Cochrane Library

Cochrane Database of Systematic Reviews

Magnesium for treating sickle cell disease (Review)

Than NN, Soe HHK, Palaniappan SK, Abas ABL, De Franceschi L

Than NN, Soe HHK, Palaniappan SK, Abas ABL, De Franceschi L.
Magnesium for treating sickle cell disease.
Cochrane Database of Systematic Reviews 2017, Issue 4. Art. No.: CD011358.
DOI: 10.1002/14651858.CD011358.pub2.

www.cochranelibrary.com

WILEY

TABLE OF CONTENTS

[Intervention Review]

Magnesium for treating sickle cell disease

Nan Nitra Than[1], Htoo Htoo Kyaw Soe[1], Senthil K Palaniappan[2], Adinegara BL Abas[1], Lucia De Franceschi[3]

[1]Department of Community Medicine, Melaka-Manipal Medical College, Melaka, Malaysia. [2]Department of Medicine, University Hospitals of Leicester, NHS trust, Leicester, UK. [3]Department of Medicine, University of Verona-AOUI Verona, Verona, Italy

Contact address: Nan Nitra Than, Department of Community Medicine, Melaka-Manipal Medical College, Jalan Batu Hampar, Bukit Baru, Melaka, 75150, Malaysia. nannitra@gmail.com.

Editorial group: Cochrane Cystic Fibrosis and Genetic Disorders Group.
Publication status and date: New, published in Issue 4, 2017.

Citation: Than NN, Soe HHK, Palaniappan SK, Abas ABL, De Franceschi L. Magnesium for treating sickle cell disease. *Cochrane Database of Systematic Reviews* 2017, Issue 4. Art. No.: CD011358. DOI: 10.1002/14651858.CD011358.pub2.

ABSTRACT

Background

Sickle cell disease is an autosomal recessive inherited haemoglobinopathy which causes painful vaso-occlusive crises due to sickle red blood cell dehydration. Vaso-occlusive crises are common painful events responsible for a variety of clinical complications; overall mortality is increased and life expectancy decreased compared to the general population. Experimental studies suggest that intravenous magnesium has proven to be well-tolerated in individuals hospitalised for the immediate relief of acute (sudden onset) painful crisis and has the potential to decrease the length of hospital stay. Some in vitro studies and open studies of long-term oral magnesium showed promising effect on pain relief but failed to show its efficacy. The studies show that oral magnesium therapy may prevent sickle red blood cell dehydration and prevent recurrent painful episodes. There is a need to access evidence for the impact of oral and intravenous magnesium effect on frequency of pain, length of hospital stay and quality of life.

Objectives

To evaluate the effects of short-term intravenous magnesium on the length of hospital stay and quality of life in children and adults with sickle cell disease. To determine the effects of long-term oral magnesium therapy on the frequency of painful crises and the quality of life in children and adults with sickle cell disease.

Search methods

We searched the Cochrane Haemoglobinopathies Trials Register, compiled from electronic database searches and handsearching of journals and conference abstract books.

Date of last search of the Cochrane Cystic Fibrosis and Genetic Disorders Group's Haemoglobinopathies Trials Register: 01 December 2016.

Date of last search of other resources (clinical trials registries): 29 March 2017.

Selection criteria

We searched for published and unpublished randomized controlled studies of oral or intravenous magnesium compared to placebo or no magnesium.

Data collection and analysis

Authors independently assessed the study quality and extracted the data using standard Cochrane methodologies.

Main results

We included five randomized placebo-controlled studies with a total of 386 participants (aged three to 53 years). Two shorter parallel studies (n = 306) compared intravenous magnesium sulphate to placebo (normal saline) for admission to hospital due to a vaso-occlusive crisis, for which we were able to analyse data. The quality of evidence was moderate for studies presenting this comparison mainly due to limitations due to risk of bias and imprecision. Two of the three longer-term studies comparing oral magnesium pidolate to placebo had a cross-over design. The third was a parallel factorial study which compared hydroxyurea and oral magnesium to each other and to placebo over a longer period of time; we only present the comparison of oral magnesium to placebo from this study. The quality of evidence was very low with uncertainty of the estimation.

The eight-hourly dose levels in the two studies of intravenous magnesium were different; one used 100 mg/kg while the second used 40 mg/kg. Only one of these studies (n = 104) reported the mean daily pain score while hospitalised (a non-significant difference between groups, moderate quality evidence). The second study (n = 202) reported a number of child- and parent-reported quality of life scores. None of the scores showed any difference between treatment groups (low quality evidence). Data from one study (n = 106) showed no difference in length of stay in hospital between groups (low quality evidence). Both studies reported on adverse events, but not defined by severity as we had planned. One study showed significantly more participants receiving intravenous magnesium experienced warmth at infusion site compared to placebo; there were no differences between groups for other adverse events (low quality evidence).

Three studies (n = 80) compared oral magnesium pidolate to placebo. None of them reported data which we were able to analyse. One study (n = 24) reported on the number of painful days and stated there was no difference between two groups (low quality evidence). None of the studies reported on quality of life or length of hospital stay. Two studies (n = 68) reported there were no differences in levels of magnesium in either plasma or red blood cells (moderate quality evidence). Two studies (n = 56) reported adverse events. One reported episodes of mild diarrhoea and headache, all of which resolved without stopping treatment. The second study reported adverse events as gastrointestinal disorders, headache or migraine, upper respiratory infections and rash; which were all evenly distributed across treatment groups (moderate quality evidence).

Authors' conclusions

Moderate to low quality evidence showed neither intravenous magnesium and oral magnesium therapy has an effect on reducing painful crisis, length of hospital stay and changing quality of life in treating sickle cell disease. Therefore, no definitive conclusions can be made regarding its clinical benefit. Further randomized controlled studies, perhaps multicentre, are necessary to establish whether intravenous and oral magnesium therapies have any effect on improving the health of people with sickle cell disease.

PLAIN LANGUAGE SUMMARY

Magnesium for treating sickle cell disease

Review question

We reviewed the evidence of the effect of intravenous (given into a vein) magnesium and oral (taken by mouth) magnesium on the frequency of painful crises (sickle cell crises with severe pain due to blockages of the blood supply to bones, joints, lungs, liver, spleen, kidney, eye or central nervous system), length of hospital stay and quality of life in people with different types of sickle cell disease.

Background

Sickle cell disease is a relatively common inherited blood disease. Its symptoms include the rapid onset of painful crises, which can lead to increased rates of hospital admission. The likely cause is that deformed sickle-shaped red blood cells put stress on blood vessels which blocks them and leads to vaso-occlusive crises. Vascular function is impaired in people with sickle cell disease. It is known that magnesium can widen blood vessels and, when regularly administered, it improves the amount of liquid in red blood cells and can help stop their shape deforming. Intravenous magnesium can cause mild to moderate side effects after administration e.g. nausea, vomiting, feeling of warmth, low blood pressure, etc.; and oral magnesium supplementation can cause mild side effects e.g. diarrhoea and abdominal cramps. We wanted to find out whether short-term intravenous magnesium and long-term oral magnesium is better than a placebo (dummy treatment with no magnesium) or no magnesium treatment, for reducing painful crises, shortening the length of stay in hospital and for improving quality of life. We were also interested in side effects of treatment with magnesium and some blood tests.

Search date

The evidence is current to: 01 December 2016.

Study characteristics

The review included five studies with a total of 386 people with sickle cell disease aged between four and 53 years. Two studies (306 people) compared intravenous magnesium to a placebo (in this case saline (salty water)) in people admitted to hospital as an emergency because of pain and lasted until they were discharged (less than four weeks). Two of the three longer-term studies compared oral magnesium pidolate with placebo and the third study compared hydroxyurea and magnesium pidolate to each other and to placebo but we have only included the results of the comparison of magnesium pidolate to placebo).

Key results

Not all the studies reported on our outcomes and we could not analyse data from most of the studies. We did find that in the people admitted to hospital as emergency cases, intravenous magnesium did not reduce pain levels, could not shorten the length of time spent in hospital and did not improve their quality of life compared to placebo. However, more people given magnesium experienced warmth where the needle was inserted than those people who were given placebo.

Oral magnesium pidolate, given over a longer period, did not reduce the severity of painful episodes and had no measurable effect on properties of sickled red cells (e.g. magnesium levels in the blood). Oral magnesium appeared to be safe and well-tolerated with only mild side effects (diarrhoea and headache). Further research is needed to compare the short-term and long-term benefits of magnesium treatment and its side effects.

Quality of the evidence

The quality of evidence for intravenous magnesium and oral magnesium in treating sickle cell disease was moderate for pain when using short-term intravenous magnesium and for levels of magnesium in the blood when taking longer-term oral magnesium supplements. The quality of evidence was low for all other outcomes we measured. All of the included studies of oral or intravenous magnesium for treating sickle cell disease had some aspects that could undermine the reliability of their results. Therefore, we have some uncertainty of these findings and further research may provide evidence that could change our conclusions.

SUMMARY OF FINDINGS FOR THE MAIN COMPARISON [Explanation]

Intravenous magnesium sulphate versus placebo for treating sickle cell disease

Patient or population: people with sickle cell disease
Settings: emergency department (Toronto, Canada); children's hospital (USA)
Intervention: intravenous magnesium sulphate

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Placebo	Intravenous magnesium sulphate				
Change in frequency of vaso-occlusive painful crises (as measured by pain diary of days with significant pain) Follow up: Not reported	The mean pain score in placebo group was **5.3**.	The mean pain score in the intervention group was **0.10 higher** (0.82 lower to 1.02 higher) than in the placebo group.	NA[4]	104 (1 study)	⊕⊕⊕◯ **moderate**[1]	There was no significant difference in the mean pain score between the intervention group and the placebo group
HRQoL score (Child-reported PedsQL generic scale) at 1 week follow up Follow up: 7-10 days and 3 months after discharge from hospital	NA[4]	The mean HRQoL score (child-reported HRQoL) in the intervention group was **2.3 lower** (7.21 lower to 2.61 higher) than in the placebo group.	NA[4]	202 (1 study)	⊕⊕◯◯ **low**[2,3]	There was no significant difference in the mean HRQoL score between the intervention group and the placebo group
HRQoL score (Child-reported PedsQL multidimensional fatigue scale) at 1 week follow up Follow up: 7-10 days	NA[4]	The mean HRQoL score (child-reported HRQoL) in the intervention group was **1.4 lower** (13.44 lower to 10. 64 higher) than in the	NA[4]	202 (1 study)	⊕⊕◯◯ **low**[2,3]	There was no significant difference in the mean HRQoL score between the intervention group and the placebo

and 3 months after discharge from hospital		placebo group.				group
HRQoL score (Child-reported PedsQL sickle cell disease module) at 1 week follow up Follow up: 7-10 days and 3 months after discharge from hospital	NA[4]	The mean HRQoL score (child-reported HRQoL) in the intervention group was 3.5**lower** (14.95 lower to 7.95 higher) than in the placebo group.	NA[4]	202 (1 study)	⊕⊕◯◯ **low**[2,3]	There was no significant difference in the mean HRQoL score between the intervention group and the placebo group
Length of hospital stay (hours) Follow up: Not reported	The mean length of hospital stay in the control group was **117.9 hours.**	The mean length of hospital stay in the intervention group was **14.7 higher** (20.51 lower to 49.91 higher) than in the placebo group.	NA[4]	104 (1 study)	⊕⊕◯◯ **low**[1,2]	There was no significant difference in the mean length of hospital stay between the intervention group and the placebo group
Adverse effects: hypotension Follow up: Not reported	**5** out of 151 participants.	**2** out of 155 participants.	**RR 2.57** (0.50 to 13.08)	306 (2 studies)	⊕⊕◯◯ **low**[1,2]	There was no significant difference in hypotension between the intervention group and the placebo group
Adverse effects: warmth at infusion site Follow up: 7-10 days and 3 months after discharge from hospital	**2** out of 102 participants.	**26** out of 100 participants.	**RR 13.26** (3.23 to 54.40)	202 (1 study)	⊕⊕◯◯ **low**[2,3]	There was significant difference in warmth at the infusion site between the intervention group and the placebo group

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: confidence interval; **HRQoL**: health-related quality of life; **NA**: not applicable; **RR**: risk ratio.

GRADE Working Group grades of evidence
High quality: further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: we are very uncertain about the estimate.

[1] Evidence downgraded due to serious limitation of risk of bias: the study lacked blinding of outcome assessment and the targeted sample size expected from protocol was not achieved.
[2] Evidence downgraded to imprecision: wide 95% CIs of the effects of outcomes of the intervention.
[3] Serious risk of bias: method of blinding of outcome assessment was not clearly described. Downgraded by one level from high.
[4] The results from the study were presented as either median or mean difference so an estimate of risk within the control group is not available.

BACKGROUND

Description of the condition

Sickle cell disease (SCD) is a hereditary red cell disorder found in populations across the world (Weatherall 2001). It is caused by a single point mutation in codon 6 of the human beta-globin (β-globin) gene that results in the substitution of valine for glutamic acid and the synthesis of pathological haemoglobin S (HbS). People with SCD can be homozygous for the pathological haemoglobin S(SS) or heterozygous with co-inherited other haemoglobin disorders such as HbC (SC) or β-thalassaemia (Sβ) (Mehta 2000). The worldwide distribution of SCD has dramatically changed in recent decades due to the survival of people with SCD in endemic areas, such as sub-Saharan countries, and the migration of populations from endemic areas to some western countries (Modell 2008; Weatherall 2001). The condition is characterized by decreased life expectancy targeting both paediatric and adult populations (Hamideh 2013; Platt 1994). Several years ago, the mean survival rate was 14.3 years for children with SCD (Diggs 1973), but this increased up to 20 years of age in children and adolescents as reported in a co-operative study of SCD (Leikin 1989). In adults with SCD life expectancy is 48 years for females and 42 years for males (Platt 1994). Mortality rates are low in children with SCD in developed countries (0.5 to 1.0 per 100,000 population) and high in some selected developing countries (15.5 per 1000 children) (Rahimy 2009).

In SCD, pathophysiological studies have shown that the dense, dehydrated red cells play a central role in acute and chronic clinical manifestations of SCD, in which production of sickle-shaped red blood cells in capillaries and small vessels leads to obstruction of the microcirculation by sickled red blood cells, causing ischemias (Belcher 2014; De Franceschi 2011; Hebbel 2011; Solovey 2001; Vinchi 2013). However, the persistent membrane damage with loss of deformability and changes in cell morphology (HbS polymerization) also favours the generation of distorted rigid cells and further contributes to vaso-occlusive events and cell destruction in the peripheral circulation (De Franceschi 2011; Kuypers 1998). Studies in both mouse models and humans with SCD have shown that vaso-occlusive crises (VOCs) result from a complex (and still only partially known) scenario, which also involves neutrophils, natural killer T-cells (NKT cells) (De Franceschi 2011; Field 2013) and platelets (Ataga 2003; Klings 2001; Okpala 2004), as well as soluble factors such as cytokines or the coagulation system (De Franceschi 2011; Sabaa 2008). In SCD, recurrent VOCs promote tissue damage, characterized by chronic inflammation and ischaemia-reperfusion cellular injury (where ischaemia means a lack of oxygen) that leads to acute and chronic multi-organ dysfunction (Reiter 2003). In young children, the main clinical manifestations of VOCs are dactylitis, osteomyelitis, osteonecrosis, splenic infarct, splenic sequestration, acute chest syndrome, stroke, papillary necrosis leading to kidney failure (Yaster 2000). In young adults with SCD the most frequent causes of hospitalizations are pain crisis, pneumonia, sepsis, acute chest syndrome and heart failure (Brousseau 2010; Caroll 2009; Platt 2000).

Recent evidence on the presence of circulating free heme and haemoglobin, related to the saturation of the physiological binding system, such as haptoglobin and haemopexin (De Caterina 1995; Lane 1996; Vinchi 2013) has opened an interesting prospective on the intravascular pro-oxidant environment and the local reduction of nitric oxide, which avidly binds free Hb promoting regional relative nitric oxide deficiency (De Franceschi 2003; Kato 2008; Rees 2010; Siciliano 2011). Thus, the two main clinical manifestations of SCD are acute VOCs and chronic haemolytic anaemia. The clinical manifestations of SCD are related to the peculiar biochemical properties of HbS, which polymerises when deoxygenated. Sickling of red blood cells has been shown to be exponentially related to the haemoglobin concentration. This demonstrates a crucial role of HbS concentration in sickling. Cyclic polymerization-depolymerization of HbS is paralleled by the abnormal activation of red cell membrane cation systems resulting in a reduction of cell ion and water content (cell dehydration), increased red cell density and further acceleration of HbS polymerizations (Brugnara 1993a; De Franceschi 1994; McNaughton-Smith 2008; Stocker 2003). Dense, dehydrated erythrocytes are likely to undergo instant polymerizations in conditions of mild hypoxia due to their high HbS concentration, and HbS polymers may be formed under normal oxygen pressure.

Analysis of membrane physiology of sickled red cells have shown abnormalities in different specialized membrane-embedded transporters that carry cations, anions and water across the erythrocyte membrane. In the last two decades, studies on the nature and properties of the pathways mediating potassium (K^+) loss in sickle cell erythrocytes have identified three major membrane systems; the Ca^{2+}-activated K^+ channel, known as the Gardos channel (Brugnara 1993a; De Franceschi 1994; De Franceschi 1996; Vandorpe 1998), operating in parallel with the conductive Cl^- pathway (Bennekou 2001) and the electroneutral potassium chloride (K-Cl) co-transport (Brugnara 1986; Su 1999). A role for the energy driven Na-K pump system has also been identified as contributing to sickled red cell abnormalities (Joiner 1993). Specific inhibitors for either the Gardos channel or the conductive Cl^- pathway have been developed for SCD, but no specific pharmacological blockers are available for the K-Cl co-transport (Brugnara 1993b; De Franceschi 1995; De Franceschi 1996), whose activity is regulated by magnesium (Mg) concentration and through a balance between kinase(s) and phosphatase(s) (De Franceschi 1997).

Description of the intervention

Studies in recent decades have highlighted the high bio-complexity of SCD, which requires the development of new therapeutic strategies. These strategies should be oriented towards multiple

targets in order to affect the range of clinical manifestations of SCD.

Magnesium is needed for more than 300 biochemical reactions in the body to maintain various cellular functions in different organs. A normal adult human body contains approximately 1000 mmol of Mg (22 g to 26 g), most of which is present in bone (up to 60%) and the remainder is present in skeletal muscle, soft tissues, extracellular fluid and intracellular fluid. Total serum Mg ranges from 0.70 to 1.10 mmol/L (Saris 2000). Generally, Mg is absorbed in the small intestine and excreted through kidneys (Rude 1998). The mean (standard deviation (SD)) daily dietary intake of Mg in a UK study was reported as 418 (120) mg for men and 343 (94) mg for women (Britton 1994). The recommended daily allowance (RDA) in adults is 4.5 mg/kg/day. The requirement is higher in pregnancy or during lactation and debilitating illness (Saris 2000). Supplementation with Mg may be indicated in a number of specific health conditions such as cardiovascular diseases, neurological disorders, bronchospasm, spinal cord injury or diabetes which are characterized by Mg cellular deficiency or a relative reduction in Mg absorption (Ladefoged 1996; Vormann 2003; Wester 1987).

Magnesium plays a key role in heart rhythm, immune system functions, bone metabolism, sugar metabolism and arterial blood pressure (Patel 2014; Galli-Tsinopoulou 2014; Orchard 2014; Saris 2000; Wester 1987). Intracellular Mg is an important co-factor for various enzymes, transporters, and nucleic acids that are essential for replication and energy metabolism (Bringhurst 2012). Magnesium also plays a crucial role in the modulation of endothelial inflammation, which may be beneficial in SCD, a pro-inflammatory disease with increased circulating levels of inflammatory cytokines IL-1b, IL-6 and TNFa, and expression of endothelial VCAM-1 (Brittain 2007; Maier 2004; Makis 2000). This modulation may be beneficial with increased circulating levels of cytokines in a pro-inflammatory SCD disease with painful crisis.

Intravenous Mg reduces VOCs through two mechanisms: a direct inhibition of calcium in the vascular smooth-muscle wall; and an endothelial-dependent release of nitric oxide (Mathew 1988; Yang 2000). Moderate adverse effects of intravenous Mg sulphate administration are vomiting, nausea, a feeling of warmth, flushing, hypotension, bradycardia, somnolence, double vision, slurred speech and weakness, in particular when it is rapidly administered. These side effects usually occur when the total plasma magnesium level reaches 3.5 to 5 mmol/L (almost double the normal Mg plasma levels) (Knochel 1997; Hermans 1996). The most severe effects of severe hypermagnesaemia (defined as Mg levels of 5 to 10 mmol/L) are neurological (e.g. fixed and dilated pupils, muscular paralysis, hyporeflexia, neuromuscular block with respiratory failure) (Rizzo 1993) and cardiovascular (e.g. junctional or sinus bradycardia, sinoatrial block, atrioventricular block, and asystole) (Berns 1976; Knochel 1997; Mauskop 1998).

