A RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-BLIND TRIAL OF INTRAVENOUS MAGNESIUM SULFATE FOR THE MANAGEMENT OF SEVERE HAND, FOOT AND MOUTH DISEASE WITH AUTONOMIC NERVOUS SYSTEM DYSREGULATION IN VIETNAMESE CHILDREN.

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1. BACKGROUND

Hand foot and mouth disease (HFMD) is a common infectious disease, primarily affecting young children, caused by a number of enteroviruses belonging to the species *Human enterovirus A*, including coxsackievirus a16 (CA16) and enterovirus 71 (EV71). Infection with EV71 is of particular concern as it can cause severe disease, sometimes resulting in death. EV71 was first detected in California, USA, in 1969, and was responsible for sporadic cases of central nervous system infections. Over the past 15 years, however, EV71 has emerged as a cause of more severe HFMD across Asia [1]. In 1997 the virus caused an unexpectedly large and severe outbreak in Sarawak, Malaysia, with high mortality, and in 1998 an epidemic occurred in Taiwan that was thought to involve millions of people [2, 3]. EV71 related HFMD has also increased markedly in Vietnam during this time; in 2011 164 deaths occurred among the 106,000 Vietnamese children diagnosed clinically with HFMD [4].

During outbreaks thousands of children can develop HFMD, and although most will have self-limited illness with fever and rash only, a small proportion rapidly develop neurological and systemic complications that can be fatal. It is unclear whether this is driven by pathogen characteristics or by host immune or host genetics factors. Neurological manifestations of EV71 infection include aseptic meningitis and acute flaccid paralysis, but the issue of most concern is brainstem encephalitis, since autonomic nervous system (ANS) dysregulation may occur, potentially with rapid progression to cardiopulmonary failure [1]. Clinical features indicating ANS dysregulation include high persistent fever, profuse sweating, mottled skin, tachycardia, tachypnoea, hypertension and hyperglycaemia [5]. The Vietnamese Ministry of Health (MOH) has developed guidelines for clinical staging of HFMD infection (Appendix 1). Briefly, Grade 1 represents classic HFMD without complications. Patients with Grade 2 disease show some evidence of central nervous system involvement, usually manifesting as myoclonus. This grade is further split into Grade 2a, in which there is a reported history of myoclonic jerks, and Grade 2b, in which myoclonic jerks are observed by medical or nursing staff. In Grade 3 disease there is evidence of autonomic dysfunction, while patients with Grade 4 disease have cardio-pulmonary compromise.

Although the mechanisms underlying the ANS dysregulation have not been clearly defined there is evidence that inflammation occurs in the medulla oblongata and cervical spinal cord, causing increased sympathetic activity and resulting in severe systemic and pulmonary hypertension, and eventually pulmonary oedema [1]. The frequency of ANS dysregulation is also unclear, since many of the signs are rather subjective and can be present anyway among children with high fever; however the presence of systemic hypertension in children with HFMD is considered to be clear evidence of

ANS dysregulation. The frequency with which this occurs in HFMD is not known however, as only severe cases are usually reported; in one study of 36 severe EV71 infection cases from Taiwan more than 36% developed systemic hypertension, while in 22 severe HFMD cases reported from China, 17 cases had hypertension [6].

Research has also revealed left ventricular dysfunction in patients with EV71 associated brainstem encephalitis/hypertension who later develop pulmonary oedema. Although no histological or virological evidence of viral myocarditis was seen, catecholamine-associated cardiotoxic effects were found on histological examination of cardiac ventricular biopsies, together with high concentrations of norepinephrine and epinephrine in plasma from these patients [7]. Thus, high plasma catecholamine concentrations secondary to brainstem encephalitis are purported to have a direct effect on cardiac function, as well as to cause pulmonary oedema by raising pulmonary pressures [1]. Alternatively, or in addition, the impact of altered cytokine profiles on the cardiopulmonary system may influence the severity of HFMD [8]. In small studies, the severity of EV71 infection has been associated with altered concentrations of a number of different cytokines, including TNF$\propto$, INFγ, IL6, and IL13, in blood and CSF, with evidence that cytokine levels correlate with the degree of injury to the brainstem and spinal ganglia [9]. Moreover, in a number of animal studies of neurogenic hypertension, associations have been demonstrated between inflammatory cytokine levels and enhanced vasomotor and cardiac sympathetic drive [10].

