonable manner [45 CFR §46.115(b)].

15 Appendix

MAGiC Study Blood Pressure Table

1. Obtain **pre-infusion systolic BP** (SBP)
2. Identify **corresponding 80% SBP** value (use table below)
3. **Monitor SBP** during infusion and up to 30/20 minutes after infusion completion
4. If SBP drops **below** the corresponding 80% SBP during monitoring:
   - Stop current **and** subsequent infusions
   - Give 10mL/kg dose of normal saline
   - Call/Page XXX-XXXX

Table 3: MAGiC Study Blood Pressure Table

Pre-infusion SBP	80% SBP
<80	63
80-84	66
85-89	70
90-94	74
95-99	78
100-104	82
105-109	86
110-114	90
115-119	94
120-124	98
125-129	102
130-134	106
135-139	110
>139	112

Bibliography

[1] J. A. Panepinto, D. C. Brousseau, C. A. Hillery, and J. P. Scott. Variation in hospitalizations and hospital length of stay in children with vaso-occlusive crises in sickle cell disease. *Pediatr Blood Cancer*, 44(2):182–6, 2005.

[2] D. C. Brousseau, J. P. Scott, C. A. Hillery, and J. A. Panepinto. The effect of magnesium on length of stay for pediatric sickle cell pain crisis. *Acad Emerg Med*, 11(9):968–72, 2004.

[3] B. Rochelson, O. Dowling, N. Schwartz, and C. N. Metz. Magnesium sulfate suppresses inflammatory responses by human umbilical vein endothelial cells (huvecs) through the nfkappab pathway. *J Reprod Immunol*, 73(2):101–7, 2007.

[4] W. B. Weglicki, T. M. Phillips, A. M. Freedman, M. M. Cassidy, and B. F. Dickens. Magnesium-deficiency elevates circulating levels of inflammatory cytokines and endothelin. *Mol Cell Biochem*, 110(2):169–73, 1992.

[5] J. A. Maier, D. Bernardini, Y. Rayssiguier, and A. Mazur. High concentrations of magnesium modulate vascular endothelial cell behaviour in vitro. *Biochim Biophys Acta*, 1689(1):6–12, 2004.

[6] C. A. Hillery and J. A. Panepinto. Pathophysiology of stroke in sickle cell disease. *Microcirculation*, 11(2):195–208, 2004.

[7] R. P. Hebbel. Beyond hemoglobin polymerization: the red blood cell membrane and sickle disease pathophysiology. *Blood*, 77(2):214–37, 1991.

[8] H. F. Bunn. Pathogenesis and treatment of sickle cell disease. *N Engl J Med*, 337(11):762–9, 1997.

[9] J. A. Panepinto, K. M. O'Mahar, M. R. DeBaun, F. R. Loberiza, and J. P. Scott. Health-related quality of life in children with sickle cell disease: child and parent perception. *Br J Haematol*, 130(3):437–44, 2005.

[10] O. S. Platt, B. D. Thorington, D. J. Brambilla, P. F. Milner, W. F. Rosse, E. Vichinsky, and T. R. Kinney. Pain in sickle cell disease. rates and risk factors. *N Engl J Med*, 325(1):11–6, 1991.

[11] M. de Montalembert, M. Belloy, F. Bernaudin, F. Gouraud, R. Capdeville, R. Mardini, N. Philippe, J. P. Jais, J. Bardakdjian, R. Ducrocq, M. Maier-Redelsperger, J. Elion, D. Labie, and R. Girot. Three-year follow-up of hydroxyurea treatment in severely ill children with sickle cell disease. the french study group on sickle cell disease. *J Pediatr Hematol Oncol*, 19(4):313–8, 1997.

[12] J. P. Scott, C. A. Hillery, E. R. Brown, V. Misiewicz, and R. J. Labotka. Hydroxyurea therapy in children severely affected with sickle cell disease. *J Pediatr*, 128(6):820–8., 1996.

[13] C. J. McCartney, A. Sinha, and J. Katz. A qualitative systematic review of the role of n-methyl-d-aspartate receptor antagonists in preventive analgesia. *Anesth Analg*, 98(5):1385–400, table of contents, 2004.