Oral Mg is an important regulator of different ion transport and membrane systems, either directly in exchange systems such as the Na-Mg exchange or the K-Cl co-transport (De Franceschi 2001; De Franceschi 2006; Rivera 2005); or indirectly as an essential co-factor for sodium-stimulated phosphorylation of the sodium potassium adenosine triphosphate metabolism (ATP) (Na-K ATPase) (Flatman 1981). Oral Mg supplementation with different preparations such as Mg sulphate, Mg glutamate or Mg pidolate can cause some mild side effects such as diarrhoea and abdominal cramps (De Franceschi 1997).

How the intervention might work

Red blood cell dehydration in SCD is related to cyclic HbS polymerisation which is associated with reduced red cell Mg content. The reduced red cell Mg content is most likely related to abnormal red cell membrane permeability to double-charged ions such as Mg and calcium (Ca) during deoxygenation events and to the abnormal activation of the Na-Mg exchange (De Franceschi 1997; De Franceschi 2000; Rivera 2005). The role of Mg content in erythrocytes for regulating K-Cl co-transport has been demonstrated in normal human controls and people with SCD (Brugnara 1987; Canessa 1987). Studies on the level of Mg content are variable; some studies showed normal circulating levels of Mg (Akenami 1999; Oladipo 2005), while others reported low levels (Olukoga 1990; Zehtabchi 2004). However, plasma levels do not reflect the intracellular Mg content and the amount of free Mg, which is important in the context of cellular functions (De Franceschi 2001; De Franceschi 2006).

In SCD, red cell dehydration is related to abnormally high red cell membrane permeability during deoxygenation and the loss of potassium through the activation of two important pathways: the Gardos channel (Vandorpe 1998); and the K-Cl co-transporter (Brugnara 1986; Brugnara 1995). The activity of the K-Cl co-transport is affected by cell Mg content, oxidative stress and cyclic phosphorylation and dephosphorylation events (Brugnara 1993b; De Franceschi 1995; De Franceschi 1997; De Franceschi 2000); increased red cell Mg levels can inhibit the K-Cl cotransport activity (Brugnara 1993b; De Franceschi 1995; De Franceschi 1997; De Franceschi 2000). Thus, changes in red cell Mg content in SCD can reduce the abnormal activation of K-Cl co-transport, preventing at least one of the components of sickle red cell dehydration (Brugnara 1987; Brugnara 1995).

Several uncontrolled studies have reported beneficial effects of the intravenous administration of Mg sulphate or the oral administration of Mg citrate in people with SCD (Anstall 1959; Lehmann 1963).

In one study, intravenous Mg sulphate was administered in children with SS type of SCD being treated for painful crises; it was observed that the length of hospital stay decreased (Brousseau 2004). As there were no previous studies of Mg supplementation in children in with SCD, the dosage of intravenous Mg sulphate was lower than the dose used for other conditions (40 mg/kg) (Buchanan 2004). In another study, intravenous Mg sulphate was

administered in people with SS or HbS β-thalassaemia experiencing sickle cell pain crisis. It was seen that treatment decreased the duration of the VOC and subsequently decreased in length of hospital stay (Badaki 2014).

It has been observed that oral Mg supplements, such as Mg pidolate, have significantly improved red cell hydration by reducing the number of dense sickle erythrocytes, absolute reticulocyte count and immature reticulocytes, while erythrocyte Mg and potassium content were significantly increased (De Franceschi 1997; De Franceschi 2000; Zehtabchi 2004). One study used oral Mg as a short-term treatment (less than four weeks) at a dose of 0.6 morphine equivalent (meq)/kg/day (504 mg/day) (De Franceschi 1997) and another unblinded trial study used Mg pidolate in people with SCD homozygous for HbS (SS) as a long-term treatment at a dose of 540 mg/day for six months (De Franceschi 2000). The later study found that there was significantly reduced number of painful days during the six-month period.

Why it is important to do this review

This review aims to bring together evidence for the impact of intravenous Mg to reduce the pain and improve the quality of life in people with different SCD genotypes who are admitted to hospital for a painful crisis. It also aims to find out the effects of longer-term oral Mg on the frequency of painful crises and on the quality of life of people who are in a steady state of SCD, in order to ameliorate red cell dehydration and hematological phenotype. This review hopes to establish the clinical value of a pharmaceutical approach.

OBJECTIVES

To evaluate the effects of intravenous Mg on the length of hospital stay and quality of life in children and adults with SCD.

To determine the effects of oral Mg therapy on the frequency of painful crises and the quality of life in children and adults with SCD.

METHODS

Criteria for considering studies for this review

Types of studies

Randomized controlled studies and quasi-randomized controlled studies (controlled clinical studies).

Types of participants

Children and adults with SCD regardless of genotype such as sickle cell anaemia (HbSS), haemoglobin SC disease (HbSC) and S beta-thalassaemia (Sβ+ and Sβ 0) and of both sexes were eligible for inclusion.

Types of interventions

Intravenous administration of Mg to children and adults (of any genotype) at any dose and any duration at hospital admission for acute pain crisis compared to either placebo or no supplementation (we regarded short term as less than four weeks).

Oral administration of Mg to children and adults (of any genotype) at any dose and any duration (we regarded short term as less than four weeks and long term as from six months and less than two years) with at least one painful crisis in previous one year compared to either placebo or no supplementation.

Types of outcome measures

Primary outcomes

1. Change in frequency of vaso-occlusive painful crises as measured by
   i) pain diary of days with significant pain
   ii) number of painful days (where the pain is strong enough to require analgesics)
2. Quality of life (measured using a validated scale)
3. Length of hospital stay

Secondary outcomes

1. Change in dense red cells in SCD
   i) change in mean corpuscular haemoglobin concentration (MCHC)
   ii) change in red cell distribution width (RDW)
   iii) change in percentage of dense red cells (determined by phthalate density method)
2. Amelioration of hematological phenotype
   i) haematological parameters (complete blood count (full blood count) including erythrocyte indices, reticulocyte percentage)
   ii) biochemical parameters (blood urea nitrogen (BUN), creatinine, alanine amino transferase (ALT), aspartate amino transferase (AST) and blood chemistries using standard assays on a chemistry analyser)
3. Effects on K-Cl co-transport, red cell K+ and Mg2+ content (as determined with atomic absorption spectrometry (De Franceschi 1999))
   i) erythrocytes
   ii) potassium
   iii) sodium

4. Adverse effects

i) mild, e.g. abdominal cramps, unpleasant flushing, nausea, vomiting, diarrhoea

ii) moderate, e.g. vomiting , nausea, feeling warmth, flushing, hypotension, bradycardia

iii) severe, e.g. cardiac arrhythmias, somnolence, double vision, slurred speech and weakness

Search methods for identification of studies

We searched for all relevant published and unpublished studies without restrictions on language, year or publication status.

Electronic searches

We identified relevant studies from the Cystic Fibrosis & Genetic Disorders Group's Haemoglobinopathies Trials Register using the terms: (sickle cell OR (haemoglobinopathies AND general)) AND magnesium.

The Haemoglobinopathies Trials Register is compiled from electronic searches of the Cochrane Central Register of Controlled Trials (CENTRAL) (updated each new issue of the Cochrane Library) and weekly searches of MEDLINE. Unpublished work is identified by searching the abstract books of five major conferences: the European Haematology Association conference; the American Society of Hematology conference; the British Society for Haematology Annual Scientific Meeting; the Caribbean Health Research Council Meetings; and the National Sickle Cell Disease Program Annual Meeting. For full details of all searching activities for the register, please see the relevant section of the Cochrane Cystic Fibrosis and Genetic Disorders Group website.

Date of last search: 01 December 2016.

Using the search terms supplied in the appendices, we searched the clinical trial registries clinicaltrials.gov (clinicaltrials.gov/), WHO International Clinical Trials Registry Platform (WHO ICTRP) (http://www.who.int/ictrp/en/) and *meta*Register of Current Controlled Trials (*m*RCT) (www.controlled-trials.com/mrct/) (Appendix 1).

Date of last search: 29 March 2017.

We searched the PubMed database (www.ncbi.nlm.nih.gov/pubmed) for potentially relevant trials from 1946 to date (Appendix 1).

Date of last search: 01 April 2017.

Searching other resources

We also checked the references of the retrieved relevant articles to identify any additional studies. We made personal contact with experts and researchers in the field to ask for additional studies including unpublished and ongoing studies.

Data collection and analysis

Selection of studies

Two authors (NNT and HHKS) independently examined the titles and abstracts of the articles from electronic searches to remove any obviously irrelevant reports. The same two authors independently retrieved and reviewed full texts of the remaining articles to allow us to decide whether to include or exclude these according to the review's inclusion criteria. We were not blinded about the information of articles such as the journal of publication, the names of authors, the institution or the study results. We linked the multiple reports of the same study together. There was no disagreement about the inclusion of a study. However, if there are any disagreements in future, the two review authors will resolve the issue by discussion or by consulting a third review author (ALA). The review authors corresponded with other investigators, when required, to obtain further details of the study and missing information to clarify study eligibility and to allow a decision on the selection of studies. We listed the excluded studies with the reasons for exclusion in the Characteristics of excluded studies table.

Data extraction and management

Two authors (NNT and HHKS) independently extracted the data from the studies which fulfilled the inclusion criteria using a standard data collection form and checked these for consistency. There were no disagreements; however, if there are any disagreements in future, we will resolve these by discussion. If required, we will consult with a third review author (ALA) to reach a consensus. We corresponded with original author(s) if the information in the published article was missing or not clear. One author (HHKS) entered data into the RevMan software (RevMan 2014), and a second author (ALA) checked for any errors.

We extracted the following information as far as possible:

- source (study ID (created by review author), report ID (created by review author), review author ID (created by review author), citation (journal or conference, year of publication, etc.), contact details);
- eligibility (confirmed eligibility for review or reason for exclusion);
- methods (study design, study setting, time and duration of study, sequence generation, allocation sequence concealment, blinding, other concerns about bias);
- participants (total number, eligibility criteria (inclusion and exclusion criteria), age and sex of participants);
- interventions (total number of intervention groups for each intervention and comparison group of interest: dose of intervention; routes of delivery; timing of administration; frequency of administration; duration of intervention; co-interventions (if any));
- outcomes (for each outcome of interest: outcome definition (diagnostic method, name of scale, definition of threshold); units

of measurement (if relevant); for scales, upper and lower limits, and whether a high or low score is favourable);

- results (number of participants allocated to each intervention group, for each outcome of interest: sample size; missing participants; summary data for each intervention group (mean and standard deviation (SD) for continuous data, 2 x 2 table for dichotomous data, etc);
- miscellaneous (key conclusions of the study authors, references to other relevant studies; funding source; correspondence required).

We extracted data from multiple reports of the same study directly into a single data collection form. We planned to group the outcome data into those measured at one month, up to three months, up to six months, up to one year and over one year. The data we are currently able to present are from the 'up to one month' period (Brousseau 2015; Goldman 2013). For any studies included in future updates of the review, we will also record any outcome data which measured at other time periods (Higgins 2011a).

Assessment of risk of bias in included studies

Two authors (NNT and HHKS) independently assessed the risk of bias of included studies in seven domains as listed below. In each domain, we assigned a judgment of 'low risk', 'high risk' or 'unclear risk' of bias according to the criteria described in the *Cochrane Handbook of Systematic Reviews of Interventions* (Higgins 2011b).

- Sequence generation: low risk of bias (if the investigators included random component in sequence generation process such as using random number table, computer-generated random number, coin tossing, shuffling cards or envelopes, etc.); high risk of bias (if the investigators included a non-random component in sequence generation process such as the use of date of birth, date of admission, hospital or clinic record number, etc.); or unclear risk of bias (If there is no sufficient information about sequence generation process to judge whether high or low risk).
- Allocation concealment: low risk of bias (If the investigators used the methods such as central allocation, sequentially numbered drug containers of identical appearance, sequentially numbered opaque or sealed envelopes so that participants and investigator enrolling could not foresee the assignment); high risk of bias (If the investigators used an open random allocation schedule, non-opaque envelops and any other quasi-randomized methods such as alternation or rotation, case record number, etc. so that participants and investigator enrolling could possibly foresee the assignment); or unclear risk of bias (if method of concealment is not described or there is no sufficient information to judge whether high or low risk).
- Blinding of participants and personnel: low risk of bias (if blinding was done to the study participants or personnel and the method of blinding is described); high risk of bias (if no blinding or incomplete blinding was done to study participants and personnel); or unclear risk of bias (if there is no sufficient information to judge whether high or low risk).
- Blinding of outcome assessment: low risk of bias (if blinding was done to outcome assessors and method of blinding was described); high risk of bias (if blinding was not done to outcome assessors or if blinding was done, but likely it was broken); or unclear risk of bias (if there is no sufficient information to judge whether high or low risk).
- Incomplete outcome data: low risk of bias (no attrition, the number of dropouts or withdrawals was balanced in intervention groups with similar reasons); high risk of bias (imbalance of dropouts or withdrawals in intervention groups, the number and reasons of dropouts or withdrawals was not described); or unclear risk of bias: If there is no sufficient information of attrition or exclusions to judge whether high or low risk.
- Selective outcome reporting: low risk of bias (all the study's pre-specified outcomes of interest were reported); high risk of bias (not all the study's pre-specified outcomes of interest were reported, use of not pre-specified measurement for assessing outcomes, incomplete reporting of outcome data); or unclear risk of bias (if there is no sufficient information to judge whether high or low risk).
- Other sources of bias: low risk of bias (the study appears to be free of other source of bias); high risk of bias (presence of other source of bias); or unclear risk of bias (if there is no sufficient information to judge whether high or low risk).

The two review authors were not blinded to the names of study authors, institutions, journals and results of included studies. We discussed any discrepancies when assessing the risks of bias for a study. If we were not able to reach a consensus, we consulted a third review author (ALA). We contacted principal investigators to provide additional information which we thought was missing or unclear to allow us to make a risk of bias judgement.

Measures of treatment effect

We reported dichotomous data (adverse events) using the risk ratio (RR) with 95% confidence intervals (CIs). We reported continuous data (pain scores, QoL and length of hospital stay) using the mean difference (MD) with 95% CIs for outcomes measured using the same scale between studies. If different studies had used different scales for measuring the same outcome, the review authors would have reported the standardized mean difference (SMD).

Unit of analysis issues

If the authors had included cluster-randomized studies and if there had been little heterogeneity between all included studies, we would have pooled the results from both individually randomized studies and cluster-randomized studies.

For cross-over studies, if we believed there was a carryover effect or where second-period data were not available, we planned to in-

clude only data from the first period in the meta-analysis (Elbourne 2002). We contacted principle investigator of the De Montalembert cross-over study to clarify where data were presented separately by time period, but investigators were unable to provide information regarding the time point (De Montalembert 2003). As we were not able to clarify whether these data are from the first or second period of time we have presented the results narratively and in the additional tables.

In future updates of the review, if we include studies where there is no carry-over effect and second period data are available, we will collect the mean difference of measurements on the experimental intervention (E) and on the control intervention (C) of each participant and its standard error (SE). We will include the effect estimate and its SE in the meta-analysis using the generic inverse variance method in Review Manager (RevMan 2014).

If, in future updates of the review, we identify and include studies with more than two intervention groups, we will determine the relevant groups and, if necessary, combine the relevant groups to create a single pair-wise comparison.

Dealing with missing data

Some of the studies we included have only been published in abstract form or presented at meetings or conferences. We therefore corresponded with the investigators to request full reports; we are still awaiting responses to allow us to draw our conclusions. We investigated attrition rates including dropouts, withdrawals and loss to follow up. For the missing or unclear data, or missing data with no reported reason for dropout, we contacted the study investigator to provide further information, but are still awaiting responses (Higgins 2011c).

Assessment of heterogeneity

We assessed the clinical, methodological and statistical heterogeneity of the included studies. For clinical heterogeneity, we assessed the variability in intervention, participants and outcomes studied. For methodological heterogeneity, we assessed the sequence generation, allocation concealment, blinding, and differences in outcome assessment. We used the Chi² test to determine statistical heterogeneity and we assessed P value less than 0.10 as statistically not significant. We were able to combine data from two studies for one adverse event (hypotension) and I^2 was 0% (no important heterogeneity) (Analysis 1.5) (Deeks 2011; Higgins 2003).

Assessment of reporting biases

We were able to compare four of the protocols of included studies with the final report of methods and results sections to identify the selective outcome reporting (Brousseau 2015; CHAMPS 2011; Goldman 2013; Mueller 2005). We plan to use funnel plot in future updates (if we include at least 10 studies) to access the possibility of publication bias in studies, as recommended in chapter 10 of the *Cochrane Handbook of Systematic Reviews of Interventions* (Sterne 2011). If there is asymmetry, the possible causes of asymmetry may include true heterogeneity, high risk of bias or poor methodological quality, publication bias and selective outcome reporting.

Data synthesis

The authors presented the available data from two of the included studies in a meta-analysis using the RevMan software (RevMan 2014). We used a fixed-effect model since we only combined data in a single instance; and the level of heterogeneity was not important. If, in future updates of the review, we are able to combine data from multiple studies and there is less than moderate heterogeneity among the included studies, we will continue to analyse the data using a fixed-effect model. However, we will use a random-effects model if we identify at least moderate heterogeneity (where the I^2 value is at 50% or more, which we regard as statistically significant heterogeneity).

Subgroup analysis and investigation of heterogeneity

If, in future, the authors are able to include sufficient studies (at least 10) in a meta-analysis and they identify at least 50% heterogeneity, we plan to undertake the following subgroup analyses:

1. types of SCD (HbSS, HbSC disease, S β-thalassaemia);
2. demographic status (age less than 18 years or more than 18 years; male versus female);
3. different doses of Mg supplementation (any dose used in oral or parenteral administration);
4. different forms of Mg supplements (Mg pidolate, Mg sulphate, etc).

Sensitivity analysis

If we are able to include sufficient studies (at least 10) in a meta-analysis, we plan to perform a sensitivity analysis where we exclude the studies judged to have a high risk of bias (e.g. high or unclear risk of bias in allocation concealment, blinding, incomplete outcome data). We will perform the meta-analysis twice; firstly, including all studies and then including only studies that do not have a high risk of bias. We will also perform the meta-analysis twice using firstly a fixed-effect model and then a random-effects model to assess how robust the findings are to the choice of analysis method. Finally, we will also perform sensitivity analysis to investigate the robustness of our conclusions when combining cluster-randomized studies with individually randomized studies. We will report any sensitivity analyses of data by producing a summary table.

Summary of findings tables

In a post hoc change, we produced summary of findings tables using GRADE Profiler (GRADEpro 2014). We included one table

for the comparison of intravenous Mg sulphate versus placebo (Summary of findings for the main comparison) and one table for the comparison of oral Mg versus placebo (Summary of findings 2). For the intravenous Mg table, we presented the outcomes: change in frequency of painful VOCs (measured by pain diary day of significant pain); length of hospital stay; Child self-reported QoL; hypotension; and warm at infusion site for intravenous Mg (Summary of findings for the main comparison). For the oral Mg table we presented the outcomes: change in frequency of painful VOCs (measured by number of painful days); QoL; length of hospital stay; change in dense red cells; biochemical parameters; and adverse events (Summary of findings 2).