Management of ANS dysregulation presents particular challenges. In one report from Taiwan, use of the phosphodiesterase-3 inhibitor, milrinone, was said to control hypertension and support myocardial function in a group of 24 children with severe HFMD compared to historical controls. [11]. In addition, milrinone has also been shown to decrease mortality in HFMD patients with pulmonary edema in a small open-label randomized clinical trial in Viet Nam [12]. This has now become the recommended therapy for severe HFMD with ANS dysregulation in Vietnam, with MOH Guidelines setting down indications for when milrinone should be commenced in suspected HFMD cases. Currently the MOH Guidelines define the intervention level for use of milrinone at a systolic pressure exceeding the 99th centile for age plus 5 mm Hg, which approximates to the internationally accepted definition of Stage 2 hypertension in children (Appendix 2)[13]. However, anecdotally, clinical failures still occur despite high dose intravenous milrinone (see below), and a number of children go on to require haemofiltration and ventilatory support, the next steps recommended in the MOH guidelines [4]. Secondly, there is very little clinical or safety data available with respect to milrinone use in children, apart from a few small studies following cardiac surgery. Adverse effects reported in adults include ectopic activity and potentially life-threatening ventricular arrhythmias [14]. Recent reports in children suggest that milrinone use is an independent risk factor for clinically

significant tachyarrhythmias after congenital heart surgery, and may be associated with development of acute renal failure[15].

Autonomic disinhibition is also postulated to occur in severe tetanus - elevated concentrations of circulating catecholamines have been observed and urinary epinephrine and norepinephrine excretion are increased proportional to disease severity [16, 17]. Beta-blockers, calcium channel blockers and morphine sulphate have all been used to regulate tetanus-associated sympathetic activity but with limited success. However, in a randomized controlled trial comparing magnesium sulphate ($MgSO_4$) with placebo in patients with severe tetanus, use of magnesium was associated with significantly reduced requirements for drugs to control muscle spasms and cardiovascular instability [18]. In addition, in *in vitro* studies magnesium has been shown to reduce catecholamine secretion from peripheral nerve endings and the adrenal medulla [19]. In patients undergoing tracheal intubation or surgery for phaeochromocytoma use of magnesium was associated with a reduction in systolic blood pressure and plasma catecholamine concentrations [20] . Magnesium is also used widely in conditions such as eclampsia, severe asthma and pulmonary hypertension, and there are isolated reports of rapid and effective control of life-threatening autonomic hyperreflexia in patients with spinal cord lesions [21-25]. Intrapartum use of $MgSO_4$ in women with preeclampsia was associated with reduced cytokine levels in the women and their babies[26]. Similarly, use of magnesium in a small number of patients with aneurysmal subarachnoid haemorrhage was associated with reduced serum levels of certain inflammatory cytokines [27].

Formal safety data relating to use of $MgSO_4$ in paediatrics are limited, but from its use in children with severe asthma, and in a small number of neonates with uncontrolled pulmonary hypertension, adverse effects appear to be infrequent. In a case series involving 8 EV71 confirmed HFMD cases with ANS dysregulation managed at HTD over a 4 month period in early 2012 $MgSO_4$ was added when hypertension remained poorly controlled despite high dose milrinone (up to 0.75 ug/kg/minute) - in all cases the blood pressure reduced within 30-60 minutes and remained stable subsequently on a continuous magnesium infusion for 48-72 hours. No patient required haemofiltration, although 2 of 8 cases were ventilated because of respiratory distress. Brain MRI was performed later in 4 of these cases and in the 2 children with neurological sequelae abnormalities were found (involving the medulla in both cases, and with extensive atrophic changes in one child). Longer term neurological sequelae have not been investigated to date [28]. Magnesium continues to be used for these severe HFMD patients and up to now a total of 24 cases that failed on mirilnone have been treated with MgSO4 at HTD. Only two of these cases required ventilation (2/24, 8%) because of respiratory distress, and there were no other severe adverse events. By comparison, during 2011 before MgSo4 was being used on the PICU, of the 35 cases treated with milrinone alone

9 required ventilation (9/35, 26%, unpublished data). None of the 24 patients who received MgSO4 had a plasma magnesium level above 2.5 mmol/l, and the dosage regimen used was the same as that planned for this study.

In summary, although HFMD has become one of the major contributors to childhood morbidity and mortality in Vietnam, current management strategies rely on guidelines that are based on expert opinion and only two small clinical studies [5, 11, 12, 29]. The efficacy of milrinone, an expensive drug with a significant toxicity profile, for management of ANS dysregulation is unclear, and $MgSO_4$, an alternative therapeutic agent that is cheap, safe and easily available may be effective.

We hypothesize that intervention with magnesium sulphate early, when ANS dysregulation first becomes apparent, may control cardiovascular instability and prevent progression to severe disease. Therefore we plan to carry out a randomized controlled trial comparing magnesium sulphate with placebo in children with HFMD, autonomic instability and systemic hypertension.