[14] S. Demirkaya, O. Vural, B. Dora, and M. A. Topcuoglu. Efficacy of intravenous magnesium sulfate in the treatment of acute migraine attacks. *Headache*, 41(2):171–7, 2001.

[15] M. E. Bigal, C. A. Bordini, S. J. Tepper, and J. G. Speciali. Intravenous magnesium sulphate in the acute treatment of migraine without aura and migraine with aura. a randomized, double-blind, placebo-controlled study. *Cephalalgia*, 22(5):345–53, 2002.

[16] Y. Cete, B. Dora, C. Ertan, C. Ozdemir, and C. Oktay. A randomized prospective placebo-controlled study of intravenous magnesium sulphate vs. metoclopramide in the management of acute migraine attacks in the emergency department. *Cephalalgia*, 25(3):199–204, 2005.

[17] L. De Franceschi, D. Bachir, F. Galacteros, G. Tchernia, T. Cynober, S. Alper, O. Platt, Y. Beuzard, and C. Brugnara. Oral magnesium supplements reduce erythrocyte dehydration in patients with sickle cell disease. *J Clin Invest*, 100(7):1847–52, 1997.

[18] K Hugh-Jones, H Lehmann, and JM McAlister. Some experiences in managing sickle-cell anaemia in children and young adults, using alkalis and magnesium. *British Medical Journal*, 2:226–229, 1964.

[19] J. C. Bernini, Z. R. Rogers, E. S. Sandler, J. S. Reisch, C. T. Quinn, and G. R. Buchanan. Beneficial effect of intravenous dexamethasone in children with mild to moderately severe acute chest syndrome complicating sickle cell disease. *Blood*, 92(9):3082–9, 1998.

[20] T. C. Griffin, D. McIntire, and G. R. Buchanan. High-dose intravenous methylprednisolone therapy for pain in children and adolescents with sickle cell disease. *N Engl J Med*, 330(11):733–7., 1994.

[21] A. Haenni, K. Johansson, L. Lind, and H. Lithell. Magnesium infusion improves endothelium-dependent vasodilation in the human forearm. *Am J Hypertens*, 15(1 Pt 1):10–5, 2002.

[22] EP Orringer, JF Casella, KI Ataga, M Koshy, P Adams-Graves, L Luchtman-Jones, T Wun, M Watanabe, F Shafer, A Kutlar, M Abboud, M Steinberg, B Adler, P Swerdlow, C Terregino, S CSaccente, B Files, S Ballas, R Brown, S Wojtowicz-Praga, and JM Grindel. Purified poloxamer 188 for treatment of acute vaso-occlusive crisis of sickle cell disease: A randomized controlled trial. *JAMA*, 286(17):2099–106, 2001.

[23] P. J. Pearson, P. R. Evora, J. F. Seccombe, and H. V. Schaff. Hypomagnesemia inhibits nitric oxide release from coronary endothelium: protective role of magnesium infusion after cardiac operations. *Ann Thorac Surg*, 65(4):967–72, 1998.

[24] Zhi-Wei Yang, A Gebrewold, M Nowakowski, BT Altura, and BM Altura. Mg2+-induced endothelium-dependent relaxation of blood vessels and blood pressure lowering: role of no. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.*, 278:R628–R639, 2000.

[25] M. H. Ascarelli, V. Johnson, W. L. May, R. W. Martin, and J. N. Martin. Individually determined postpartum magnesium sulfate therapy with clinical parameters to safely and cost-effectively shorten treatment for pre-eclampsia. *Am J Obstet Gynecol*, 179(4):952–6., 1998.

[26] J. A. Maier, C. Malpuech-Brugere, W. Zimowska, Y. Rayssiguier, and A. Mazur. Low magnesium promotes endothelial cell dysfunction: implications for atherosclerosis, inflammation and thrombosis. *Biochim Biophys Acta*, 1689(1):13–21, 2004.

[27] R. Mathew and B. M. Altura. Magnesium and the lungs. *Magnesium*, 7(4):173–87, 1988.