RESULTS

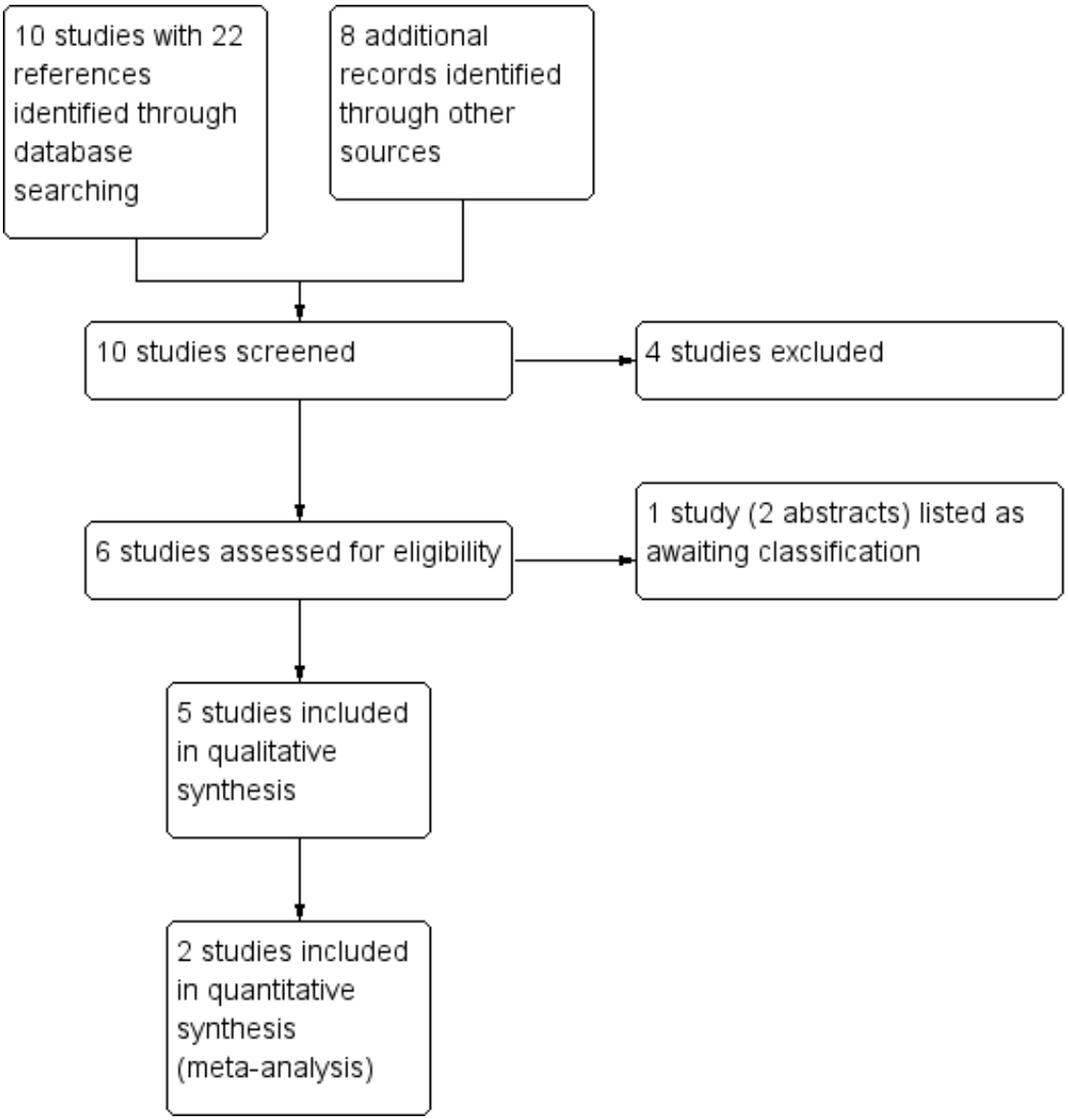
Description of studies

See Characteristics of included studies and Characteristics of excluded studies.

Results of the search

We identified 10 studies with 22 references in our searches, five studies (16 references) were included (Brousseau 2015; CHAMPS 2011; De Montalembert 2003; Goldman 2013; Mueller 2005). Four studies were excluded each with one full-text article (Brousseau 2004; De Franceschi 1997; De Franceschi 2000; Hankins 2007). One study (with two abstracts) is listed as 'Awaiting classification' and will be either included or excluded when we have further information (Voskaridou 2000). This process is detailed in the PRISMA diagram (Figure 1).

Figure 1. Study flow diagram.



Included studies

There are three included studies for oral Mg treatment (CHAMPS 2011; De Montalembert 2003; Mueller 2005) and two included studies for intravenous Mg in treating people with SCD (Brousseau 2015; Goldman 2013). The number of participants in the studies of oral Mg ranged from 12 children (Mueller 2005) to 24 children (De Montalembert 2003); and in the studies of intravenous IV Mg ranged from 104 children (Goldman 2013) to 202 participants (Brousseau 2015).

Trial design

All five included studies were randomized; three were of parallel design (Brousseau 2015; CHAMPS 2011' Goldman 2013) and two were of cross-over design (De Montalembert 2003; Mueller 2005). Four of the studies had two treatment arms (Brousseau 2015; De Montalembert 2003; Goldman 2013; Mueller 2005), but one compared four separate treatment groups (factorial design) (CHAMPS 2011). Two studies were short-term studies of people admitted to the emergency department for a VOC (Brousseau 2015; Goldman 2013), but the remaining three studies were of longer duration (at least six months of treatment) and outpatient-based (CHAMPS 2011; De Montalembert 2003; Mueller 2005). One study was conducted in Europe (De Montalembert 2003), one in Canada (Goldman 2013) and three in the USA (Brousseau 2015; CHAMPS 2011; Mueller 2005).

Participants

The numbers of participants in the two intravenous Mg studies (202 (Brousseau 2015) and 104 (Goldman 2013)) were greater than in the oral Mg studies (which ranged from 12 (Mueller 2005) to 24 children (De Montalembert 2003)). Three studies included only paediatric participants (i.e. up to 18 years of age) (De Montalembert 2003; Goldman 2013; Mueller 2005) and two studies included both adults and children; one with an age range of five to 53 years, although 73% of participants were under the age of 16 (CHAMPS 2011) and the final study recruited participants aged between four and 21 years; 60% were in the stratified age group of 12 to 21 years old (Brousseau 2015). All five studies reported on the gender split of participants and in all studies there were more males than females. In four of the studies the split was almost equal (CHAMPS 2011; Goldman 2013; Mueller 2005), but in one study there were significantly more males (71%) than females (De Montalembert 2003). For the three long-term studies, all participants had experienced at least one VOC in the previous 12 months (CHAMPS 2011; De Montalembert 2003; Mueller 2005). One study described participants as having SCD (De Montalembert 2003); two studies specified participants had HbSC (CHAMPS 2011; Mueller 2005); the fourth study included a range of participants: 61 (58.6%) had homozygous sickle cell anaemia; 33 (31.7%) had sickle haemoglobin C disease; and 10 (9.6%) had sickle beta thalassaemia (Goldman 2013) and the fifth study reported that 92 participants (91%) in the Mg group had HgbSS and 98 participants (95%) in the control group had HgbSS (Brousseau 2015).

There is a discrepancy in the stated number of participants in Goldman study between the two publications relating to this study (MAST study- NCT00313963); the 2011 paper states that 107 participants were randomized and 104 included in the study, but the 2013 paper states that 106 participants were included (Goldman 2013). There is also a discrepancy in the number of participants described in the MAGiC study (Brousseau 2015). The primary paper states that 202 participants were enrolled (Brousseau 2015); however a paper by Brandow reporting secondary data analysis of this study looking at opoid used (in emergency department and during hospital admission) reported 204 participants were enrolled. We have contacted the authors of both studies for further clarification.

Interventions

Study	Treatment	Control	Trial design
Brousseau 2015	Intravenous Mg sulphate Dosage: 40 mg/kg (max 2.4 g) . Infused at a concentration of 40 mg/mL, 8-hourly, total of 6 doses	Placebo (normal saline)	Randomized parallel study
Goldman 2013	Intravenous Mg sulphate Dosage: 8-hourly doses of 100 mg/kg, maximum of 2 g per dose	Placebo (saline)	Randomized parallel study

(*Continued*)

CHAMPS 2011	Oral Mg pidolate Liquid Dosage: 0.3 mmol/kg/day in twice daily doses	Placebo or hydroxyurea (data from comparison of Mg versus hydroxyurea not eligible for inclusion in this review)	Randomized parallel study (factorial design)
De Montalembert 2003	Oral Mg pidolate Blisters containing 1500 mg of pyrolidone carboxylate of Mg Dosage: 1, 2, 3 or 4 blisters per day depending on weight	Placebo	Randomized cross-over study
Mueller 2005 (NCT 00040456)	Oral Mg pidolate Liquid Dosage: 0.6 meq Mg pidolate/kg body weight per day divided into 2 daily doses	Placebo	Randomized cross-over study

All five studies compared the active intervention to a placebo control group (Brousseau 2015; CHAMPS 2011; De Montalembert 2003; Goldman 2013; Mueller 2005); one study additionally compared oral Mg pidolate to oral hydroxyurea (these data are not presented in this review as we are only comparing Mg to placebo or no treatment) (CHAMPS 2011).

Two studies treating participants for a painful crisis compared intravenous Mg sulphate to saline (Brousseau 2015; Goldman 2013). The doses were both given every eight hours, but differed in volume; one study administered 40 mg/kg to a maximum of 2.4 g with a maximum total of six doses until discharge (Brousseau 2015) and the second administered 100 mg/kg to a maximum of 2 g per dose (Goldman 2013).

Three studies used oral Mg pidolate as the active intervention (CHAMPS 2011; De Montalembert 2003; Mueller 2005). One study used once-daily blisters whose tablets containing 1500 mg of pyrolidone carboxylate of Mg; the number of tablets (and therefore the amount of treatment drug) depended on the weight of the participant (De Montalembert 2003). Two studies gave twice-daily doses of Mg pidolate in liquid form (CHAMPS 2011; Mueller 2005). The doses were 0.6 meq Mg pidolate/kg body weight per day (Mueller 2005) and 0.3 mmol/kg/day (CHAMPS 2011).

Outcomes

Three of the studies reported on the review's primary outcome of pain (De Montalembert 2003; Goldman 2013; Mueller 2005); but the study of intravenous Mg sulphate for the relief of a VOC had a slightly different focus than the longer-term studies and only looked at the mean daily pain intensity and cumulative analgesic requirement (Brousseau 2015; Goldman 2013). Two studies reported on the number of painful days (De Montalembert 2003; Mueller 2005), one of these studies additionally reported on pain intensity and the amount of analgesic administered (De Montalembert 2003). One study reported on the review's second primary outcome HRQoL (Brousseau 2015); length of hospital stay (third primary outcome) was reported by two the short-term studies (Brousseau 2015; Goldman 2013). The secondary outcome measures of changes in dense red cell and amelioration of hematological phenotype outcome measures were not reported by any of the included studies (De Montalembert 2003; Goldman 2013; Mueller 2005; CHAMPS 2011). Effects of Mg2+ content was reported by one study (De Montalembert 2003). Three studies reported adverse events but not split according to severity as we had planned to report (Brousseau 2015; Goldman 2013; Mueller 2005).

Excluded studies

We excluded four studies since none of them were randomized (Brousseau 2004; De Franceschi 1997; De Franceschi 2000; Hankins 2007) (see Characteristics of excluded studies).

Studies awaiting classification

There is only one controlled clinical study which is listed as awaiting classification (Voskaridou 2000). The study was published as an abstract only which does not allow us to adequately assess the design against our inclusion criteria. The study included 10 adults who were diagnosed with β^S/β thalassaemia disease. Five adults received 0.6 mE/kg/day of oral of Mg aspartate for six months (after informed consent) and a further five adults received a placebo

for six months. The outcomes measured were an arbitrary scoring of clinical severity; follow up of main red cell parameters along with the level of intracellular and serum Mg and the density of red cell distribution.

Risk of bias in included studies

Overall, we judged the included studies to have a moderate risk of bias (Figure 2; Figure 3).

Figure 2. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

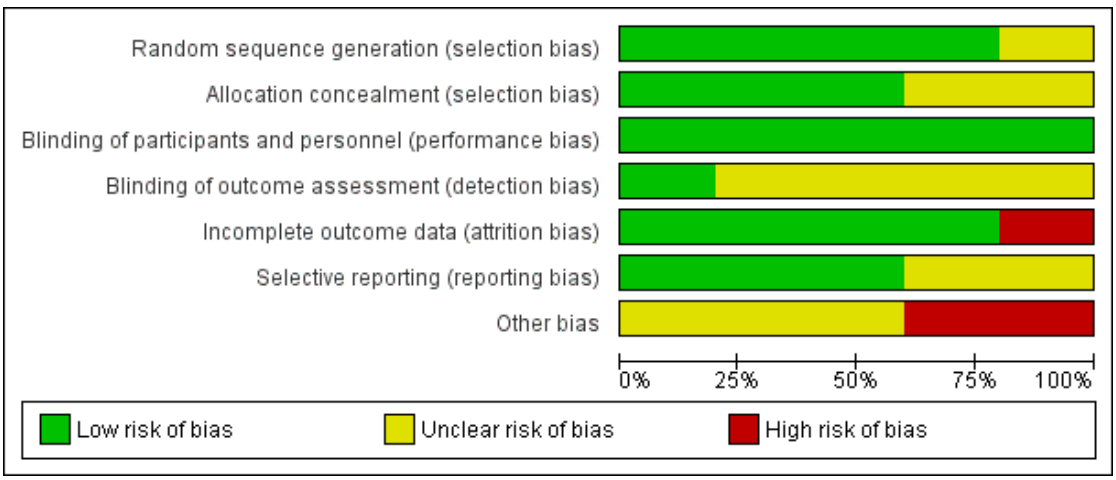
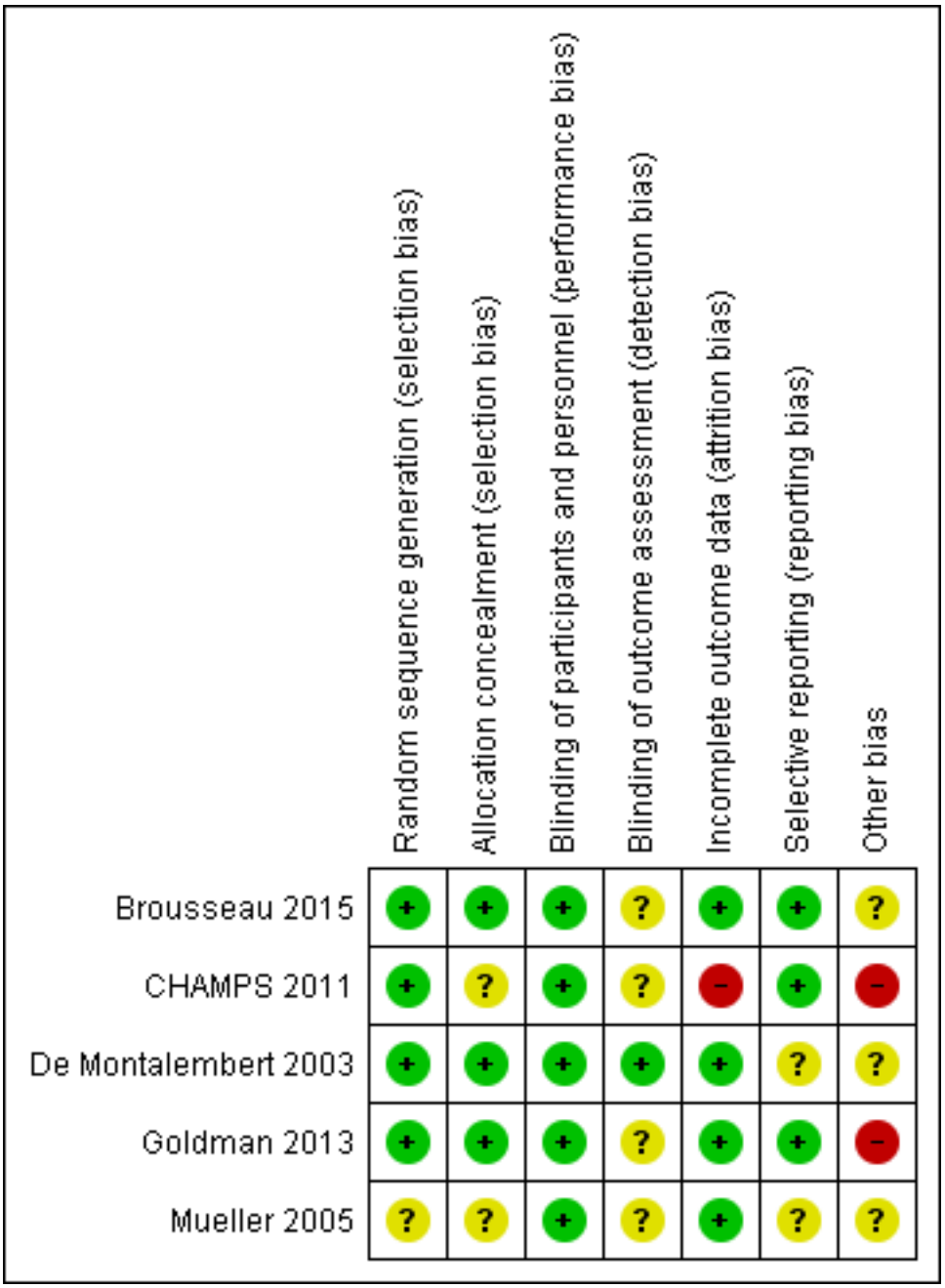


Figure 3. Risk of bias summary: review authors' judgements about each risk of bias item for each included study.



Allocation

Sequence generation

Two of the included studies were judged to have an unclear risk of bias for sequence generation as neither study described the method of randomization (De Montalembert 2003; Mueller 2005). The De Montalembert study states that participants were randomly allocated to one of the two arms - either six months of Mg pidolate followed by a three-month washout period and then six months of placebo or the reverse order (De Montalembert 2003). The Mueller study states that participants were randomized to either receive oral Mg pidolate or placebo for six months followed by a washout period and six months of the other agent with a further washout period (Mueller 2005).

We judged three studies to have a low risk of bias for sequence generation (Brousseau 2015; CHAMPS 2011; Goldman 2013). In Brousseau study, randomization tables were provided by the data co-ordinating centre and used randomly varying block sizes for equal allocation stratified by age group, study site and hydroxyurea use during past three months (Brousseau 2015). In the Goldman study, participants were also randomly assigned to treatment group by the research pharmacy using preset randomization tables (blocks of four) (Goldman 2013). In the CHAMPS study the allocation sequence was generated using a sequential allocation algorithm (CHAMPS 2011).

Allocation concealment

The two studies which had an unclear risk of bias for sequence generation also had an unclear risk of bias for allocation concealment, again due to a lack of clear information (CHAMPS 2011; Mueller 2005).The two studies with a low risk of bias for sequence generation, were also graded as having a low risk of bias for allocation concealment (Brousseau 2015; Goldman 2013). In Brousseau study, a central randomization service was used where the randomization tables were provided by PECARN data co-ordinating centres to the research pharmacy preparing the drugs at each study site (Brousseau 2015). In the Goldman study, the sequence was generated by the research pharmacy (Goldman 2013). Following contact with the de Montalembert study authors, it was confirmed that treatments were allocated by a central randomization service, therefore we graded this study as having a low risk of bias also (De Montalembert 2003).

Blinding

The De Montalembert study was described as being double-blind, but there was no description of the placebo or any further information in the abstract to allow us to make a clear judgement; therefore, we contacted the lead author who confirmed that there was a full blinding of participants and personnel as treatment and placebo were not distinguishable and we have graded this study as having an low risk of bias for blinding of participants, personnel and outcome assessors (De Montalembert 2003). We graded the remaining four studies as having a low risk of bias for participants and personnel as they are all described as being double-blind and give descriptions of placebo treatments that were indistinguishable from the study medication (Brousseau 2015; Goldman 2013; Mueller 2005; CHAMPS 2011). However, we graded these four studies as having an unclear risk of bias for blinding of outcome assessors since, although they were described as being double blind and in some cases it was stated (e.g. on a trial registry) that blinding included outcome assessors, no further details were given (Brousseau 2015; Goldman 2013; Mueller 2005; CHAMPS 2011).

Incomplete outcome data

We graded one included study as having a high risk of attrition bias (CHAMPS 2011). The CHAMPS study enrolled 64 participants and 44 of these were randomized; 36 participants completed eight weeks of the study but only 22 participants completed the full 44 weeks providing data for the primary end point (CHAMPS 2011). We graded one included study as having an low risk of attrition bias (De Montalembert 2003). The number of participants in the De Montalembert study was stated in the abstract as 24 with no mention of the number randomized or if there were any withdrawals, but the lead author later confirmed that 24 participants were randomized and there were no withdrawals (De Montalembert 2003). However, we judged the remaining three studies as having low risk of attrition bias (Brousseau 2015; Goldman 2013; Mueller 2005). In Brousseau study, 208 children were randomized and 204 children received study drug (Brousseau 2015). In the Goldman study, 106 children were randomly assigned to treatment arms in the study, 104 participants were included (two withdrew consent) and 98 provided data (Goldman 2013). In the Mueller study, only five out of 12 participants were evaluated for efficacy; however, reasons were given for the seven participants who dropped out: three for non-compliance, two for study violation (pharmacy dispensed wrong formulation) and two withdrew citing personal reasons (Mueller 2005).

Selective reporting

The study protocol for the De Montalembert study was not available, we therefore judged this study to have an unclear risk of bias for selective outcome reporting (De Montalembert 2003).

The remaining four included studies had protocols available from the online trial registry clinicaltrials.gov showing a history of changes:

- Brousseau 2015 (NCT01197417 - since 31 August 2010);
- CHAMPS 2011 (NCT00532883 - since 20 September 2007);
- Goldman 2013 (NCT00313963 - since 10 April 2006); and
- Mueller 2005 (NCT00040456 - since 26 June 2002).

To date, the Mueller study has only been presented as a poster with no full paper publication (Mueller 2005). We have contacted the primary investigator of the study for information to allow us to assess reporting bias, but we are still waiting for the reply. In the study protocol, the stated primary outcome was to evaluate whether treatment with oral Mg pidolate decreases the number of painful crises; secondary outcomes were tolerance of long-term treatment with oral Mg pidolate and the effects of Mg pidolate therapy on K-Cl co-transport activity, red blood cell (RBC) hydration status and the intracellular Mg content of erythrocytes. Only limited adverse event data were reported in the poster presentation of this study, but efficacy data were due at a later date (Mueller 2005). We judged this study to have an unclear risk of bias due to insufficient information to allow a judgement.

Three of the studies had corresponding full paper publications and we judged them to have a low risk of bias as they reported their pre-specified outcome measures (Brousseau 2015; CHAMPS 2011; Goldman 2013).