2. STUDY OBJECTIVES

***PRIMARY OBJECTIVE**:*

- To evaluate the effects of $MgSO_4$ on control of hypertension and progression to severe disease (shock, respiratory compromise or death) in children with severe HFMD and ANS dysregulation.

SECONDARY OBJECTIVES:

- To describe the clinical signs of autonomic dysfunction observed in children with severe HFMD, measure levels of biomarkers of sympathetic activity including plasma and urine catecholamine levels and levels of inflammatory cytokines, and to assess the impact of $MgSO_4$ on these parameters.
- To examine relationships between measures of cardiac output (CO) and systemic vascular resistance (SVR) with clinical signs of autonomic dysfunction, and to assess the impact of $MgSO_4$ on these parameters.
- To evaluate the effects of $MgSO_4$ on long-term outcome from severe HFMD in survivors, by assessing neurodevelopmental status at 6 months.

3. STUDY DESIGN

We will perform a randomized, placebo-controlled, double blind trial of intravenous $MgSO_4$ in Vietnamese children with HFMD and signs of ANS dysregulation with systemic hypertension. This

clinical trial forms one part of a large research programme on HFMD that is in progress in Ho Chi Minh City – potential interactions with two other studies that are currently planned are described in the section on contemporaneous studies (section 8.8).

Children with clinical suspicion HFMD admitted to the Paediatric Intensive Care Unit (PICU) at the Hospital for Tropical Diseases (HTD) or the High Dependency Unit (HDU) on the Infectious Diseases Ward at Children's Hospital Number 1 (CH1) in Ho Chi Minh City will be eligible for enrolment to the study if they develop systemic hypertension and at least one other clinical sign of ANS dysregulation. Trained study physicians will assess potential patients for the following inclusion and exclusion criteria (see Appendix 3 for all definitions).

3.1. INCLUSION CRITERIA

- Age 6 months to 15 years
- Clinical suspicion of HFMD requiring PICU/HDU admission
- Considered severe enough to warrant invasive blood pressure monitoring by PICU/HDU staff
- Development of hypertension defined as follows:
  - For children aged 1 year and over, at least 3 consecutive systolic blood pressure recordings above the 95th centile for age, gender and length (USA guidelines for defining Stage 1 hypertension in children, (Appendix 2)) measured invasively over a period of 20 minutes provided the child is not distressed or crying [30, 31].
  - For children aged 6 months to 1 year, systolic BP > 100 mm Hg measured invasively on at least 3 occasions over a period for 20 minutes provided the child is not distressed or crying
- Plus one or more of the following criteria:
  - Tachypnoea for age
  - Irregular or labored breathing, but with SpO2 above 92% in air and normal ABG (pH, pCO2, pO2, HCO_3 all within the normal range for the local laboratory)
  - Resting heart rate > 150 bpm
  - Mottled skin
  - Profuse sweating
  - Refractory fever
  - Hyperglycemia
- Informed consent

3.2. EXCLUSION CRITERIA

- Past history of hypertension, chronic renal, cardiac or pulmonary disease, or any neurological disorder
- Hypertensive emergency
- Already commenced milrinone or any other inotropic agents
- Respiratory distress with SpO2<92% in air or PaCO2>45 mm Hg
- AV block or any arrhythmia
- Acute renal failure

3.3. INTERVENTION

Following written informed consent a loading dose of 50mg/kg of either $MgSO_4$ or visually matched placebo will be given over 20 minutes followed by a maintenance infusion for 72 hours according to response, aiming for Mg levels 2-3 times normal in the treatment arm. All staff involved in clinical care will be blind to the treatment allocation, and Mg levels will be monitored and adjusted by doctors responsible for monitoring Mg/Ca laboratory result from another clinical facility as detailed in the section on dose adjustment below.

3.4. STUDY ENDPOINTS

Current Vietnamese MOH guidelines specify that milrinone should be given to children with HFMD and ANS dysregulation when the systolic blood pressure is sustained at a level exceeding a value approximating to the 99th percentile for age (gender and length are not considered) plus 5 mm Hg – i.e. Stage 2 hypertension – with the intention that treatment should commence within 1-2 hours [32]. Enrolment to this study is designed to be early, when hypertension is first identified (at Stage 1), so that in the event of treatment failure the MOH treatment guidelines can be applied. A small number of patients who present with Stage 2 hypertension may also be enrolled; in such cases very stringent blood pressure criteria will ensure that if the blood pressure does not improve within 30 minutes of commencing the study drug, milrinone will be added – i.e. all Stage 2 patients will be on milrinone within 1 hour of presentation unless the blood pressure falls to Stage 1 levels.