[28] H. Teragawa, M. Kato, T. Yamagata, H. Matsuura, and G. Kajiyama. Magnesium causes nitric oxide independent coronary artery vasodilation in humans. *Heart*, 86(2):212–6, 2001.

[29] H. Teragawa, H. Matsuura, K. Chayama, and T. Oshima. Mechanisms responsible for vasodilation upon magnesium infusion in vivo: clinical evidence. *Magnes Res*, 15(3-4):241–6, 2002.

[30] L. Ciarallo, A. H. Sauer, and M. W. Shannon. Intravenous magnesium therapy for moderate to severe pediatric asthma: results of a randomized, placebo-controlled trial [see comments]. *J Pediatr*, 129(6):809–14, 1996.

[31] L. Ciarallo, D. Brousseau, and S. Reinert. Higher-dose intravenous magnesium therapy for children with moderate to severe acute asthma [in process citation]. *Arch Pediatr Adolesc Med*, 154(10):979–83, 2000.

[32] RJ Scarfone, JM Loiselle, MD Joffe, CC Mull, S Stiller, K Thompson, and EJ Gracely. A randomized trial of magnesium in the emergency department treatment of children with asthma. *Ann Emerg Med*, 36(6):572–8, 2000.

[33] V Crosby, A Wilcock, and R Corcoran. The safety and efficacy of a single dose (500 mg or 1 g) of intravenous magnesium sulfate in neuropathic pain poorly responsive to strong opioid analgesics in patients with cancer. *Journal of Pain and Symptom Management*, 19(1):35–9, 2000.

[34] L. De Franceschi, D. Bachir, F. Galacteros, G. Tchernia, T. Cynober, D. Neuberg, Y. Beuzard, and C. Brugnara. Oral magnesium pidolate: effects of long-term administration in patients with sickle cell disease. *Br J Haematol*, 108(2):284–9, 2000.

[35] W. B. Weglicki and T. M. Phillips. Pathobiology of magnesium deficiency: a cytokine/neurogenic inflammation hypothesis. *Am J Physiol*, 263(3 Pt 2):R734–7, 1992.

[36] M. Aslan, T. M. Ryan, B. Adler, T. M. Townes, D. A. Parks, J. A. Thompson, A. Tousson, M. T. Gladwin, R. P. Patel, M. M. Tarpey, I. Batinic-Haberle, C. R. White, and B. A. Freeman. Oxygen radical inhibition of nitric oxide-dependent vascular function in sickle cell disease. *Proc Natl Acad Sci U S A*, 98(26):15215–20, 2001.

[37] M. T. Gladwin, V. Sachdev, M. L. Jison, Y. Shizukuda, J. F. Plehn, K. Minter, B. Brown, W. A. Coles, J. S. Nichols, I. Ernst, L. A. Hunter, W. C. Blackwelder, A. N. Schechter, G. P. Rodgers, O. Castro, and F. P.

Ognibene. Pulmonary hypertension as a risk factor for death in patients with sickle cell disease. *N Engl J Med*, 350(9):886–95, 2004.

[38] K. A. Nath, Z. S. Katusic, and M. T. Gladwin. The perfusion paradox and vascular instability in sickle cell disease. *Microcirculation*, 11(2):179–93, 2004.

[39] J. Ou, Z. Ou, D. W. Jones, S. Holzhauer, O. A. Hatoum, A. W. Ackerman, D. W. Weihrauch, D. D. Gutterman, K. Guice, K. T. Oldham, C. A. Hillery, and Jr. Pritchard, K. A. L-4f, an apolipoprotein a-1 mimetic, dramatically improves vasodilation in hypercholesterolemia and sickle cell disease. *Circulation*, 107(18):2337–41, 2003.

[40] M. T. Gladwin, J. H. Crawford, and R. P. Patel. The biochemistry of nitric oxide, nitrite, and hemoglobin: role in blood flow regulation. *Free Radic Biol Med*, 36(6):707–17, 2004.