The primary outcome of the Brousseau study was length of hospital stay (hours); the secondary outcomes were morphine use during hospitalizations, adverse events (during infusion and development of acute chest syndrome) and health-related QoL (both during and after study drug infusion) (Brousseau 2015). The primary outcome of the Goldman study was also length of hospital stay; the secondary outcomes were a reduction of mean daily pain score during an admission for a sickle cell pain crisis, adverse events during admission and the cumulative volume of a narcotic drug required to manage the crises during admission (Goldman 2013). The primary outcome of the CHAMPS study was the proportion of hyperdense red blood cells (red blood cells with density greater than 41 g/dL); secondary outcomes were to document the toxicity of both drugs (Mg and hydroxyurea) and their effects on hematological parameters and red cell metabolism in people with SCD (CHAMPS 2011).

Other potential sources of bias

We judged only one study to have a low risk of potential bias where the author later confirmed that the authors compared the data observed during the placebo periods (the first period for half of the participants and the second period for the remaining participants) and those observed during the treatment periods (the first period for half of the participants and the second period for the remaining participants) (De Montalembert 2003).

We judged two of the included studies to have a high risk for other potential sources of bias (CHAMPS 2011; Goldman 2013). In the Goldman study, the targeted sample size expected from protocol was not achieved. The estimated enrolment ranged from 120 to 126 (see history of changes of NCT00313963 on clinicaltrials.gov). The earlier abstract of the Goldman study published in 2011 reported 107 individuals were randomized and 106 participated in the study. The full study publication states that a total of 159 people were approached to participate in the study, 106 (67%) consented and two participants (2%) withdrew their consent to participate in the study (Goldman 2013). In the Wang study, the original sample size calculation was 188 participants across four treatment arms (see the NCT00532883 record registered on 01 September 2005 on clinicaltrials.gov), but the enrolment of participants in this Phase II trial between January 2007 and August 2009 was only 44 (according to the last-updated version of the NCT00532883 record from January 2010). The full Wang paper states that 64 participants enrolled and 44 of these were randomized; a total of 36 participants completed the eight weeks of study (the primary end point evaluation), but only 22 participants completed 44 weeks of treatment. The study was terminated early because of the slow enrolment (CHAMPS 2011).

The authors judged there to be an unclear risk from other potential sources of bias for two studies (Brousseau 2015; Mueller 2005). In the Brousseau study, blood Mg levels were not measured before and after intravenous Mg therapy, so Mg toxicity was not monitored (Brousseau 2015). Also the targeted sample size calculation for the Brousseau study was 104 per arm with a total of 208 (see history of changes on clinicaltrials.gov for NCT01197417). In the original full publication, 410 potential participants were approached and 208 were randomized. However, there are differences in the number of participants described in the secondary data analysis of the MAGiC study by Brandow, where 204 participants were stated as being enrolled. Due to the lack of measurement of Mg blood levels, we judged this study have an unclear for other potential sources of bias (Brousseau 2015). The Mueller study expected to enrol 20 participants; 100 eligible potential participants were contacted and invited to participate in the study, but according to the current recruiting status on clinicaltrials.gov, the number of participants recruited is 12 and the study was terminated due to lack of accrual. A full paper has not been published and the results for 12 participants have only been presented narratively without data analyses (Mueller 2005).

Effects of interventions

See: **Summary of findings for the main comparison** Intravenous magnesium sulphate versus placebo for treating sickle cell disease; **Summary of findings 2** Oral magnesium pidolate versus placebo/no intervention for treating sickle cell disease

Intravenous Mg sulphate versus control

Two studies (n = 306) compared intravenous Mg sulphate to saline for treating people admitted to hospital with a VOC (Brousseau 2015; Goldman 2013).

Primary outcomes

1. Change in frequency of vaso-occlusive painful crises

a. pain diary of days with significant pain

In the Goldman study, a total of 98 participants provided data for 104 painful episodes. The mean daily pain intensity during hospitalisation was measured for the Mg sulphate and placebo groups using the Faces Pain Scale-Revised, which has been validated for children between four and 18 years of age (Hicks 2001). There was no significant difference between the groups, MD 0.10 (95% CI -0.82 to 1.02) (Analysis 1.1).

This outcome was not reported by the second included study (Brousseau 2015).

b. number of painful days (where the pain is strong enough to require analgesics)

The secondary data analysis of MAGiC study by Brandow reported the standard opoid treatment for acute pain events and LOS. The mean total initial dose of opoid was 0.043 (0.029) mg/kg/hour and was administered over a median of 8.5 (interquartile range (IQR) 6.4 to 13.1) hours. The median time to first IV opoid was 1.02 (IQR 0.65 to 1.33) hours; the median time to first oral opioid was 28.3 (IQR 12.2 to 61.1) hours. These data and route of administrations for opoid used were not separately mentioned either with Mg or placebo in this paper by Brandow (Brousseau 2015).

This outcome was not reported by Goldman (Goldman 2013).

2. Quality of life (measured using a validated scale)

The Brousseau study assessed health-related QoL with both child-reported and parent-reported scales - the PedsQL generic core scales, PedsQL multi-dimensional fatigue scales and PedsQL SCD (Brousseau 2015). There was no significant difference in any child self-reported scores (from ED to pre-discharge and one week follow up) between the Mg and placebo groups (Analysis 1.2). Likewise for the parent self-reported scores from the different scales, there were no differences shown between the Mg and the placebo groups (Analysis 1.3). This outcome was not reported by the second included study (Goldman 2013).

3. Length of hospital stay

Brousseau reported the median length of stay which included the total time until discharge from the hospital regardless of the time of last opoid (Brousseau 2015). There was no difference in length of stay between the Mg and placebo groups. A post hoc analysis evaluating whether Mg had an effect on the length of stay, if the child presented early in the course of the pain crisis, also reported no difference in this outcome between the treatment or the placebo groups (Table 1).

Goldman also reported the length of hospital stay counted as the number of hours from the first study dose until the physician's decision to discharge the participant (Goldman 2013). This time correlates most closely with the termination of intravenous analgesia. There was no statistically significant difference between the Mg group and the control (saline) group in length of hospital stay, MD 14.70 hours (95% CI -20.51 to 49.91) (Analysis 1.4).

Secondary outcomes

1. Change in dense red cells

Neither included study reported any measure of the change in dense red cells (Brousseau 2015; Goldman 2013).

2. Amelioration of hematological phenotype

Neither included study reported on this outcome (Brousseau 2015; Goldman 2013).

3. Effects on K-Cl co-transport, red cell K+ and Mg2+ content

Neither included study reported on this outcome (Brousseau 2015; Goldman 2013).

4. Adverse effects

Both studies (n = 306) reported a number of adverse events but did not categorise them as mild, moderate or severe as we planned to report (Brousseau 2015; Goldman 2013). We therefore present adverse event data in summary and not by severity. When entered into analysis, there was only one significant difference adverse event (warmth at infusion site), but there were no significant differences for other adverse events between the treatment and control arms (Analysis 1.5).

Oral magnesium pidolate versus control

Three studies (n = 80) compared oral Mg pidolate to control (CHAMPS 2011; De Montalembert 2003; Mueller 2005). The CHAMPS paper stated a minimum and maximum number of

participants at baseline and each visit for those receiving hydroxyurea with Mg or with Mg placebo which ranged from 20 to 23 at baseline, 13 to 17 at week 8 and 12 to 13 at week 24; it also stated the same for those participants receiving placebo hydroxyurea with either Mg or Mg placebo which ranged from 19 to 21 at baseline, 17 to 19 at week 8 and 13 to 14 at week 24 (CHAMPS 2011). There is no meta-analysis performed and results are summarised narratively for outcomes comparing oral Mg and placebo group.

Primary outcomes

1. Change in frequency of vaso-occlusive painful crises

a. pain diary of days with significant pain

Only one cross-over study (n = 24) described the change in frequency of VOC (number of painful days) (De Montalembert 2003). The mean (SD) (range) number of days of pain during the six-months period in the Mg group was 8.6 (12.4) (0 to 41) days and in the placebo group was 10.5 (12.4) (0 to 54) days. There was no significant difference in the number of painful days between the Mg treatment group and placebo, MD 1.90 days (95% CI -1.05 to 4.85) (low quality evidence) (Analysis 2.1).
The remaining two studies in this comparison did not report on this outcome (CHAMPS 2011; Mueller 2005).

b. number of painful days (where the pain is strong enough to require analgesics)

This outcome was not reported by any of the included studies for this comparison (CHAMPS 2011; De Montalembert 2003; Mueller 2005).

2. Quality of life

This outcome was not reported by any of the included studies for this comparison (CHAMPS 2011; De Montalembert 2003; Mueller 2005).

3. Length of hospital stay

This outcome was not reported by any of the included studies for this comparison (CHAMPS 2011; De Montalembert 2003; Mueller 2005).

Secondary outcomes

1. Change in dense red cells in SCD

This outcome was not reported by any of the included studies for this comparison (CHAMPS 2011; De Montalembert 2003; Mueller 2005).

2. Amelioration of hematological phenotype

This outcome was not reported by any of the included studies for this comparison (CHAMPS 2011; De Montalembert 2003; Mueller 2005).

3. Effects on K-Cl co-transport, red cell K+ and Mg2+ content

Only the CHAMPS study reported on K-Cl co-transport activity and measured chloride-dependent net K efflux (CHAMPS 2011). No values for the SDs were available from this study for K-Cl co-transport content effects which would have allowed us to undertake a meta-analysis; we have therefore presented the available information in the additional tables (Table 2).

a. Erythrocytes

The CHAMPS study determined the Mg content of erythrocytes by atomic absorption spectrophotometry (CHAMPS 2011). The mean Mg levels at week eight were slightly higher in the Mg group compared with those receiving placebo (Table 2).
De Montalembert measured the RBC Mg levels every three months (De Montalembert 2003). The mean (SD) (range) values for RBC Mg during the six months in the Mg group were 7.19 (2.02) (4.81 to 14.8) mM/kg Hb and in the placebo group were 6.73 (1.14) (4.95 to 9.32) mM/kg Hb (P value = 0.24). When analysed, there was no significant difference Mg in erthrocytes between the two groups, MD 0.46 mM/kg Hb (95% CI -0.29 to 1.21) (low quality evidence) (Analysis 2.2).
The third included study planned to report on "change in the intracellular Mg content of erythrocytes" as one of the secondary outcomes, but the available poster presentation did not present data for this outcome (Mueller 2005).

b. potassium

In the CHAMPS study, the potassium content of erythrocytes was determined by atomic absorption spectrophotometry (CHAMPS 2011). Again the published report did not provide values for the SDs for K+ content effect, so we are not able to perform a meta-analysis. Available results are presented in the additional tables (Table 2). The two remaining included studies for this comparison

did not report on this outcome (De Montalembert 2003; Mueller 2005).

c. sodium

In the CHAMPS study, the sodium content of erythrocytes was determined by atomic absorption spectrophotometry (CHAMPS 2011). As before, no SDs were reported and we were not able to undertake a meta-analysis; the available results are presented in the additional tables (Table 2). The two remaining included studies for this comparison did not report on this outcome (De Montalembert 2003; Mueller 2005).

4. Adverse effects

Adverse events were not reported in the De Montalembert study (De Montalembert 2003). In the Mueller study, which has only been published as a scientific meeting abstract, investigators report some narrative information on adverse events. The report states that there were seven events probably related to the study drug or placebo, but only one episode of diarrhoea was severe or worse (described by the paper as grade 3 or higher). There were a further eight events which were possibly related to the study drug or placebo: diarrhoea in three participants (none severe or disabling (described as grade 3 or 4)); and headache in five participants (two were disabling (grade 4) and three were severe (grade 3)). All adverse events resolved without stopping treatment (Mueller 2005). In the CHAMPS study, a total of 38 participants reported 293 adverse events while receiving study drugs. Of these, 22 events in 10 participants were reported as serious adverse events, but no differences were observed across the four treatment groups in the distribution of these events. Fifteen of the reported serious adverse events (in nine participants) and 111 of the adverse events (in 27 participants) were described as VOCs, but there were no significant differences among groups. Participants also reported 42 occurrences of gastrointestinal disorders, 22 episodes of headache or migraine, 18 upper respiratory infections and nine episodes of rash. There were no deaths during the 26.3 participant years of follow-up (CHAMPS 2011).

ADDITIONAL SUMMARY OF FINDINGS [*Explanation*]

Oral magnesium versus placebo for treating sickle cell disease						
Patient or population: people with sickle cell disease **Settings**: outpatient (Europe) **Intervention**: oral magnesium pidolate						
Outcomes	**Illustrative comparative risks* (95% CI)**		**Relative effect (95% CI)**	**No of Participants (studies)**	**Quality of the evidence (GRADE)**	**Comments**
	Assumed risk	**Corresponding risk**				
	Placebo/no intervention	**Magnesium**				
Change in frequency of vaso-occlusive painful crises (as measured by pain diary days with significant pain) Follow up: Not reported	NA[3]	The mean number of painful days in the intervention group was **1.9 higher** (1.05 lower to 4.85 higher) than in the placebo group.	NA[3]	24 (1 study)	⊕⊕◯◯ **low**[1,2]	Cross-over randomized controlled study. There was no significant difference in the mean number of painful days between the intervention group and the placebo group
Quality of life	Not reported					
Length of hospital stay	Not reported					
Change in dense red cells in sickle cell disease	Not reported					
Effects of K-Cl co-transport red cell K+ and Mg2+ content (RBC magnesium) mM/kg Hb Follow up: Not reported	NA[3]	The mean RBC magnesium in the intervention group was **0.46 higher** (0.29 lower to 1.21 higher) than in the	NA[3]	24 (1 study)	⊕⊕⊕◯ **moderate**[2]	Cross-over randomized controlled study. There was no significant difference in mean

		placebo group.			RBC magnesium between the intervention group and the placebo group
Adverse events: diarrhoea and headache Follow up: every 2 weeks then every 4 weeks.		9 events that were considered probably related to drug (or placebo)	12 (1 study)	⊕⊕⊕◯ **moderate**[4]	Cross-over randomized control study (Mueller 2005). This study reported adverse events as total for both intervention and placebo period
Adverse events: including serious adverse events, vaso-occlusive crisis, gastrointestinal disorder, headache or migraine, upper respiratory tract infection, rash Follow up: 26.3 patient years of follow up		293 adverse events while receiving study drugs.	36 (1 study)	⊕⊕⊕◯ **moderate**[5]	Phase II multi-centre double-blinded trial, factorial design (CHAMPS 2011). This study reported adverse events for four treatment arms. No differences were observed across the four treatment groups

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **K-Cl**: potassium chloride; **NA**: not applicable; **RBC**: red blood cell; **RR:** Risk Ratio.

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1]Evidence downgraded to imprecision: wide 95% CIs of the effects of outcomes of the intervention.
[2]Evidence downgraded due to reporting bias: the study protocol was not available.
[3]The results from the study were presented as mean difference so an estimate of risk within the control group is not available.

[4]Evidence downgraded due to unclear risk of selection bias (random sequence generation and allocation concealment) and reporting bias.
[5]High risk of attrition bias: incomplete outcome data.

DISCUSSION

Summary of main results

There were five randomized controlled studies (n = 386) included in this review (Brousseau 2015; CHAMPS 2011; De Montalembert 2003; Goldman 2013; Mueller 2005). Two parallel studies examined short-term intravenous magnesium (Mg) (Brousseau 2015; Goldman 2013) and three studies examined longer-term oral Mg; one of these was of parallel design with four arms comparing oral Mg pidolate to hydroxyurea and respective placebos (only the Mg pidolate versus placebo comparison was eligible for inclusion in this review) (CHAMPS 2011) and two were of cross-over design (De Montalembert 2003; Mueller 2005).

Only one (n = 104) of the two studies reporting on intravenous Mg use reported analysable data for pain score (Goldman 2013). There was no difference found between Mg sulphate or placebo groups for pain score at up to one month, mean difference (MD) 0.10 (95% confidence interval (CI) -0.82 to 1.02) (Analysis 1.1). The second study of intravenous Mg (n = 202) reported analysable change data for health-related quality of life (HRQoL) scores between arrival at the emergency department to pre- discharge and one week follow-up, we found there was no significant difference between Mg and placebo groups in child self-reported scores (Analysis 1.2) or parent self-reported score (Analysis 1.3). Both studies reported on the length of hospital stay, but data were only analysable from one study (n = 104) (Goldman 2013) and showed no difference between the groups, MD 14.70 hours (95% CI -20.51 to 49.91) (Analysis 1.4). Both studies also reported a number of adverse events but did not categorise them as mild, moderate or severe as we had planned to report. We therefore present adverse event data in general and not by severity. There was significant association of warmth at infusion site between the intravenous Mg sulphate group and the placebo group, but there was no significant difference in hypotension, nausea or vomiting, pain at the infusion site, pruritus, tachycardia, drowsiness and acute chest syndrome (Analysis 1.5). From our analysis, there was no clear benefit, but some harm (warmth at infusion site) identified with intravenous Mg sulphate compared to placebo (Brousseau 2015; Goldman 2013).

One cross-over study (n = 24) comparing oral Mg pidolate to placebo reported on one of our primary outcomes (change in frequency of painful crisis) and one secondary outcome (Mg levels in red blood cells); there was no significant difference reported in painful days and Mg levels in RBC between the Mg treatment group and placebo group in the six-month study (De Montalembert 2003). The parallel CHAMPS study, (n = 44) reported effects of oral Mg pidolate and placebo on potassium chloride (K-Cl) co-transport, sodium and potassium ion transport measures but we were not able to analyse these due to insufficient information (CHAMPS 2011). We were not able to analyse any adverse event data. One study reported no adverse events (De Montalembert 2003) and a second study reported limited narrative on adverse events all of which resolved without stopping treatment (Mueller 2005). The third study reported that no differences were observed across the four treatment groups in the distribution of adverse events such as vaso-occlusive pain crises (VOCs), gastrointestinal disorders, headache or migraine, upper respiratory infection or rash; there were no deaths reported (CHAMPS 2011).

Overall completeness and applicability of evidence

Two parallel studies of intravenous Mg sulphate reported on our all primary outcomes of pain score and length of stay in hospital in hours and quality of life (Brousseau 2015; Goldman 2013). Only one of the studies comparing oral Mg pidolate to placebo measured our primary outcome of change in the frequency of painful crises as measured by pain diary days (analysable data available) (De Montalembert 2003). None of the oral studies reported on length of stay in hospital or quality of life (CHAMPS 2011; De Montalembert 2003; Mueller 2005). In reporting adverse effects, none of the studies categorised these as mild, moderate or severe as we had originally planned to report. We therefore presented adverse event data in general and not by the severity of the event. We were able to analyse all the primary outcomes for the studies of intravenous Mg sulphate, but the data were limited. We were not able to analyse outcomes which we expected to for oral Mg pidolate. The most common reason for this was that the interventions have so far mainly been evaluated in pilot studies instead of randomized controlled trials (RCTs); these non-randomized studies are not eligible for inclusion in our review. Furthermore, not all the outcome measures, which we defined a priori, were assessed, e.g. none of the included RCTs reported any change in dense red cells and haematological effects from Mg treatment. Although we planned to investigate whether oral Mg reduces the number of dense erythrocytes and improves the erythrocyte membrane transport abnormalities in people with sickle cell disease (SCD), we failed to do so because complete data were not reported. In the included studies, both oral Mg pidolate and intravenous Mg sulphate were given at a lower dose than the maximally-tolerated dose, perhaps limiting our ability to identify any biological effects on red blood cell density. Our review provides a basis for performing clinical efficacy studies using oral Mg or intravenous Mg sulphate for treating SCD, which may improve painful episodes, reduce red cell dehydration and length of hospital stay.

Quality of the evidence

There is a moderate level of evidence for the change in frequency of vaso-occlusive painful crises, which demonstrated no significant difference between intravenous Mg sulphate and the control group in treating SCD. However, there is low quality evidence for

the effect of intravenous Mg sulphate on length of hospital stay, adverse events (hypotension, warm at infusion site) and HRQoL (Summary of findings for the main comparison). With regards to treatment with oral Mg for treating SCD, there is low quality evidence for the assessment of painful crisis and moderate quality evidence in the effects on K-Cl co-transport (red cell Mg content) with oral Mg (Summary of findings 2).

Four studies were judged to be at a low risk of selection bias, with adequate methods to generate a random sequence and to conceal group allocation (Brousseau 2015; CHAMPS 2011; De Montalembert 2003; Goldman 2013); the remaining study was judged to have an unclear risk of selection bias (Mueller 2005). Blinding of participants and personnel was only undertaken in four studies (Brousseau 2015; CHAMPS 2011; De Montalembert 2003; Goldman 2013), while we judged a single study to have an unclear risk for this domain (Mueller 2005). Only one study had a low risk of detection bias, where the outcome assessors were blinded (Mueller 2005), the remaining studies had an unclear risk of detection bias. Two studies were judged to have an unclear risk of attrition bias (Goldman 2013; Mueller 2005), one study had a high risk of attrition bias (CHAMPS 2011) and two studies had a low risk of attrition bias (Brousseau 2015; De Montalembert 2003). Three studies were judged to be at low risk of selective reporting bias as we were able to confirm that they reported on each planned outcome (Brousseau 2015; CHAMPS 2011; Goldman 2013). However, there was no available protocol for either of the remaining studies and so it was unclear if the other two studies had a risk of bias from this domain (De Montalembert 2003; Mueller 2005).