The Primary Endpoint will be a composite endpoint indicating disease progression within 72 hours of study enrolment. It is defined as occurrence of any of the following within 72 hours of commencing the study drug infusion:

- Specific BP criteria necessitating addition of milrinone as detailed in the Section “Criteria for addition of milrinone” below.
- Need for mechanical ventilation

- Development of shock
- Death.

Secondary endpoints will include these parameters singly, plus a number of other clinical endpoints including the following: requirement for other inotropic agents (eg dobutamine) during hospital admission; duration of hospitalization; presence of neurological sequelae at discharge in survivors; neurodevelopmental status assessed 6 months after discharge.

Safety endpoints will include the number of adverse events (AEs) and severe adverse events (SAEs) that occur in the two treatment arms during hospitalization.

Exploratory endpoints: A number of exploratory endpoints will be assessed including serial measurements of cardiac output (CO) and systemic vascular resistance (SVR), catecholamine levels, and cytokine levels. We will assess the impact of $MgSO_4$ on these parameters, provided the data collected appear reliable and consistent within individual participants. Because these children represent the severe end of the disease spectrum of HFMD, we will take samples for analysis of host genetics factors. Long-term neurodevelopmental sequelae will be examined to explore any effect of magnesium sulfate treatment on this population.

4. SAMPLE SIZE

A formal sample size calculation requires both an estimate of the risk of disease progression in the placebo arm, as well as quantification of the hypothesized treatment effect of $MgSO_4$ on the primary endpoint.

During 2011 approximately one third of patients with Grade 3 HFMD managed at HTD (48/140, 34%) and CH1 (48/148, 32%) required milrinone for ANS dysregulation with hypertension, and in 9/48 (19%) of the HTD cases and 10/48 (21%) of the CH2 cases milrinone alone failed to control the hypertension. Following introduction of the MOH management guidelines to commence intra-arterial BP monitoring early (at the first sign of autonomic disturbance), among 16 children managed in this way at HTD over a 2 month period, 12/16 patients developed Stage 1 hypertension, and 7 of these 12 cases developed Stage 2 hypertension and were treated with milrinone. Based on this observation (i.e. that 7/12 children with Stage 1 hypertension progressed), for the sample size calculation for this study we estimated a progression rate of 50% in the control arm.

With respect to the hypothesized treatment effect there is little direct information for the actual scenario we plan to investigate, i.e. the influence of $MgSO_4$ commenced at Stage 1 hypertension on subsequent control of blood pressure and progression to severe disease. In the series of 24 EV71

confirmed HFMD cases with ANS dysregulation and poorly controlled hypertension despite high dose milrinone described earlier, when $MgSO_4$ was added to the treatment regimen in all cases the blood pressure reduced within 30-60 minutes and thereafter remained stable on a continuous $MgSO_4$ infusion. In the tetanus study mentioned above (in which the study drug was given to patients with severe tetanus requiring a tracheostomy) requirement for additional therapy to treat ANS dysfunction was reduced from 14/97 (14%) in the placebo group to 3/97 (3%) in the $MgSO_4$ group, although the need for assisted ventilation was similar in the two groups. Thus indirect evidence suggests that the effect size of the proposed intervention may be large; we therefore estimate that use of $MgSO_4$ could reduce the risk of progression by at least 50%.

Based on 1:1 randomization, an anticipated relative reduction in the risk of progression of 50% (from 50% in the control arm to 25% in Mg recipients), 90% power and a two-sided 5% significance level, 85 patients per treatment group are required. To allow for some violations of our assumptions and losses to follow-up, we plan to recruit 190 patients (95 patients per treatment arm) into the study.

5. STUDY PROCEDURES

Vietnamese MOH guidelines for HFMD management indicate that all suspected cases with Grade 2b or more severe disease should be admitted to a PICU or HDU facility for close observation. The guidelines also indicate that a peripheral arterial line should be inserted for invasive blood pressure monitoring if there are any signs of autonomic dysregulation. For patients with Grade 3 disease (i.e. the group eligible for inclusion in this study) pulse, blood pressure, respiratory rate and pattern, oxygen saturations, temperature etc. should be monitored at least hourly, the blood sugar should be checked 6 hourly, and the child should receive intravenous sedation with phenobarbitone, as well as a dose of 1g/kg intravenous immunoglobulin (IVIG) as soon as possible, with a second dose of IVIG 24 hours later.