[41] S. T. Miller, L. A. Sleeper, C. H. Pegelow, L. E. Enos, W. C. Wang, S. J. Weiner, D. L. Wethers, J. Smith, and T. R. Kinney. Prediction of adverse outcomes in children with sickle cell disease. *N Engl J Med*, 342(2):83–9., 2000.

[42] B. Balkaran, G. Char, J. S. Morris, P. W. Thomas, B. E. Serjeant, and G. R. Serjeant. Stroke in a cohort of patients with homozygous sickle cell disease. *J Pediatr*, 120(3):360–6, 1992.

[43] T. R. Kinney, L. A. Sleeper, W. C. Wang, R. A. Zimmerman, C. H. Pegelow, K. Ohene-Frempong, D. L. Wethers, J. A. Bello, E. P. Vichinsky, F. G. Moser, D. M. Gallagher, M. R. DeBaun, O. S. Platt, and S. T. Miller. Silent cerebral infarcts in sickle cell anemia: a risk factor analysis. the cooperative study of sickle cell disease. *Pediatrics*, 103(3):640–5, 1999.

[44] O. S. Platt, D. J. Brambilla, W. F. Rosse, P. F. Milner, O. Castro, M. H. Steinberg, and P. P. Klug. Mortality in sickle cell disease. life expectancy and risk factors for early death. *N Engl J Med*, 330(23):1639–44, 1994.

[45] J. Stuart, P. C. Stone, N. O. Akinola, J. R. Gallimore, and M. B. Pepys. Monitoring the acute phase response to vaso-occlusive crisis in sickle cell disease. *J Clin Pathol*, 47(2):166–9, 1994.

[46] A. Solovey, Y. Lin, P. Browne, S. Choong, E. Wayner, and R. P. Hebbel. Circulating activated endothelial cells in sickle cell anemia. *N Engl J Med*, 337(22):1584–90, 1997.

[47] A. Pathare, S. A. Kindi, S. Daar, and D. Dennison. Cytokines in sickle cell disease. *Hematology*, 8(5):329–37, 2003.

[48] A. Pathare, S. Al Kindi, A. A. Alnaqdy, S. Daar, H. Knox-Macaulay, and D. Dennison. Cytokine profile of sickle cell disease in oman. *Am J Hematol*, 77(4):323–8, 2004.

[49] J. M. Hibbert, L. L. Hsu, S. J. Bhathena, I. Irune, B. Sarfo, M. S. Creary, B. E. Gee, A. I. Mohamed, I. D. Buchanan, A. Al-Mahmoud, and J. K. Stiles. Proinflammatory cytokines and the hypermetabolism of children with sickle cell disease. *Exp Biol Med (Maywood)*, 230(1):68–74, 2005.

[50] J. D. Holtzclaw, D. Jack, S. M. Aguayo, J. R. Eckman, J. Roman, and L. L. Hsu. Enhanced pulmonary and systemic response to endotoxin in transgenic sickle mice. *Am J Respir Crit Care Med*, 169(6):687–95, 2004.

[51] M. J. Stuart and B. N. Setty. Sickle cell acute chest syndrome: pathogenesis and rationale for treatment. *Blood*, 94(5):1555–60, 1999.

[52] J. D. Belcher, C. J. Bryant, J. Nguyen, P. R. Bowlin, M. C. Kielbik, J. C. Bischof, R. P. Hebbel, and G. M. Vercellotti. Transgenic sickle mice have vascular inflammation. *Blood*, 101(10):3953–9, 2003.

[53] S. H. Embury, N. M. Matsui, S. Ramanujam, T. N. Mayadas, C. T. Noguchi, B. A. Diwan, N. Mohandas, and A. T. Cheung. The contribution of endothelial cell p-selectin to the microvascular flow of mouse sickle erythrocytes in vivo. *Blood*, 104(10):3378–85, 2004.

[54] N. M. Matsui, L. Borsig, S. D. Rosen, M. Yaghmai, A. Varki, and S. H. Embury. P-selectin mediates the adhesion of sickle erythrocytes to the endothelium. *Blood*, 98(6):1955–62, 2001.