We assessed sample-size calculations of other potential sources of bias and regarded one included study as having a low risk of bias due to sample-size calculation (Brousseau 2015). In first study, the total numbers of participants needed per arm was stated in the protocol as being 91; however, in full article it reported that 410 people were approached to take part in the study, 208 were randomized with 204 (98%) receiving the study drug and just four (2%) not doing so. The number of participants included in analysis were 100 (99%) from one arm and 102 (99%) from the second arm. In each arm one participant (1%) withdrew with the reason given as there being no outcome data (Brousseau 2015). Two studies were judged to have a high risk of bias relating to sample size calculations (CHAMPS 2011; Goldman 2013). The historical changes in the study archive for the the Goldman study, give the estimated enrolment ranging from 120 to 126 participants, but the full article states that a total of 159 individuals were approached for the study, 106 (67%) consented and two participants (2%) then withdrew their consent (Goldman 2013). In the CHAMPS study, the original sample size calculation was 188 participants across four treatment arms, but the full article states that 64 participants were enrolled and 44 of these were randomized; only 22 participants completed 44 weeks of the study. The study was terminated early because of the slow enrolment (CHAMPS 2011). Finally, the two remaining studies was judged to have an unclear risk bias due to sample sizes; one stated that the expected sample size of 20 participants, but was terminated early due to the lack of accrual; the full article is not yet available (Mueller 2005). The second study has no details with regards to sample size calculations (De Montalembert 2003).

Potential biases in the review process

This review complies with the comprehensive methodology which is applicable for finding and assessing all relevant studies. We extracted information from RCTs using standard Cochrane methods for data extraction and analysis with reference to the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011a). We contacted the study authors to obtain further data when we were limited by the reported data and requested protocols of their studies to allow the inclusion and further analysis of studies. Our review has some limitations, while we were able to access the protocols of four of the included studies and their history of changes from a clinical trials registry; we were unable to obtain much of the required primary data to include in our systematic analysis.

Agreements and disagreements with other studies or reviews

There are no other reviews or studies on this topic to compare with this Cochrane Review.

AUTHORS' CONCLUSIONS

Implications for practice

In this review, there is a lack of promising effects from either of the included parallel RCTs using intravenous Mg to treat people with SCD admitted to hospital for a painful crisis (where pain started 24 hours before arrival at hospital) in reducing the painful crisis at hospital and improving HRQoL after discharge from hospital.

There is also a lack of promising evidence from any RCT, either of parallel or cross-over design, to support the use of oral Mg to treat people with SCD (with at least one vaso-occlusive crisis in previous 12 months) in reducing the frequency of painful crises, reducing red cell dehydration by changing haematological and biochemical parameters and improving HRQoL. One oral Mg study was terminated early because of slow enrolment, but narrative results showed that no significant toxicity was observed in the Mg treatment arm.

Our findings are supported by low quality evidence for intravenous Mg and low quality evidence for oral Mg. We are therefore unable

to recommend that intravenous Mg sulphate or oral Mg pidolate are used to treat children or adults who are experiencing painful episodes or those with stable disease.

Implications for research

Neither intravenous Mg sulphate or oral Mg pidolate appear to be effective in treating painful crises in people with SCD. However, these conclusions are based on limited data and information from two studies of intravenous Mg sulphate and three studies of oral Mg pidolate, all with a small number of participants. Due to the limited current evidence, further RCTs may be warranted to determine whether intravenous Mg sulphate can reduce the length of hospital stay and improve the quality of life in people with SCD; and whether oral Mg can improve painful episodes and reduce red cell dehydration in people with SCD. Furthermore, data to justify proposing oral Mg pidolate as a potential therapeutic strategy for preventing erythrocyte dehydration in haemoglobin S disease is needed. Such studies must be sufficiently well-designed and large enough to allow important differences to be detected. Since no definitive conclusions can be made about the clinical efficacy of Mg therapy, future research must consider further health-related outcomes (including reporting on reducing sickle cell painful crises), as well as improving long-term HRQoL outcomes. In addition, studies may address specific considerations including timing and safety of commencement of intravenous Mg sulphate and oral Mg pidolate as well as dosage for therapeutic benefits in management guidelines.

ACKNOWLEDGEMENTS

First and foremost, we would like to dedicate our acknowledgement of gratitude to Mrs Nikki Jahnke, Managing Editor at the Cochrane Cystic Fibrosis and Genetic Disorder Group (CFGD) for her kind assistance throughout the development of this review. We also would like to thank Professor Datuk Abdul Razzak, Chief Executive of Melaka Manipal Medical College and Professor Jaspal Singh Sahota, Dean of Melaka Manipal Medical College. Finally, our gratitude goes to Associate Professor Dr Aung Win Thein, Department of Surgery and Professor Dr Soe Moe, Community Medicine Department, Melaka Manipal Medical College for their support and encouragement in writing this protocol.

This project was supported by the National Institute for Health Research, via Cochrane Infrastructure funding to the Cochrane Cystic Fibrosis and Genetic Disorders Group. The views and opinions expressed therein are those of the authors and do not necessarily reflect those of the Systematic Reviews Programme, NIHR, NHS or the Department of Health.

REFERENCES

References to studies included in this review

Brousseau 2015 *{published data only}*

Badaki-Makun O, Scott JP, Panepinto JA, Casper TC, Hillery CA, Dean JM, et al. Intravenous magnesium for pediatric sickle cell vaso-occlusive crisis: methodological issues of a randomized controlled trial. *Pediatric Blood & Cancer* 2014;**61**(6):1049–54. CFGD Register: SC240a; CRS: 5500125000000731; PUBMED: 24443249]

Brandow AM, Nimmer M, Simmons T, Casper TC, Cook LJ, Scott JP, et al. Higher dose of opioids in the emergency department and earlier initiation of oral opioids after hospitalization are associated with shorter length of stay in children with sickle cell disease treated for acute pain. *Blood* 2015;**126**(23):525. CENTRAL: 1134742; CFGD Register: SC240e; CRS: 5500050000000496; EMBASE: 72171271]

Brandow AM, Nimmer M, Simmons T, Charles Casper T, Cook LJ, Chumpitazi CE, et al. Impact of emergency department care on outcomes of acute pain events in children with sickle cell disease. *American Journal of Hematology* 2016;**91**(12):1175–80. CFGD Register: SC240h; CRS: 5500135000001642; PUBMED: 27517842]

Brousseau DC, Scott JP, Badaki O, Darbari DS, Chumpitazi C, Airewele GE, et al. A multi-center randomized controlled trial of intravenous magnesium for sickle cell pain crisis in children [abstract]. *Blood* 2014;**124**(21):Abstract no: 88. CFGD Register: SC240c; CRS: 5500135000001417]

* Brousseau DC, Scott JP, Badaki-Makun O, Darbari DS, Chumpitazi CE, Airewele GE, et al. A multicenter randomized controlled trial of intravenous magnesium for sickle cell pain crisis in children. *Blood* 2015;**126**(14): 1651–7. [CFGD Register: SC240d; CRS: 24443249; DOI: 10.1182/blood-2015-05-647107]

Estepp JH. MAGiC: VOC remains but kids with SCA appear. *Blood* 2015;**126**(14):1637–8. CFGD Register: SC240f; CRS: 5500135000001643; PUBMED: 26429963]

McArdle J, Tiller G. Intravenous magnesium not effective in sickle cell pain crises. *Journal of Paediatrics and Child Health* 2016;**52**(10):970. CENTRAL: 1215835; CFGD Register: SC240g; CRS: 5500135000001641; PUBMED: 27739204]

Panepinto JA, Beverung LM, Casper TC, Cook LJ, Brousseau DC. The impact of an acute vaso-occlusive crisis on patient reported outcomes: the MAGIC study [abstract]. *Blood* 2014;**124**(21):441. CFGD Register: SC240b; CRS: 5500135000001416]

CHAMPS 2011 *{published data only}*

* Wang W, Brugnara C, Snyder C, Wynn L, Rogers Z, Kalinyak K, et al. The effects of hydroxycarbamide and magnesium on haemoglobin SC disease: results of the multi-centre CHAMPS trial. *British Journal of Haematology* 2011;**152**(6):771–6. CENTRAL: 801819; CFGD Register: SC210b; CRS: 5500125000000521; PUBMED: 21275961]

Wang WC, Snyder C, Brugnara C, Telen MJ, Steinberg MH, Wynn LW, et al. Effects of hydroxyurea (HU) and magnesium pidolate (Mg) in hemoglobin SC disease (HbSC): the "CHAMPS'trial [abstract]. *Blood* 2009; **114**(22):Abstract no:819. CENTRAL: 744106; CFGD Register: SC210a; CRS: 5500100000003436]

De Montalembert 2003 *{published data only}*

De Montalembert M, Roudot-Thoraval F, Galacteros F, De Franceschi L, Brugnara C, Beuzard Y. Treatment with oral magnesium pidolate in children with sickle cell disease [abstract]. *British Journal of Haematology* 2003;**121**(Suppl): 43. CENTRAL: 593125; CFGD Register: SC177a; CRS: 5500100000003076]

de Montalembert M, Roudot-Thoraval F, Galacteros F, de Franceschi L, Brugnara C, Beuzard Y. Treatment with oral magnesium pidolate in children with sickle cell disease. *Blood* 2002;**100**(2):454a. CENTRAL: 593400; CFGD Register: SC177b; CRS: 5500050000000497]

Goldman 2013 *{published data only}*

* Goldman RD, Mounstephen W, Kirby-Allen M, Friedman JN. Intravenous magnesium sulphate for vaso-occlusive episodes in sickle cell disease. *Pediatrics* 2013;**132**(6): e1634–41. CENTRAL: 959808; CFGD Register: SC253a; CRS: 5500050000000103; EMBASE: 2013809378]

Goldman RD, Mounstephen W, Kirby-Allen M, Ma N, Friedman JN. Randomized double-blind placebo controlled trial of intravenous magnesium for children with sickle cell disease vaso-occlusive crisis - The mast project. *Paediatrics & Child Health* 2011;**16**:17A. CENTRAL: 1032732; CFGD Register: SC253b; CRS: 5500050000000498; EMBASE: 70784028]

Mueller 2005 *{published data only}*

Mueller BU. Oral Magnesium Pidolate, Hemoglobin SC disease, MG pidolate. NCT00040456. clinicaltrial.gov (accessed 26th March 2015).

Mueller BU, Dinu M, Kurth S, Bryant R, Mullen E, Yan J, et al. The safety of oral magnesium pidolate in children with hemoglobin SC disease [abstract]. 28th Annual Meeting of the National Sickle Cell Disease Program; 2005 April 9-13; Cincinnati, Ohio. 2005:147. CENTRAL: 592986; CFGD Register: SC181; CRS: 5500100000002949]

References to studies excluded from this review

Brousseau 2004 *{published data only}*

Brousseau DC, Scott JP, Hillery CA, Panepinto JA. The effect of magnesium on length of stay for pediatric sickle cell pain crisis. *Academic Emergency Medicine* 2004;**11**(9): 968–71.

De Franceschi 1997 *{published data only}*

De Franceschi L, Bachir D, Galacteros F, Tchernia G, Cynober T, Alper S, et al. Oral magnesium supplements reduce erythrocyte dehydration in patients with sickle cell disease. *Journal of Clinical Investigation* 1997;**100**(7): 1847–52.

De Franceschi 2000 *{published data only}*

De Franceschi L, Bachir D, Galacteros F, Tchernia G, Cynober T, Neuberg D, et al. Oral magnesium pidolate: effects of long-term administration in patients with sickle cell disease. *British Journal of Haematology* 2000;**108**(2): 284–9.

Hankins 2007 *{published data only}*

Hankins JS, Wynn LW, Brugnara C, Hillery CA, Li CS, Wang WC. Phase I study of magnesium pidolate in combination with hydroxycarbamide for children with sickle cell anaemia. *British Journal of Haematology* 2007;**140** (1):80–5.

References to studies awaiting assessment

Voskaridou 2000 *{published data only}*

Nodaros K, Voskaridou E, Atta-Politis J, Loukopoulos D. Administration of magnesium aspartate in patients with HbS/ß-thalassaemia normalizes the RBC density distribution and removes dense sickle cells from the peripheral blood [abstract]. *Blood* 1999;**94**(10 Suppl 1): 200a. CFGD Register: SC109a]

Voskaridou E, Nodaros K, Atta-Politis J, Loutradi A, Loukopoulos D. Administration of magnesium aspartate in patients with HbS/b-thalassaemia results in normalization of the red cell density distribution curves and removal of the dense sickle cells from the peripheral blood [abstract]. 5th Congress of the European Haematology Association (EHA), 2000 June 25-28; Birmingham, UK. 2000. CENTRAL: 302987; CFGD Register: SC109b; CRS: 5500100000001715]

Additional references

Akenami 1999

Akenami FO, Aken'Ova YA, Osifo BO. Serum zinc, copper and magnesium in sickle cell disease at Ibadan, southwestern Nigeria. *African Journal of Medical Sciences* 1999;**28**(3-4): 137–9.

Anstall 1959

Anstall HB, Hayward GH, Huntsman RG, Weitzman D, Lehmann H. The effect of magnesium on blood coagulation in human subjects. *Lancet* 1959;**1**(7077):814–5.

Ataga 2003

Ataga KI, Orringer EP. Hypercoagulability in sickle cell disease: a curious paradox. *American Journal of Medicine* 2003;**115**(9):721–8.

Badaki 2014

Badaki-Makun O, Scott JP, Panepinto JA, Casper TC, Hillery CA, Dean JM, et al. Intravenous magnesium for paediatric sickle cell vaso-occlusive crisis: methodological

issues of a randomised controlled trial. *Pediatric Blood & Cancer* 2014;**61**(6):1049–54.

Belcher 2014
Belcher JD, Chen C, Nguyen J, Milbauer L, Abdulla F, Alayash AI, et al. Heme triggers TLR4 signalling leading to endothelial cell activation and vaso-occlusion in murine sickle cell disease. *Blood* 2014;**123**(3):377–90. [DOI: 10.1182/blood-2013-04-495887]

Bennekou 2001
Bennekou P, de Franceschi L, Pedersen O, Lian L, Asakura T, Evans G, et al. Treatment with NS3623, a novel Cl-conductance blocker, ameliorates erythrocyte dehydration in transgenic SAD mice: a possible new therapeutic approach for sickle cell disease. *Blood* 2001;**97**(5):1451–7.

Berns 1976
Berns AS, Kollmeyer KR. Magnesium-induced bradycardia. *Annals of Internal Medicine* 1976;**85**(6):760–1.

Bringhurst 2012
Bringhurst FRDM, Krane SM, Kronenberg HM. *Harrison's Principles of Internal Medicine*. 18th Edition. New York: McGraw-Hill, 2012.

Brittain 2007
Brittain JE, Parise LV. Cytokines and plasma factors in sickle cell disease. *Current Opinion in Hematology* 2007;**14**(5):438-43.

Britton 1994
Britton J, Pavord I, Richards K, Wisniewski A, Knox A, Lewis S, et al. Dietary magnesium, lung function, wheezing, and airway hyperreactivity in a random adult population sample. *Lancet* 1994;**344**(8919):357–62.

Brousseau 2010
Brousseau DC, Owens PL, Mosso AL, Panepinto JA, Steiner CA. Acute care utilization and rehospitalization for sickle cell disease. *JAMA* 2010;**303**(13):1288–94. [DOI: 10.1001/jama.2010.378]

Brugnara 1986
Brugnara C, Bunn HF, Tosteson DC. Regulation of erythrocyte cation and water content in sickle cell anaemia. *Science* 1986;**232**(4748):388–90.

Brugnara 1987
Brugnara C, Tosteson DC. Inhibition of K transport by divalent cations in sickle erythrocytes. *Blood* 1987;**70**(6):1810-5.

Brugnara 1993a
Brugnara C, de Franceschi L, Alper SL. Inhibition of Ca (2+)-dependent K+ transport and cell dehydration in sickle erythrocytes by clotrimazole and other imidazole derivatives. *Journal of Clinical Investigation* 1993;**92**(1):520–6.

Brugnara 1993b
Brugnara C, de Franceschi L. Effect of cell age and phenylhydrazine on the cation transport properties of rabbit erythrocytes. *Journal of Cellular Physiology* 1993;**154**(2):271–80.

Brugnara 1995
Brugnara C. Red cell dehydration in pathophysiology and treatment of sickle cell disease. *Current Opinion in Hematology* 1995;**2**(2):132–8.

Buchanan 2004
Buchanan GR, DeBaun MR, Quinn CT, Steinberg MH. Sickle cell disease. *American Society of Hematology Education Program* 2004;**1**:35–47.

Canessa 1987
Canessa M, Fabry ME, Nagel RL. Deoxygenation inhibits the volume-stimulated, Cl-dependent K1 efflux in SS and young AA cells: a cytosolic Mg11 modulation. *Blood* 1987;**70**(6):1861-6.

Caroll 2009
Carroll CP, Haywood C Jr, Fagan P, Lanzkron S. The course and correlates of high hospital utilization in sickle cell disease: Evidence from a large, urban Medicaid managed care organization. *American Journal of Hematology* 2009;**84**(10):666–70. [DOI: 10.1002/ajh.21515]

De Caterina 1995
De Caterina R, Libby P, Peng HB, Thannickal VJ, Rajavashisth TB, Gimbrone MA Jr, et al. Nitric oxide decreases cytokine-induced endothelial activation. Nitric oxide selectively reduces endothelial expression of adhesion molecule and pro inflammatory cytokines. *Journal of Clinical Investigation* 1995;**96**(1):60–8.

De Franceschi 1994
De Franceschi L, Saadane N, Trudel M, Alper SL, Brugnara C, Beuzard Y. Treatment with oral clotrimazole blocks Ca(2+)-activated K+ transport and reverses erythrocyte dehydration in transgenic SAD mice. A model for therapy of sickle cell disease. *Journal of Clinical Investigation* 1994;**93**(4):1670–6.

De Franceschi 1995
De Franceschi L, Beuzard Y, Brugnara C. Sulfhydryl oxidation and activation of red cell K(+)-Cl- cotransport in the transgenic SAD mouse. *American Journal of Physiology* 1995;**269**(4 Pt 1):C899–906.

De Franceschi 1996
De Franceschi L, Beuzard Y, Jouault H, Brugnara C. Modulation of erythrocyte potassium chloride cotransport, potassium content, and density by dietary magnesium intake in transgenic SAD mouse. *Blood* 1996;**88**(7):2738–44.

De Franceschi 1999
De Franceschi L, Brugnara C, Rouyer-Fessard P, Jouault H, Beuzard Y. Formation of dense erythrocytes in SAD mice exposed to chronic hypoxia: evaluation of different therapeutic regimens and of a combination of oral clotrimazole and magnesium therapies. *Blood* 1999;**94**(12):4307–13.

De Franceschi 2001
De Franceschi L, Villa-Moruzzi E, Fumagalli L, Brugnara C, Turrini F, Motta R, et al. K-Cl cotransport modulation by intracellular Mg in erythrocytes from mice bred for low

and high Mg levels. *American Journal of Physiology. Cell Physiology* 2001;**281**(4):C1385–95.

De Franceschi 2003
de Franceschi L, Baron A, Scarpa A, Adrie C, Janin A, Barbi S, et al. Inhaled nitric oxide protects transgenic SAD mice from sickle cell disease-specific lung injury induced by hypoxia/reoxygenation. *Blood* 2003;**102**(3):1087–96.

De Franceschi 2006
De Franceschi L, Villa-Moruzzi E, Biondani A, Siciliano A, Brugnara C, Alper SL, et al. Regulation of K-Cl cotransport by protein phosphatase 1alpha in mouse erythrocytes. *Pflugers Archiv : European Journal of Physiology* 2006;**451**(6):760–8.

De Franceschi 2011
De Franceschi L, Cappellini MD, Olivieri O. Thrombosis and sickle cell disease. *Seminars in Thrombosis and Hemostasis* 2011;**37**(3):226–36. [DOI: 10.1055/s-0031-1273087]

Deeks 2011
Deeks J, Higgins J, Altman D on behalf of the Cochrane Statistical Methods Group. Chapter 9 Analysing data and undertaking meta-analysis. In: Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.

Diggs 1973
Diggs LM. Anatomic lesions in sickle cell disease. In: Abramson H, Bertles JF, Wethers DL editor(s). *Sickle Cell Disease: Diagnosis, Management, Education and Research*. St Louis: Mosby, 1973:189–229. [ISBN–10: 0801600375]

Elbourne 2002
Elbourne DR, Altman DG, Higgins JPT, Curtin F, Worthington HV, Vail A. Meta-analyses involving cross-over trials: methodological issues. *International Journal of Epidemiology* 2002;**31**(1):140–9.

Field 2013
Field JJ, Lin G, Okam MM, Majerus E, Keefer J, Onyekwere O, et al. Sickle cell vaso-occlusion causes activation of iNKT cells that is decreased by the adenosine A2A receptor agonist regadenoson. *Blood* 2013;**121**(17):3329–34. [DOI: 10.1182/blood-2012-11-465963]

Flatman 1981
Flatman PW, Lew VL. The magnesium dependence of sodium-pump-mediated sodium-potassium and sodium-sodium exchange in intact human red cells. *Journal of Physiology* 1981;**315**:421–46.

Galli-Tsinopoulou 2014
Galli-Tsinopoulou A, Maggana I, Kyrgios I, Mouzaki K, Grammatikopoulou MG, Stylianou C, et al. Association between magnesium concentration and HbA_{1c} in children and adolescents with type 1 diabetes mellitus. *Journal of Diabetes* 2014;**6**(4):369–77. [DOI: 10.1111/1753-0407.12118]

GRADEpro 2014 [Computer program]
www.gradepro.org. GRADEpro. Version 3.6.1. McMaster University, 2014.

Hamideh 2013
Hamideh D1, Alvarez O. Sickle cell disease related mortality in the United States (1999-2009). *Pediatric Blood and Cancer* 2013;**60**(9):1482–6. [DOI: 10.1002/pbc.24557]

Hebbel 2011
Hebbel RP. Reconstructing sickle cell disease: a data-based analysis of the "hyperhemolysis paradigm" for pulmonary hypertension from the perspective of evidence-based medicine. *American Journal of Hematology* 2011;**86**(2):123–54. [DOI: 10.1002/ajh.21952]

Hermans 1996
Hermans C, Lefebvre C, Devolgelaer JP, Lambert M. Hypocalcaemia and chronic alcohol intoxication: transient hypoparathyroidism secondary to magnesium deficiency. *Clinical Rheumatology* 1996;**15**(2):193–6.

Hicks 2001
Hicks CL, von Baeyer CL, Spafford PA, van Korlaar I, Goodenough B. The Faces Pain Scale-Revised: toward a common metric in pediatric pain measurement. *Pain* 2001;**93**(2):173-83.

Higgins 2003
Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003;**327**(7414):557–60.

Higgins 2011a
Higgins JPT, Deeks JJ (editors). Chapter 7: Selecting studies and collecting data. In: Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.

Higgins 2011b
Higgins JPT, Altman DG, Sterne JAC on behalf of the Cochrane Statistical Methods Group and the Cochrane Bias Methods Group (editors). Chapter 8: Assessing risk of bias in included studies. In: Higgins JPT, Green S (editors). Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.

Higgins 2011c
Higgins JPT, Deeks JJ, Altman DG on behalf of the Cochrane Statistical Methods Group. Chapter 16: Special topics in statistics. In: Higgins JPT, Green S (editors). Cochrane Handbook of Systematic Reviews of Interventions. Version 5.1 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.

http://www.who.int/ictrp/en/
World health Organisation. International Clinical Trials Registry Platform:WHO RCTRP. www.who.int/ictrp/en/ (accessed 28 April 2016).

Joiner 1993
Joiner CH, Morris CL, Cooper ES. Deoxygenation-induced cation fluxes in sickle cells. III. Cation selectivity and response to pH and membrane potential. *American Journal of Physiology* 1993;**264**(3 Pt 1):C734-44.

Kato 2008
Kato GJ, Gladwin MT. Evolution of novel small-molecule therapeutics targeting sickle cell vasculopathy. *JAMA* 2008; **300**(22):2638–46. [DOI: 10.1001/jama.2008.598]

Klings 2001
Klings ES, Farber HW. Role of free radicals in the pathogenesis of acute chest syndrome in sickle cell disease. Respiratory Research 2001; Vol. 2, issue 5:280–5.

Knochel 1997
Knochel JP. Disorders of magnesium metabolism. *Harrison's Principles of Internal Medicine*. 14th Edition. New York: McGraw Hill, 1997. [ISBN: 0070202915]

Kuypers 1998
Kuypers FA, Yuan J, Lewis RA, Snyder LM, Kiefer CR, Bunyaratvej A, et al. Membrane phospholipid asymmetry in human Thalassemia. *Blood* 1998;**91**(8):3044–51.

Ladefoged 1996
Ladefoged K, Hessov I, Jarnum S. Nutrition in short-bowel syndrome. *Scandinavian Journal of Gastroenterology. Supplement* 1996;**216**:122–31.

Lane 1996
Lane PA. Sickle cell disease. *Pediatric Clinics of North America* 1996;**43**(3):639–64.

Lehmann 1963
Lehmann H. Treatment of sickle cell anaemia. *British Medical Journal* 1963;**1**(5338):1158–9.

Leikin 1989
Leikin SL, Gallagher D, Kinney TR, Sloane D, Klug P, Rida W. Mortality in children and adolescents with sickle cell disease. *Pediatrics* 1989;**84**(3):500–8.

Maier 2004
Maier JA, Malpuech-Brugere C, Zimowska W, et al. Low magnesium promotes endothelial cell dysfunction: Implications for atherosclerosis, inflammation and thrombosis. *Biochimica Biophysica Acta* 2004;**1689**:13-21.

Makis 2000
Makis AC, Hatzimichael EC, Bourantas KL. The role of cytokines in sickle cell disease. *Annals of Hematology* 2000; **79**(8):407-13.

Mathew 1988
Mathew R, Altura BM. Magnesium and the lungs. *Magnesium* 1988;**7**:173-87.

Mauskop 1998
Mauskop A, Altura BM. Role of magnesium in the pathogenesis and treatment of migraines. *Clinical Neuroscience* 1998;**5**(1):24–7.

McNaughton-Smith 2008
McNaughton-Smith GA, Burns JF, Stocker JW, Rigdon GC, Creech C, Arrington S, et al. Novel inhibitors of the Gardos channel for the treatment of sickle cell disease. *Journal of Medicinal Chemistry* 2008;**51**(4):976–82. [DOI: 10.1021/jm070663s]

Mehta 2000
Mehta AB, Hoffbrand AV. Chapter 17: Hemolytic anemias V. Inherited defects of hemoglobin- sickle cell disease. *Hematology at a Glance*. 1st Edition. Malden, Massachusetts: Blackwell Publishing, 2000:50.

Modell 2008
Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. *Bull World Health Organ* 2008;**86**(6):480–7.

Okpala 2004
Okpala, I. The intriguing contribution of white blood cells to sickle cell disease - a red cell disorder. *Blood Reviews* 2004;**18**(1):65–73.

Oladipo 2005
Oladipo OO, Temiye EO, Ezeaka VC, Obomanu P. Serum magnesium, phosphate and calcium in Nigerian children with sickle cell disease. *West African Medical Journal* 2005;**24**(2):120-3.

Olukoga 1990
Olukoga AO, Adewoye HO, Erasmus RT, Adedoyin MA. Erythrocyte and plasma magnesium in sickle-cel anaemia. *East African Medical Journal* 1990;**67**(5):348–54.

Orchard 2014
Orchard TS, Larson JC, Alghothani N, Bout-Tabaku S, Cauley JA, Chen Z, et al. Magnesium intake, bone mineral density, and fractures: results from the Women's Health Initiative Observational Study. American Journal of Clinical Nutrition 2014 Feb 05 [Epub ahead of print].

Patel 2014
Patel BM, Raghunathan S, Porwal U. Cardioprotective effects of magnesium valproate in type 2 diabetes mellitus. *European Journal of Pharmacology* 2014;**728C**:128–34. [DOI: 10.1016/j.ejphar.2014.01.063]

Platt 1994
Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Steinberg MH, et al. Mortality in sickle cell disease. Life expectancy and risk factors for early death. *New England Journal of Medicine* 1994;**330**(23):1639–44.

Platt 2000
Platt OS. The acute chest syndrome of sickle cell disease. *New England Journal of Medicine* 2000;**342**(25):1904–7.

Pocock 1975
Pocock SJ, Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. *Biometrics* 1975;**31**(1):103-15.

Rahimy 2009
Rahimy MC, Gangbo A, Ahouignan G, Alihonou E. Newborn screening for sickle cell disease in the Republic of Benin. *Journal of Clinical Pathology* 2009;**62**(1):46–8.

Rees 2010
Rees DC, Williams TN, Gladwin MT. Sickle-cell disease. *Lancet* 2010;**376**(9757):2018–31. [DOI: 10.1016/S0140-6736(10)61029-X]

Reiter 2003
Reiter CD, Gladwin MT. An emerging role for nitric oxide in sickle cell disease vascular homeostasis and therapy. *Current Opinion in Hematology* 2003;**10**(2):99–107.

RevMan 2014 [Computer program]
The Nordic Cochrane Centre, The Cochrane Collaboration. Review Manager (RevMan). Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014.

Rivera 2005
Rivera A, Ferreira A, Bertoni D, Romero JR, Brugnara C. Abnormal regulation of Mg2+ transport via Na/Mg exchanger in sickle erythrocytes. *Blood* 2005;**105**(1):382–6.

Rizzo 1993
Rizzo MA, Fisher M, Lock JP. Hypermagnesemic pseudocoma. *Archives Internal Medicine* 1993;**153**(9):1130–2.

Rude 1998
Rude RK. Magnesium deficiency: A cause of heterogeneous disease in humans. *Journal of Bone and Mineral Research* 1998;**13**(4):749–58.

Sabaa 2008
Sabaa N, de Franceschi L, Bonnin P, Castier Y, Malpeli G, Debbabi H, et al. Endothelin receptor antagonism prevents hypoxia-induced mortality and morbidity in a mouse model of sickle-cell disease. *Journal of Clinical Investigation* 2008;**118**(5):1924–33. [DOI: 10.1172/JCI33308]

Saris 2000
Saris NE, Mervaala E, Karppanen H, Khawaja JA, Lewenstam A. Magnesium: an update on physiological, clinical, and analytical aspects. *Clinica Chimica Acta* 2000;**294**(1-2):1–26.

Siciliano 2011
Siciliano A, Malpeli G, Platt OS, Lebouef C, Janin A, Scarpa A, et al. Abnormal modulation of cell protective systems in response to ischemic/reperfusion injury is important in the development of mouse sickle cell hepatopathy. *Haematologica* 2011;**96**(1):24–32. [DOI: 10.3324/haematol.2010.028506]

Solovey 2001
Solovey AA, Solovey AN, Harkness J, Hebbel RP. Modulation of endothelial cell activation in sickle cell disease: a pilot study. *Blood* 2001;**97**(7):1937–41.

Sterne 2011
Sterne J, Egger M, Moher D on behalf of the Cochrane Bias Methods Group. Chapter 10: Addressing reporting biases. In: Higgins JPT, Green S (editors). Cochrane Handbook forSystematic Reviews of Interventions Version 5.1 [updated March 2011]. The CochraneCollaboration, 2011. Available from www.cochrane-handbook.org.

Stocker 2003
Stocker JW, De Franceschi L, McNaughton-Smith GA, Corrocher R, Beuzard Y, Brugnara C. ICA-17043, a novel Gardos channel blocker, prevents sickled red blood cell dehydration in vitro and in vivo in SAD mice. *Blood* 2003;**101**(6):2412–8.

Su 1999
Su W, Shmukler BE, Chernova MN, Stuart-Tilley AK, de Franceschi L, Brugnara C, et al. Mouse K-Cl cotransporter KCC1: cloning, mapping, pathological expression, and functional regulation. *American Journal of Physiology* 1999;**277**(5 Pt 1):C899–912.

Vandorpe 1998
Vandorpe DH, Shmukler BE, Jiang L, Lim B, Maylie J, Adelman JP, et al. cDNA cloning and functional characterization of the mouse Ca2+-gated K+ channel, mIK1. Roles in regulatory volume decrease and erythroid differentiation. *Journal of Biological Chemistry* 1998;**273**(34):21542–53.

Vinchi 2013
Vinchi F, De Franceschi L, Ghigo A, Townes T, Cimino J, Silengo L, et al. Hemopexin therapy improves cardiovascular function by preventing heme-induced endothelial toxicity in mouse models of hemolytic diseases. *Circulation* 2013;**127**(12):1317–29. [DOI: 10.1161/CIRCULATIONAHA.112.130179]

Vormann 2003
Vormann J. Magnesium: nutrition and metabolism. *Molecular Aspects of Medicine* 2003;**24**(1-3):27–37.

Weatherall 2001
Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. *Bulletin of the World Health Organization* 2001;**79**(8):704–12.

Wester 1987
Wester PO. Magnesium. *American Journal of Clinical Nutrition* 1987;**45**(5 Suppl):1305–12.

Yang 2000
Yang ZW, Gebrewold A, Nowakowski M, Altura BT, Altura BM. Mg2-induced endothelium-dependent relaxation of blood vessels and blood pressure lowering: Role of NO. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 2000;**278**:R628–R639.

Yaster 2000
Yaster M, Kost-Byerly S, Maxwell LG. The management of pain in sickle cell disease. *Pediatric Clinics of North America* 2000;**47**(3):699–710.

Zehtabchi 2004
Zehtabchi S, Sinert R, Rinnert S, Chang B, Heinis C, Altura RA, et al. Serum ionized magnesium levels and ionized calcium-to-magnesium ratios in adult patients with sickle cell anaemia. *American Journal of Hematology* 2004;**77**(3):215–22.

* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Brousseau 2015

Methods	Randomized, parallel, double-blind placebo-controlled study set in the emergency department. Multicentre: 8 sites (PECARN). Location: USA. Folllow up: by telephone 8 - 10 days post-discharge, in clinic 1 - 3 months post-discharge
Participants	People with HbSS or HbS thalassaemia, aged 4 - 21 years, presenting with VOC to emergency department and being admitted for pain management were assessed for eligibility Participants randomized within 12 hours of receiving IV opioids in emergency department 807 accessed for eligibility, 540 eligible for the study: 410 approached, 214 consented to participate, 208 randomized and finally 202 children (100 in Mg group and 102 in Placebo group) analysed for the outcomes Age group 4 - 11 years (N (%)): Mg group 41 (41%), placebo group 40 (39%) Age group 12 - 21 years (N (%)): Mg group 60 (59%), placebo group 63 (61%) Gender (N (%)): Mg group 49 (49%) females, placebo group 56 (54%)
Interventions	First dose at emergency department or inpatient floor at admission **Intervention**: IV MgSO4 40 mg/kg (maximum 2.4 g) for 20 minutes every 8 hours for a total 6 of doses or until discharge (whichever came first) **Control**: IV saline placebo for 20 minutes every 8 hours for a total 6 of doses or until discharge (whichever came first) All participants additionally received standard therapy for VOC during hospital stay, other SCD-specific medications administered at discretion of blinded treating physician
Outcomes	**Primary efficacy outcome**: length of stay in hospital (from start of first medication dose to discharge or 12-hours post final IV opoid) **Secondary efficacy outcome**: number of m Eq/kg body weight (IV and oral) administered. **Participant-reported outcomes**: HRQoL (measured by PedsQL™ generic core scales, Sickle Cell Disease module and fatigue module); days of school missed; days of continued pain; days of work missed (for parents) **Safety outcome measures**: hypotension; weakness and warm sensation associated with infusions; re-hospitalisation; Mg toxicity (serum levels). Used MedDRA classification for adverse events HRQoL assessed at enrolment, time of last study medication, 1 week post-discharge and 3 - 4 months post-discharge. Short-term outcomes assessed at 1 week post-discharge. Blood samples taken prior to first infusion, 1 hour after start of 4th infusion and 1 - 3 months post-discharge
Notes	In Brandow study (secondary data analysis of MAGiC study), all participants additionally received standard therapy for VOC in emergency department and during hospital stay, other SCD-specific medications administered at discretion of blinded treating physician.

Brousseau 2015 (*Continued*)

	The mean total initial dose of opoid was 0.043 (0.029) mg/kg/hr; administered over a median of 8.5 (IQR 6.4 -13.1) hours. The median time to first IV opoid was 1.02 (IQR 0.65 - 1.33) hours. The median time to first oral opioid was 28.3 (IQR 12.2 - 61.1) hours. The opoids used were not separately mentioned either with Mg or placebo arm ClinicalTrials.gov identifier: NCT01197417. Magnesium in Sickle Cell Crisis (MAGiC) study Awaiting response from the authors to provide further details of missing data and results of outcome measures

Risk of bias ***Risk of bias***

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Randomization tables were provided by the data co-ordinating centre and used randomly varying block sizes for equal allocation stratified by age group (4 - 11 years and 12 - 21 years), study site and hydroxyurea use during past 3 months
Allocation concealment (selection bias)	Low risk	Central randomization service was used. Randomization tables were provided by PECARN data coordinating centres to the study site research pharmacy where the study drug was prepared according to the assigned study arm
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Quote: "Double blind, placebo controlled trial. Children randomized to placebo received an equivalent volume (1 mL/kg) of normal saline over 20 minutes." 92% of children answered the question related to blinding: 47% Mg group versus 21% placebo correctly identified their treatment allocation, 53% Mg versus 79% placebo guessed incorrectly or stated that they did not know
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Double-blind, placebo-controlled study but the method of blinding of outcome assessment was not described. There was insufficient information to make a judgement
Incomplete outcome data (attrition bias) All outcomes	Low risk	The number of withdrawals was balanced across intervention groups with similar reasons (no outcome data). One participant in the Mg group did not have base line haemoglobin values. One participant in the placebo group did not have a hemoglobin

Brousseau 2015 (*Continued*)

		or white blood cell values
Selective reporting (reporting bias)	Low risk	Study prespecified outcomes of interest were reported.
Other bias	Unclear risk	Blood Mg levels were not measured before and after IV Mg therapy The targeted sample size calculation for this study was 104 per arm with a total of 208 (see history of changes on clinicaltrials.gov for NCT01197417). In the full publication, 410 potential participants were approached and 208 were randomized The number of enrolled participants described in the secondary data analysis of the MAGiC study by Brandow was a total of 204 individuals and did not mentioned separately which treatment arm they were in (Mg arm or placebo arm)

CHAMPS 2011

Methods	Prospective, randomized, double-blind, Phase II study. Parallel factorial design with 4 arms (HC + Mg placebo, Mg + HC placebo, HC + Mg, or HC placebo + Mg placebo) Duration: 44 weeks. Multicentre: 14 sites. Location: USA.
Participants	Informed consent at baseline evaluations. Eligible participants: HbSC and at least 1 VOC in previous 12 months, but none within the previous 4 weeks. 44 participants were randomized, 36 reached the primary end point and 22 participants completed the full 44 weeks Baseline: minimum 20 to maximum 23 (Mg group), minimum 19 to maximum 21 (placebo group) Week 8: minimum 13 to maximum 17 (Mg group), minimum 17 to maximum 19 (placebo group) Week 24: minimum 12 to maximum 13 (Mg group), minimum 13 to maximum 14 (placebo group) Participants were evenly distributed across the 4 treatment groups with regard to baseline characteristics including age, gender, HbF, and hyperdense cells (P = 0.13 - 0.68) Age: mean (range) 13.6 (5 - 53) years (73% under 16 years of age) Gender split: 57% male, 43% female.
Interventions	4 arms: HC + Mg placebo, Mg + HC placebo, HC + Mg, or HC placebo + Mg placebo Mg pidolate 0.6 m Eq/kg/day. Oral Mg pidolate (1 mmol/ml) was formulated by Xcelience (Tampa,FL, USA) along with an indistinguishable placebo liquid, and administered at a dose of 0.3 mmol/kg/d, twice daily HC (and HC placebo) administered orally at a dose of 20 mg/kg/d

CHAMPS 2011 (*Continued*)

Outcomes	Primary outcome: to estimate the maximum tolerated dose of Mg pidolate in combination with HC Seondary outcomes: toxicity of combination of HC with Mg pidolate; haematological parameters; red cell metabolism Measured at baseline and then at bi-weekly intervals for the first 8 weeks, then and every 4 weeks for a total of 44 weeks. At each visit, an interim history was obtained and standard blood counts were performed. Evaluations at baseline and weeks 8, 16, 24, and 44 weeks included measures of erythrocyte density, cation content, K-Cl co-transport, Gardos channel activity, Na/Mg exchange, endothelial adhesion, HbF levels, and plasma Mg
Notes	ClinicalTrials.gov identifier: NCT00143572. Although the original sample size calculations called for the randomization of 188 participants across the 4 treatment groups, the study was closed early due to slow enrolment, with 64 participants enrolled and 44 participants randomized

Risk of bias ***Risk of bias***

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Quotes: " ... due to small numbers within strata, sequential allocation algorithm was used (Pocock 1975)."
Allocation concealment (selection bias)	Unclear risk	There was insufficient information to make a judgement.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Described as double-blind. Masking: double-blind (participant, caregiver, investigator, outcome assessor). Placebo described as being indistinguishable from study medication
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Study was described as double blind (participant, caregiver, investigator, outcome assessor); however, the method of blinding was not described. There was insufficient information to make a judgement
Incomplete outcome data (attrition bias) All outcomes	High risk	64 participants enrolled and 44 participants randomized, 36 participants completed 8 weeks of the study and only 22 participants completed the full 44 weeks providing data for the primary end point
Selective reporting (reporting bias)	Low risk	Prespecified outcome measures from clinical trials.gov: NCT00532883 Primary outcome: distribution of density