[55] J. S. Mohan, G. Y. Lip, D. Bareford, and A. D. Blann. Platelet p-selectin and platelet mass, volume and component in sickle cell disease: relationship to genotype. *Thromb Res*, 117(6):623–9, 2006.

[56] K. C. Wood, R. P. Hebbel, and D. N. Granger. Endothelial cell p-selectin mediates a proinflammatory and prothrombogenic phenotype in cerebral venules of sickle cell transgenic mice. *Am J Physiol Heart Circ Physiol*, 286(5):H1608–14, 2004.

[57] K. Wood, J. Russell, R. P. Hebbel, and D. N. Granger. Differential expression of e- and p-selectin in the microvasculature of sickle cell transgenic mice. *Microcirculation*, 11(4):377–85, 2004.

[58] U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), Center for Devices and Radiological Health (CDRH). *Measurement and evaluation of PRO contribute to patient-centered care, a key ingredient in provision of quality health care*, draft guidance for industry on patient-reported outcome measures: use in medical product development to support labeling claims edition, 2006.

[59] Institute of Medicine. *Crossing the quality chasm: A new health system for the twenty-first century*, 2001.

[60] G. del Zoppo, I. Ginis, J. M. Hallenbeck, C. Iadecola, X. Wang, and G. Z. Feuerstein. Inflammation and stroke: putative role for cytokines, adhesion molecules and inos in brain response to ischemia. *Brain Pathol*, 10(1):95–112, 2000.

[61] Y. Sauntharajah, C. A. Hillery, D. Lavelle, R. Molokie, L. Dorn, L. Bressler, S. Gavazova, Y. H. Chen, R. Hoffman, and J. DeSimone. Effects of 5-aza-2'-deoxycytidine on fetal hemoglobin levels, red cell adhesion, and hematopoietic differentiation in patients with sickle cell disease. *Blood*, 102(12):3865–70, 2003.

[62] Brent Ruddock. Chronic pain: switching to an alternate opioid analgesic. *CPJ/RPC*, 138(1):44–45, 2005.

[63] S Holm. A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics*, 6:65–70, 1979.

[64] P. C. O'Brien and T. R. Fleming. A multiple testing procedure for clinical trials. *Biometrics*, 35(3):549–556, Sep 1979.

[65] KKG Lan and DL DeMets. Discrete sequential boundaries for clinical trials. *Biometrika*, 70:659–653, 1983.

[66] C Jennison and BW Turnbull. *Group sequential methods with applications to clinical trials*. Chapman and Hall, Boca Raton, FL, 2000.

[67] P. Kleinbongard, A. Dejam, T. Lauer, T. Rassaf, A. Schindler, O. Picker, T. Scheeren, A. Godecke, J. Schrader, R. Schulz, G. Heusch, G. A. Schaub, N. S. Bryan, M. Feelisch, and M. Kelm. Plasma nitrite reflects constitutive nitric oxide synthase activity in mammals. *Free Radic Biol Med*, 35(7):790–6, 2003.

[68] T. Lauer, M. Preik, T. Rassaf, B. E. Strauer, A. Deussen, M. Feelisch, and M. Kelm. Plasma nitrite rather than nitrate reflects regional endothelial nitric oxide synthase activity but lacks intrinsic vasodilator action. *Proc Natl Acad Sci U S A*, 98(22):12814–9, 2001.

[69] J. D. Belcher, P. H. Marker, J. P. Weber, R. P. Hebbel, and G. M. Vercellotti. Activated monocytes in sickle cell disease: potential role in the activation of vascular endothelium and vaso-occlusion. *Blood*, 96(7):2451–9, 2000.

[70] H. Croizat. Circulating cytokines in sickle cell patients during steady state. *Br J Haematol*, 87(3):592–7, 1994.

[71] D. K. Kaul, X. D. Liu, S. Choong, J. D. Belcher, G. M. Vercellotti, and R. P. Hebbel. Anti-inflammatory therapy ameliorates leukocyte adhesion and microvascular flow abnormalities in transgenic sickle mice. *Am J Physiol Heart Circ Physiol*, 287(1):H293–301, 2004.