CHAMPS 2011 (*Continued*)

		of HbSC red cells. Secondary outcomes: to determine the effects of Mg on hematological parameters and toxicity
Other bias	High risk	The original sample size calculation was 188 participants across four treatment arms, but only 44 people were randomized before the study was terminated early because of the slow enrolment. In the full paper, Wang states that 64 participants enrolled and 44 of these were randomized; a total of 36 participants completed the eight weeks of study (the primary end point evaluation), but only 22 participants completed 44 weeks of treatment

De Montalembert 2003

Methods	Randomized, double-blind, placebo-controlled, cross-over study with 2 arms Duration: 6 months of Mg pidolate followed by 3-month washout period and 6 months of placebo (or treatment in reverse order) Location: Europe.
Participants	24 children with SCD suffering at least 1 painful crisis per year Age: mean (SD) 9 (4) years, range 3 - 18 years. Gender split: 17 male, 7 female. Baseline weight: > 15 kg.
Interventions	**Intervention**: oral Mg pidolate in blisters containing 1500 mg of pyrolidone carboxylate of Mg per blister in following doses: Weight 15 - 25 kg: 1 blister per day; Weight 25 - 40 kg: 2 blisters per day; Weight 40 - 55 kg: 3 blisters per day; Weight > 55 kg: 4 blisters per day. **Control**: placebo (no further details given).
Outcomes	Number of painful days. RBC Mg. Plasma Mg. Amount of analgesic administered. Pain intensity measured by visual analogue scale.
Notes	The study authors were asked to provide results of outcomes measured

Risk of bias		***Risk of bias***
Bias	**Authors' judgement**	**Support for judgement**

De Montalembert 2003 (*Continued*)

Random sequence generation (selection bias)	Low risk	Quote (from report) "Patients were randomly allocated to one of the two arms : 6 months of Mg pidolate followed by 6 months of placebo, or treatment in the reverse order" Quote (from correspondence): "Treatments were allocated by a central randomization service"
Allocation concealment (selection bias)	Low risk	There was insufficient information to make a judgement in the abstract but study authors confirmed that treatments were allocated by a central randomization service Quote (from correspondent): "Treatments were allocated by a central randomization service"
Blinding of participants and personnel (performance bias) All outcomes	Low risk	No details or description of placebo given in the abstract to allow us to make a clear judgement but the lead author confirmed that there was a full blinding of participants and personnel as treatment and placebo were not distinguishable Quote (from correspondent): "There was a full blinding of participants and personnel"
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Study was described as double-blind and placebo-controlled; however, the method of blinding was not described Quote (from correspondent): "There was a full blinding of participants and personnel"
Incomplete outcome data (attrition bias) All outcomes	Low risk	Quote "Twenty-four children (17M, 7G) mean age 9±4 years were included" Comments: in the abstract there was no mention of the number randomized or if there were any withdrawals, but the lead author confirmed that 24 participants were randomized and there were no withdrawals
Selective reporting (reporting bias)	Unclear risk	The study protocol was not available.
Other bias	Unclear risk	Not clear if a sample size calculation was undertaken.

Goldman 2013

Methods	Randomized, double-blind, placebo-controlled parallel study with 2 arms Single centre (emergency department). Location: Toronto, Canada.
Participants	Total of 159 children approached for study. 106 children (67%) consented and were randomized 2 (2%) withdrew consent. 104 children were included and analysed: 51 in Mg group, 53 in placebo group Age: mean (SD) 12.4 (3.8) years, range 4 - 18 years. Gender split: 56 (54%) female. Disease status: 61 (58.6%) had homozygous sickle cell anaemia; 33 (31.7%) had sickle haemoglobin C disease; 10 (9.6%) had sickle beta thalassaemia Pain started a median of 24 hours before arrival at hospital (range 4 - 240 hours; SD 37 hours) Elapsed time from last visit to emergency department until study admission (mean of 7. 3 months in MgSO4 group and 8.7 months in placebo group)
Interventions	**Intervention**: IV MgSO4 (100 mg/kg, maximum of 2 g per dose) every 8 hours until deemed ready for discharge **Control**: IV placebo (normal saline in volume equivalent to MgSO4) every 8 hours until deemed ready for discharge
Outcomes	LOS in hospital. Mean daily pain intensity. Cumulative analgesic dose required during admission. Adverse events (changes in vital signs, appearance of rash, allergic reactions, diarrhoea, fever, nausea, vomiting, pain at infusion site)
Notes	Clinical trial.gov identifier: NCT00313963 (MAST study) There were differences in the earlier published paper from 2011 In Goldman 2011, 107 participants were randomized and 104 included LOS was 82.4 hours in Mg group and 79.3 hours in placebo group (P = 0.52)

Risk of bias ***Risk of bias***

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote "Randomisation and dispensing were conducted by research pharmacy using a preset randomisation table (blocks of 4)" Study design was mentioned as 2-armed randomized, double-blind, placebo-controlled study. Participants with families providing consent were randomly assigned
Allocation concealment (selection bias)	Low risk	Quote "Families providing consent were randomly assigned by research pharmacist to receive IV MgSO4 100mg/kg, maxi-

Goldman 2013 (*Continued*)

		mum of 2 g dose) 8 hourly or IV placebo.. .....“
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Quote ”Investigators, physicians, nurses, parents, patients were blinded to the treatment arm. Study drug and placebo looked exactly the same (volume and appearance) “
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Quote ”Invstigators, physicians, nurses, parents, patients were blinded to the treatment arm.“ Comment: insufficient information to make a judgement.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Quote ”106 consented, two children (2%) withdrew from the study because of withdrawal of consent. A total of 98 unique patients who had 104 episodes in which they were recruited to the study“
Selective reporting (reporting bias)	Low risk	Pre-specified outcomes were reported.
Other bias	High risk	The targeted sample size expected from protocol was not achieved. A total of 159 individuals were approached to participate, 106 consented and 2 participants later withdrew their consent The abstract published in 2011 stated that 107 were randomized and 106 children were included

Mueller 2005

Methods	Randomized controlled cross-over study with 2 arms. Duration: 16 months in total (oral Mg pidolate or placebo for 6 months, followed by 2-month washout period, then cross over to 6 months period of alternate agent (placebo/Mg) and 2 months wash out) Multicentre: 2 major paediatric sickle cell centres. Location: Boston and Texas, USA.
Participants	Over 100 children invited to participate between January 2002 and December 2004, enrolled participants or parents/guardian gave informed consent. Only 12 participants with HbSC disease and at least 1 pain crisis within last year were enrolled Age: range 3.9 to 16.8 years. Gender split: 7 male, 5 female. Of 12 participants, 5 were evaluated for efficacy assessment, 7 dropped out of trial for several reasons: 3 for non-compliance, 2 for study violation (pharmacy dispense wrong formulation), 2 withdrew for personal reasons

Mueller 2005 (*Continued*)

Interventions	**Intervention**: a liquid containing 0.6 m Eq Mg pidolate/kg/body weight per day, divided into 2 daily doses; Mg pidolate (45 g) distributed as a pre-mixed powder containing Koolaid Tropical Punch powder (9 g), and sucrose (67 g) **Control**: a placebo liquid containing an equivalent amount of placebo to the study medication, divided into 2 daily doses; contained the same amount of sucrose and Tropical Punch powder as the intervention as well as 45 g of lactose
Outcomes	Primary outcome: change in the number of painful crises. Secondary outcomes: tolerance of long-term treatment with oral Mg pidolate; change in the intracellular Mg content of erythrocytes; effect of Mg pidolate therapy on the K-Cl co transport system activity Follow-up undertaken every 2 weeks, then every 4 weeks.
Notes	Clinical trials.gov identifier: NCT00040456 The study was terminated due to poor participant enrolment. The study was opened to 2 major sickle cell centres and had relatively non-restrictive enrolment criteria, accrual was extremely low, presumably due to sporadic and rather low disease intensity of HbSC disease We have requested further details from the study investigators (randomization, blinding, missing data and efficacy results of outcomes measure) Abstract was published 10 years ago and a search using authors' name did not reveal full publication; to date no data received from study investigators

Risk of bias ***Risk of bias***

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Described as randomized, but no details given for how the sequence was generated. Insufficient information to judge
Allocation concealment (selection bias)	Unclear risk	Insufficient information to judge.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Entry on clinicaltrials.gov NCT00040456 states there was double-blind masking (participant, caregiver, investigator). Placebo described as a liquid containing an equivalent amount of placebo to the study medication, divided into the same number of daily doses and containing the same amount of sucrose and Tropical Punch powder as the intervention as well as 45 g of lactose
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Entry on clinicaltrials.gov NCT00040456 states there was double-blind masking (participant, caregiver, investigator); however, the method of blinding the outcome asses-

Mueller 2005 (*Continued*)

		sors was not described
Incomplete outcome data (attrition bias) All outcomes	Low risk	Of 12 participants, 5 were evaluated for efficacy assessment, 7 dropped out of trial for several reasons: 3 for non-compliance, 2 for study violation (pharmacy dispense wrong formulation), 2 withdrew for personal reasons
Selective reporting (reporting bias)	Unclear risk	There was insufficient information to judge.
Other bias	Unclear risk	In this study expected to enrol 20 participants; 100 eligible potential participants were contacted and invited to participate in the study. According to the current recruiting status on clinicaltrials.gov, the number of participants recruited is 12 and the study was terminated due to lack of accrual. A full paper has not been published and the results for 12 participants have only been presented narratively without data analyses

HbF: foetal haemoglobin
HbSC: haemoglobin sickle cell
HC: hydroxyurea
HRQoL: health-related quality of life
IQR: interquartile range
IV: intravenous
LOS: length of stay
m Eq: morphine equivalent units
Mg: magnesium
MgSO4: magnesium sulphate
Na: sodium
RBC: red blood cell
SCD: sickle cell disease
SD: standard deviation
VOC: vaso-occlusive crisis

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Brousseau 2004	A prospective, non-randomized and non-blinded, single-arm convenience sample, with historical controls
De Franceschi 1997	A pilot study with 10 participants, not a randomized controlled study
De Franceschi 2000	Open-label unblinded study with no control group (all participants received magnesium pidolate)
Hankins 2007	A phase I dose-escalation pilot study of magnesium in combination with hydroxyurea not magnesium alone; no placebo or no magnesium comparator

Characteristics of studies awaiting assessment *[ordered by study ID]*

Voskaridou 2000

Methods	Placebo-controlled clinical study. Duration: 6 months.
Participants	10 adults with HbS/b thalassaemia.
Interventions	**Intervention** (n = 5): 0.6 m Eq/kg/day oral Mg aspartate. **Control** (n = 5): placebo.
Outcomes	Red blood cell Mg. Red cell distribution width. Dense red cells. Mean corpuscular haemoglobin concentration and reticulocytes Frequency of crisis.
Notes	Awaiting response from the authors to request to provide further details about randomizations, blinding and outcomes measured Two abstracts published 15 years ago (1999 and 2000) with no further published paper available

m Eq: morphine equivalent units
Mg: magnesium

DATA AND ANALYSES

Comparison 1. Intravenous magnesium sulphate versus placebo

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Pain score	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
1.1 Up to 1 month	1	104	Mean Difference (IV, Fixed, 95% CI)	0.10 [-0.82, 1.02]
2 Quality of Life (Child self-report total score)	1		Mean Difference (Fixed, 95% CI)	Subtotals only
2.1 Child self-reported PedsQL Generic Scales (Pre-discharge)	1	173	Mean Difference (Fixed, 95% CI)	1.5 [-37.50, 40.50]
2.2 Child self-reported PedsQL Generic Scales (1 week follow-up)	1	161	Mean Difference (Fixed, 95% CI)	-2.3 [-7.21, 2.61]
2.3 Child self-reported PedsQL Multidimensional Fatigue Scales (Pre-discharge)	1	175	Mean Difference (Fixed, 95% CI)	2.8 [-2.06, 7.66]
2.4 Child self-reported PedsQL Multidimensional Fatigue Scales (1 week follow-up)	1	160	Mean Difference (Fixed, 95% CI)	-1.4 [-13.44, 10.64]
2.5 Child self-reported PedsQL Sickle Cell Disease Module (Pre-discharge)	1	179	Mean Difference (Fixed, 95% CI)	-3.20 [-7.75, 1.35]
2.6 Child self-reported PedsQL Sickle Cell Disease Module (1 week follow-up)	1	160	Mean Difference (Fixed, 95% CI)	-3.5 [-14.95, 7.95]
3 Quality of Life (Parent self-report total score)	1		Mean Difference (Fixed, 95% CI)	Subtotals only
3.1 Parent self-reported PedsQL Generic Scales (Pre-discharge)	1	146	Mean Difference (Fixed, 95% CI)	-5.4 [-12.17, 1.37]
3.2 Parent self-reported PedsQL Generic Scales (1 week follow-up)	1	142	Mean Difference (Fixed, 95% CI)	-2.0 [-6.85, 2.85]
3.3 Parent self-reported PedsQL Multidimensional Fatigue Scales (Pre-discharge)	1	148	Mean Difference (Fixed, 95% CI)	-2.7 [-7.79, 2.39]
3.4 Parent self-reported PedsQL Multidimensional Fatigue Scales (1 week follow-up)	1	143	Mean Difference (Fixed, 95% CI)	-0.5 [-2.87, 1.87]
3.5 Parent self-reported PedsQL Sickle Cell Disease Module (Pre-discharge)	1	150	Mean Difference (Fixed, 95% CI)	-2.4 [-4.88, 0.08]

3.6 Parent self-reported PedsQL Sickle Cell Disease Module (1 week follow-up)	1	74	Mean Difference (Fixed, 95% CI)	-0.4 [-1.85, 1.05]
4 Length of hospital stay (hours)	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
4.1 Up to 1 month	1	104	Mean Difference (IV, Fixed, 95% CI)	14.70 [-20.51, 49.91]
5 Adverse effects (at up to 1 month)	2		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
5.1 Nausea or vomiting	1	104	Risk Ratio (M-H, Fixed, 95% CI)	1.04 [0.15, 7.10]
5.2 Hypotension	2	306	Risk Ratio (M-H, Fixed, 95% CI)	2.57 [0.50, 13.08]
5.3 Pain at the infusion site	1	104	Risk Ratio (M-H, Fixed, 95% CI)	3.64 [0.79, 16.69]
5.4 Warmth at the infusion site	1	202	Risk Ratio (M-H, Fixed, 95% CI)	13.26 [3.23, 54.40]
5.5 Pruritus	1	104	Risk Ratio (M-H, Fixed, 95% CI)	0.35 [0.01, 8.31]
5.6 Tachycardia	1	104	Risk Ratio (M-H, Fixed, 95% CI)	0.35 [0.01, 8.31]
5.7 Drowsiness	1	104	Risk Ratio (M-H, Fixed, 95% CI)	3.12 [0.13, 74.76]
5.8 Acute chest syndrome	1	202	Risk Ratio (M-H, Fixed, 95% CI)	1.17 [0.60, 2.26]

Comparison 2. Oral magnesium pidolate versus placebo

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Pain diary of days with significant pain	1		Mean Difference (Fixed, 95% CI)	Totals not selected
2 Effects on magnesium content (erythrocytes)	1		Mean Difference (Fixed, 95% CI)	Totals not selected

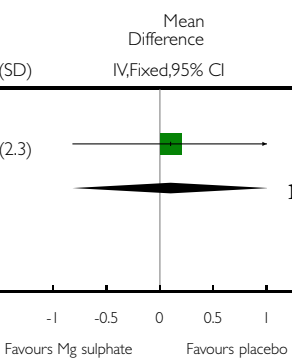
Analysis 1.1. Comparison 1 Intravenous magnesium sulphate versus placebo, Outcome 1 Pain score.

Review: Magnesium for treating sickle cell disease

Comparison: 1 Intravenous magnesium sulphate versus placebo

Outcome: 1 Pain score

Study or subgroup	Magnesium sulphate N	Mean(SD)	Placebo N	Mean(SD)	Mean Difference IV,Fixed,95% CI	Weight	Mean Difference IV,Fixed,95% CI
1 Up to 1 month							
Goldman 2013	51	5.4 (2.5)	53	5.3 (2.3)		100.0 %	0.10 [-0.82, 1.02]
Subtotal (95% CI)	**51**		**53**			**100.0 %**	**0.10 [-0.82, 1.02]**
Heterogeneity: not applicable							
Test for overall effect: Z = 0.21 (P = 0.83)							

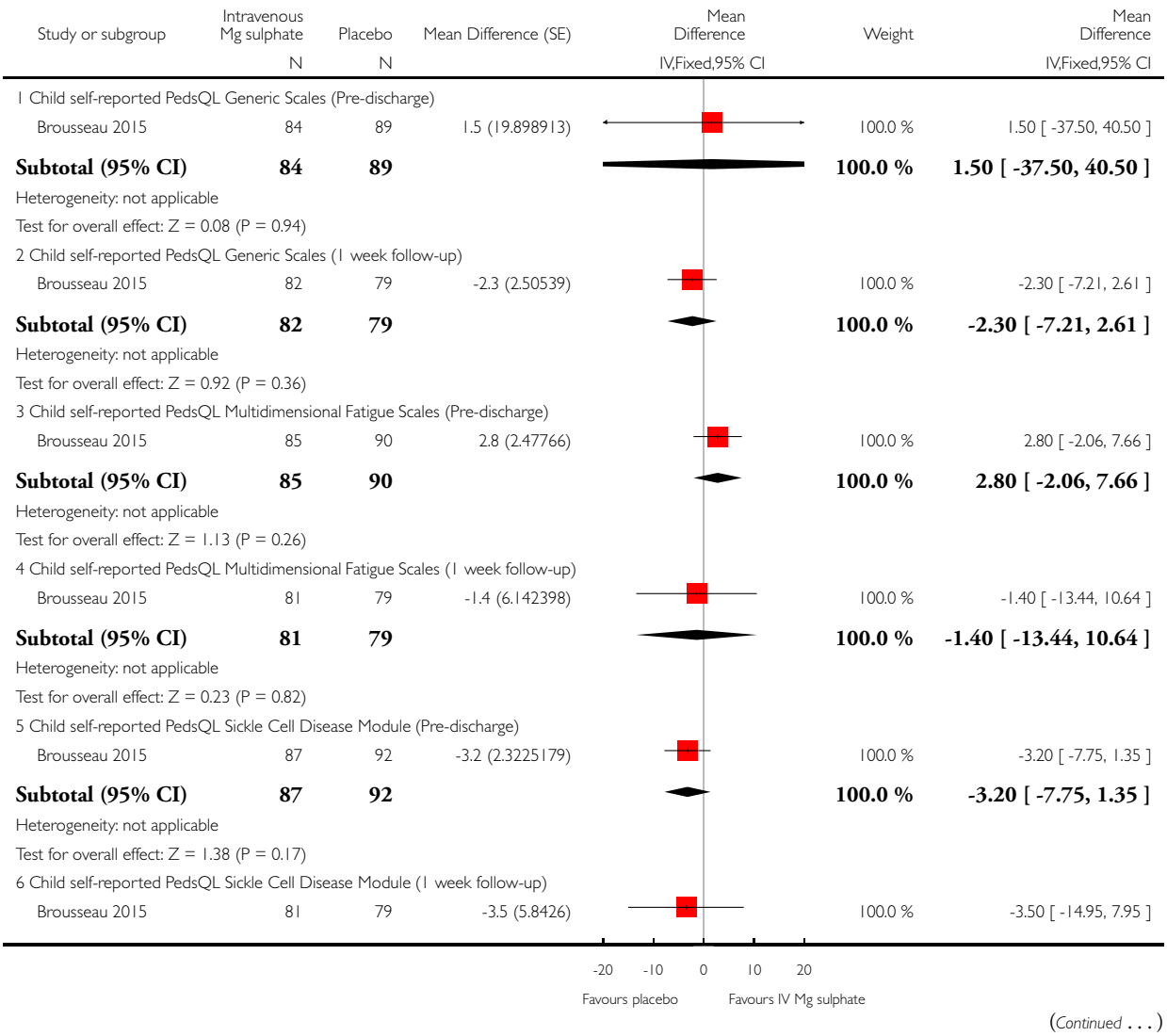


Analysis 1.2. Comparison 1 Intravenous magnesium sulphate versus placebo, Outcome 2 Quality of Life (Child self-report total score).

Review: Magnesium for treating sickle cell disease

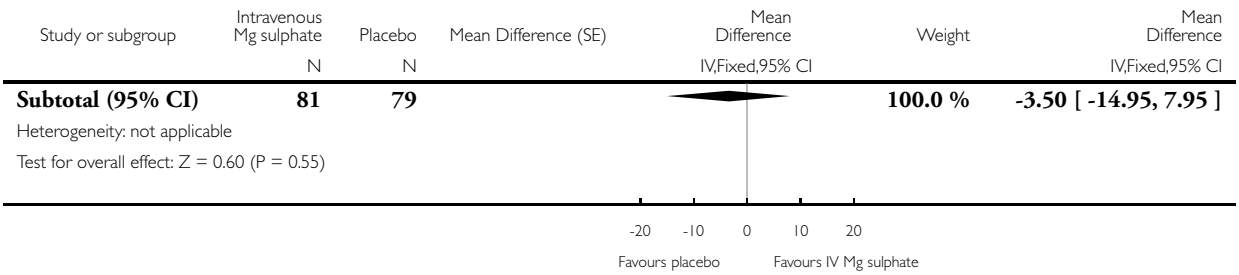
Comparison: 1 Intravenous magnesium sulphate versus placebo

Outcome: 2 Quality of Life (Child self-report total score)



(*Continued . . .*)

(. . . *Continued*)

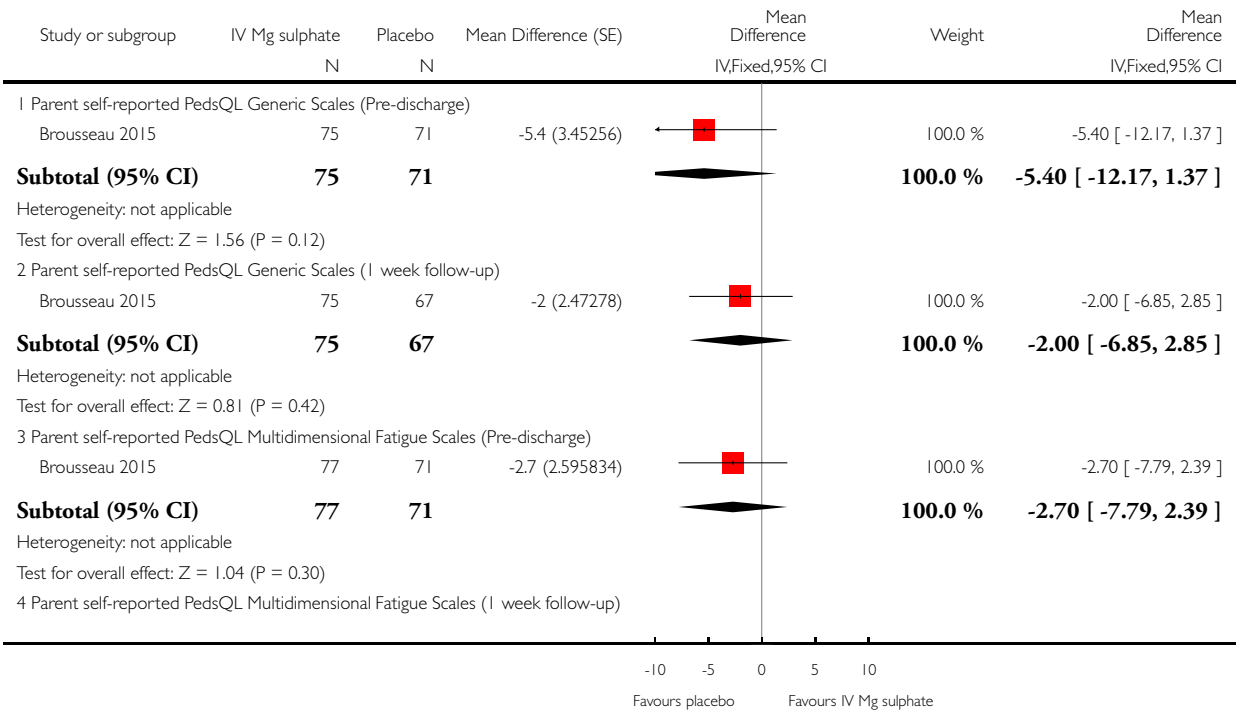


Analysis 1.3. Comparison 1 Intravenous magnesium sulphate versus placebo, Outcome 3 Quality of Life (Parent self-report total score).

Review: Magnesium for treating sickle cell disease

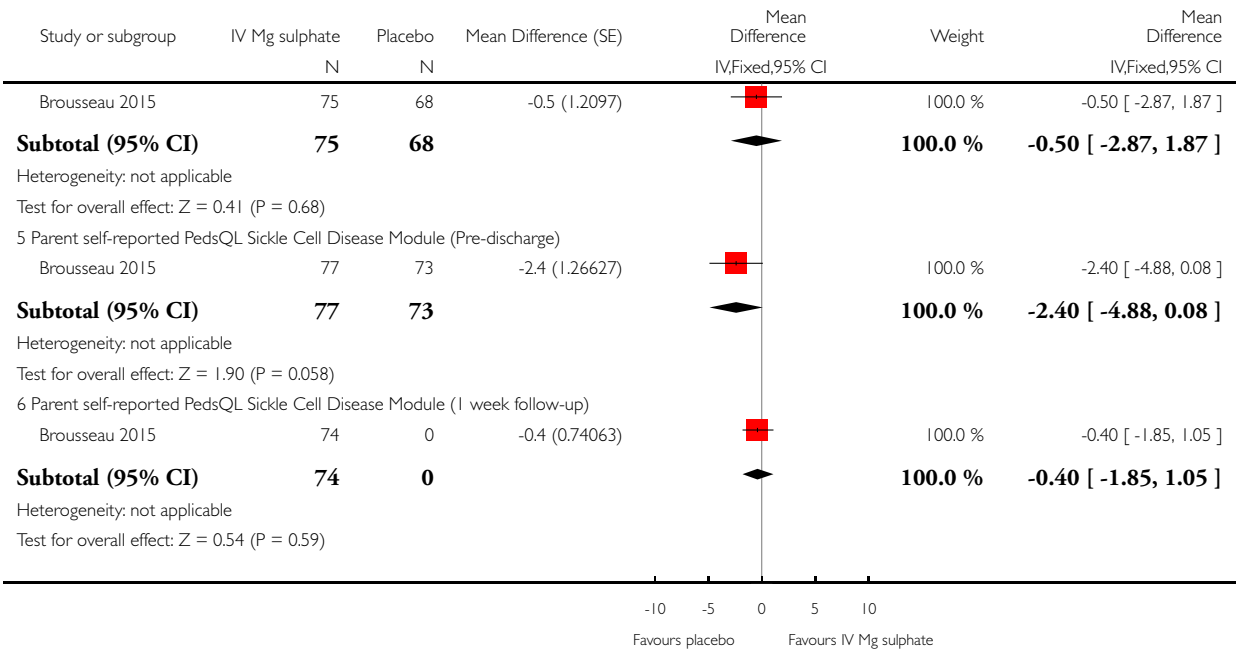
Comparison: 1 Intravenous magnesium sulphate versus placebo

Outcome: 3 Quality of Life (Parent self-report total score)



(*Continued* . . .)

(. . . *Continued*)

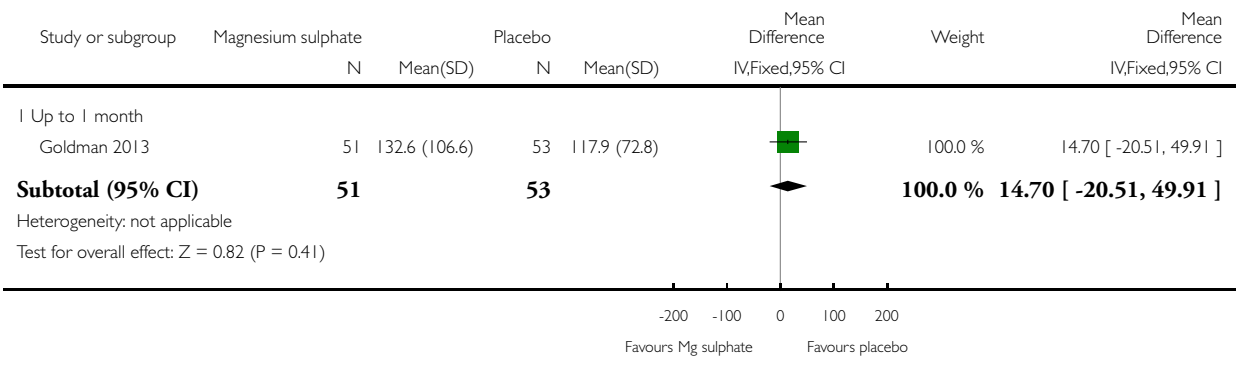


Analysis 1.4. Comparison 1 Intravenous magnesium sulphate versus placebo, Outcome 4 Length of hospital stay (hours).

Review: Magnesium for treating sickle cell disease

Comparison: 1 Intravenous magnesium sulphate versus placebo

Outcome: 4 Length of hospital stay (hours)

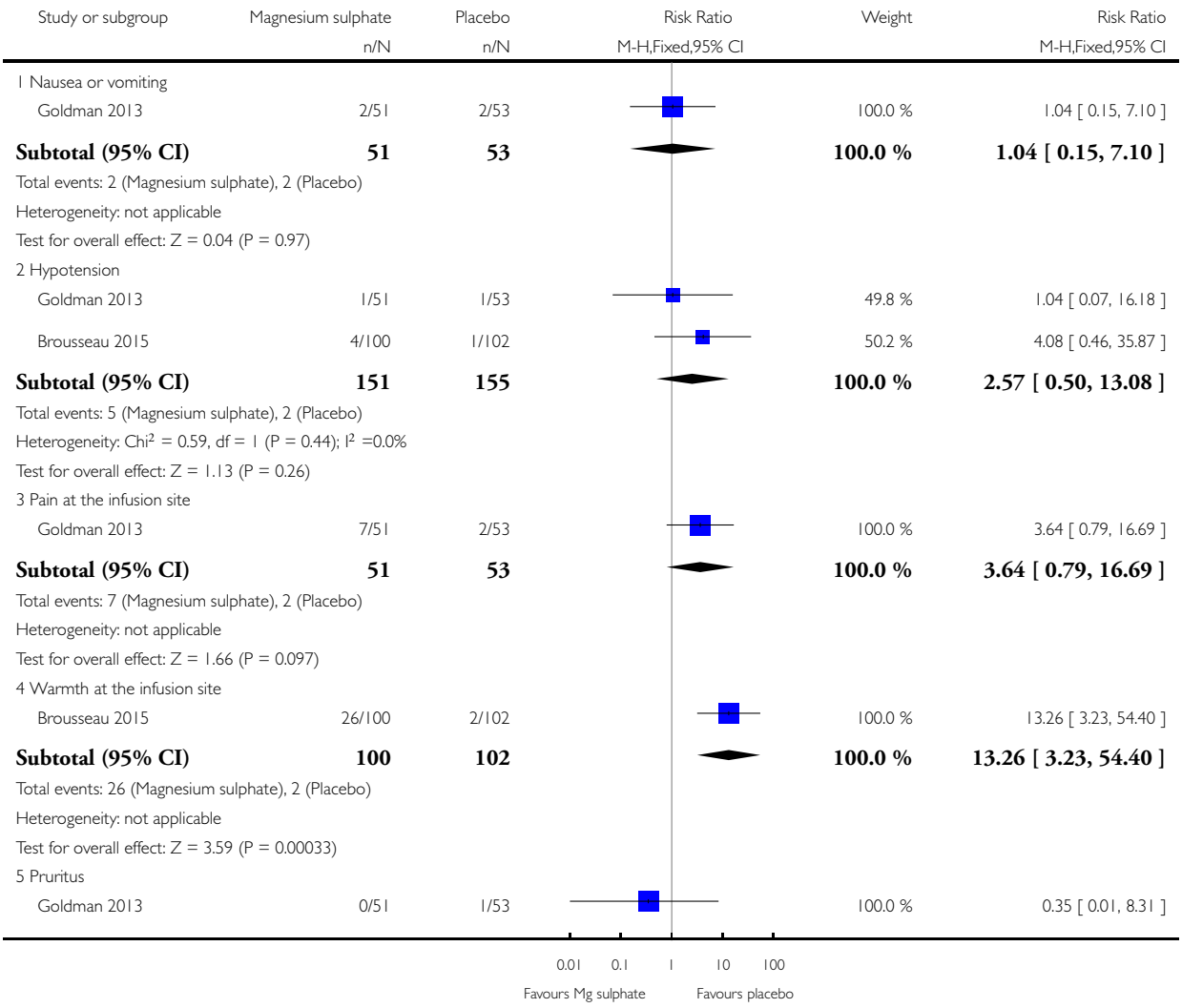


Analysis 1.5. Comparison 1 Intravenous magnesium sulphate versus placebo, Outcome 5 Adverse effects (at up to 1 month).

Review: Magnesium for treating sickle cell disease

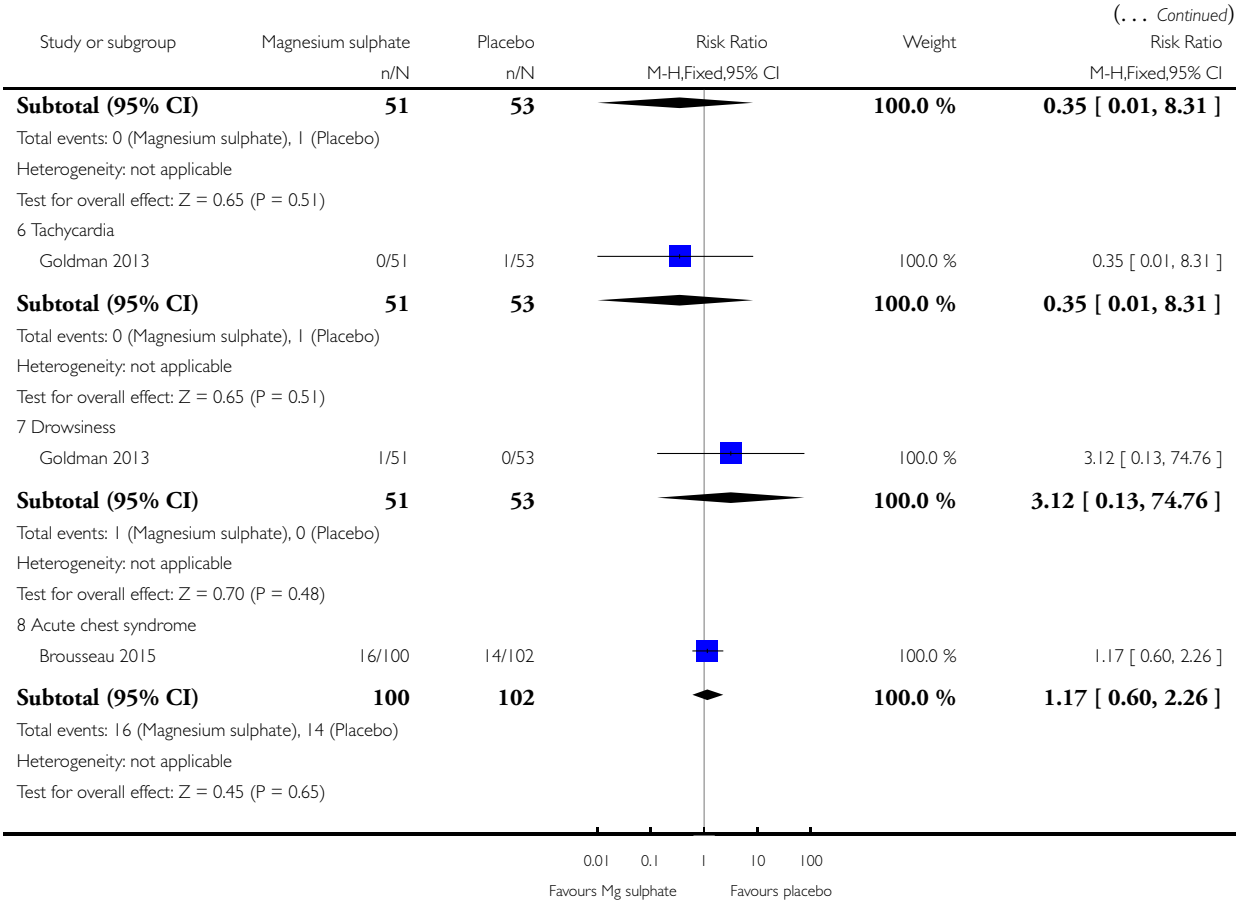
Comparison: 1 Intravenous magnesium sulphate versus placebo

Outcome: 5 Adverse effects (at up to 1 month)



(*Continued . . .*)

(. . . *Continued*)



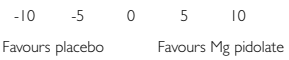
Analysis 2.1. Comparison 2 Oral magnesium pidolate versus placebo, Outcome 1 Pain diary of days with significant pain.

Review: Magnesium for treating sickle cell disease

Comparison: 2 Oral magnesium pidolate versus placebo

Outcome: 1 Pain diary of days with significant pain

Study or subgroup	Magnesium pidolate N	Placebo N	Mean Difference (SE)	Mean Difference IV,Fixed,95% CI	Mean Difference IV,Fixed,95% CI
De Montalembert 2003	24	24	1.9 (1.50499)		1.90 [-1.05, 4.85]



Analysis 2.2. Comparison 2 Oral magnesium pidolate versus placebo, Outcome 2 Effects on magnesium content (erythrocytes).

Review: Magnesium for treating sickle cell disease

Comparison: 2 Oral magnesium pidolate versus placebo

Outcome: 2 Effects on magnesium content (erythrocytes)

Study or subgroup	Magnesium pidolate N	Placebo N	Mean Difference (SE)	Mean Difference IV,Fixed,95% CI	Mean Difference IV,Fixed,95% CI
De Montalembert 2003	24	24	0.46 (0.3809)		0.46 [-0.29, 1.21]

ADDITIONAL TABLES

Table 1. Length of stay (hours)

Study: Brousseau 2015	Magnesium Group	Placebo Group	P value
LOS: median (IQR)	56.0 (27.0 - 109.0)	47.0 (24.0 - 99.0)	P = 0.24
LOS: total time until discharge from hospital	74.5 (40.0 - 124.0)	60.5 (37.0 - 122.0)	P = 0.46
LOS: early in the course of the pain crisis	58.0 (30.0 - 113.0)	47.0 (23.0 - 92.5)	P = 0.49

IQR: interquartile range
LOS: length of stay

Table 2. Effects on K-Cl cotransport, red cell K+ and Mg2+ content

Study: CHAMPS 2011	Mg			No Mg		
Ion content	N (minimum, maximum)			N (minimum, maximum)		
	(20, 23)	(13, 17)	(12, 13)	(19, 21)	(17, 19)	(13, 14)
	Baseline	Week 8	Week 24	Baseline	Week 8	Week 24
Plasma ionized Mg (mmol/l)	0.57	0.62	0.58	0.59	0.59	0.57
Plasma total Mg (mmol/L)	0.85	0.90	0.87	0.87	0.86	0.84
K-Cl co-transport (mmol/10^{13}cells x h)	13.3	13.6	13.9	13.1	14.9	13.1
Cell Na (mmol/kg Hb)	43.5	48.6	43.7	39.6	41.2	40.2
Cell K (mmol/kg Hb)	229	233	223	232	236	236

Both mean levels of plasma ionised Mg and plasma total Mg at week 8 were slightly greater compared to placebo (P value = 0.02 for ionised Mg) (P value = 0.04 for total Mg).
Cl: chloride
Hb: haemoglobin
K: potassium
Mg: magnesium
Na: sodium

APPENDICES

Appendix 1. Electronic search strategies

Database/Resource	Strategy
PubMed (1946 to date) Date of search: 01 April 2017	(**magnesium OR mgS04) AND sickle**
Clinicaltrials.gov Date of search: 29 March 2017	SEARCH 1: **magnesium AND sickle** SEARCH 2: **mgS04 AND sickle**
WHO ICTRP Date of search: 29 March 2017	SEARCH 1: **magnesium AND sickle** SEARCH 2: **mgS04 AND sickle**
ISRCTN Registry Date of search: 29 March 2017	SEARCH 1: **magnesium AND sickle** SEARCH 2: **mgS04 AND sickle**

CONTRIBUTIONS OF AUTHORS

Roles and responsibilities	**Roles and respo**
Task	**Who will undertake the task?**
Protocol stage: draft the protocol	**NNT, HHKS,SK, ALA**
Review stage: select which trials to include (2 + 1 arbiter)	**NNT**, **HHKS, ALA**
Review stage: extract data from trials (2 people)	**NNT, HHKS**
Review stage: enter data into RevMan	**NNT,SK, ALA**
Review stage: carry out the analysis	**NNT, HHKS, SK, LDF**
Review stage: interpret the analysis	**NNT, HHKS, ALA, LDF**
Review stage: draft the final review	**NNT, ALA, SK, LDF**
Update stage: update the review	**NNT, HHKS, SK, ALA, LDF**

DECLARATIONS OF INTEREST

All authors: none known.

SOURCES OF SUPPORT

Internal sources

- No sources of support supplied

External sources

- National Institute for Health Research, UK.

This systematic review was supported by the National Institute for Health Research, via Cochrane Infrastructure funding to the Cochrane Cystic Fibrosis and Genetic Disorders Group.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

In a post hoc change we included two summary of findings tables for the comparison of oral and intravenous magnesium sulphate versus placebo. We also presented the adverse events outcome in general instead of classified into mild, moderate, severe.