5.1. STUDY POPULATION AND SCREENING (FIGURE 1)

All patients aged between 6 months and 15 years admitted to the PICU at HTD or HDU at CH1 with clinically suspected HFMD, who are considered by the ward medical staff to have any indication warranting insertion of an arterial line for continuous invasive blood pressure monitoring, will be considered as potentially eligible for the study. Such patients are monitored continuously on monitors such as Nihon Kohden / Spacelabs Healthcare etc., following recommendations in the MOH guidelines. All these devices automatically store information on cardiovascular parameters electronically for periods of 24 hours

The parents/guardians of potentially eligible children will be informed about the study and given a Patient Information Sheet (PIS) to read. In the event that the child subsequently fulfills the clinical inclusion criteria a Study Doctor will go though the PIS again with the family and discuss any questions they may have before asking for consent. If the parent/guardian gives written consent then the necessary screening bloods and ECG will be performed and if no reasons for exclusion are identified then the patient will proceed to enrolment and randomization. During the consent process the parents/guardians will be informed that data stored automatically on the monitoring device for up to 2 hours prior to enrolment will be downloaded if they agree for their child to take part in the study, in order to provide an objective baseline assessment of the child's cardiovascular status before commencing the study drug. It is anticipated that this phase of recruitment, from development of appropriate inclusion criteria to commencement of study drug, should take less than 1 hour.

Patients who present to the PICU/HDU with already established Stage 2 hypertension will have arterial access established immediately and the process of explaining the study, requesting consent, checking inclusion/exclusion criteria and proceeding to enrolment and randomization if appropriate, will be carried out as quickly as possible aiming to commence the study drug within 30 minutes. These children will be very closely observed and if the blood pressure does not improve within 30 minutes of commencing the study drug, milrinone will be added – i.e. all Stage 2 patients will be on milrinone within 1 hour of presentation unless the blood pressure has settled to Stage 1 levels with the study drug.

In case worry over the child's illness could affect the parents/guardians ability to make an informed decision on study participation, the study doctor will go though the PIS with the family at least once more 12-36 hours after enrolment, or at any time they ask. This review of the PIS will be recorded. Parents/guardians of deceased children will not be re-approached.

In the event that a study participant deteriorates clinically one of the study doctors will be available during discussions with the parents/guardians about the child's progress and the plans for their ongoing care, in case the family have any issues or concerns relating to the study.

5.2. RECRUITMENT OF PARTICIPANTS (FIGURES 2, 3)

Initially we plan to start recruitment at HTD only, to ensure that all study procedures run smoothly during the first few months. We will review participant enrolment after 4-6 months, with the plan to expand recruitment to include CH1 as well. Current estimates indicate that 100-120 Grade 3 HFMD cases are admitted to PICU at HTD annually; if 75% of these cases develop Grade 1 hypertension we might recruit 60-80 eligible subjects per year. Patient numbers admitted to CH1 are generally higher than to

HTD so we anticipate that by using both sites it should be possible to achieve the planned sample size within 2 years.

Beginning of recruitment (HTD only):

Study staff working on PICU at HTD will identify parents/guardians of potentially eligible patients as soon as possible after admission. Study staff will consult the hospital chart and the parents/guardians to verify if the initial screening criteria are met, and will provide the PIS and discuss the study with relevant families at this time. Subsequently if a child develops autonomic disturbance with hypertension the study staff will talk to the family again and request consent, then follow the full pathway for inclusion/exclusion criteria, and if appropriate, proceed to enrolment and randomization. A case report form (CRF) detailing the history and examination findings at enrolment will be completed as soon as the study drug infusion has been started. A fast track pathway for children with Stage 2 hypertension at presentation to PICU is noted above. Screening and obtaining informed consent will not prolong the commencement of required treatment. Children whose parents/guardians do not consent to the study will continue with standard care.

After enrolment of the first 30 patients an independent Data and Safety Monitoring Board (DSMB) will review the data. The recommendation of the DSMB will be sent to the Ethical Committees of the Hospital for Tropical Diseases and Children's Hospital 1. Recruitment will continue at any active site during the DSMB review period.

Study continuation (HTD and CH1):

After the first DSMB review CH1 will decide when to start enrolment of patients with Stage 1 hypertension into the study –the study will be executed in a similar manner on the HDU at CH1 as during the initial phase at HTD. Following recruitment of 20 patients with Stage 2 at HTD or 100 total patients, and review of the data by the independent DSMB, CH1 may decide to recruit this patient group as well. It is envisaged that both sites will be recruiting within about 6 months of beginning the study, aiming to complete recruitment over 2 years.

5.3. RANDOMIZATION AND BLINDING

Randomization to either treatment arm will be in a 1:1 ratio, stratified according to the hospital where recruitment takes place. A randomization list using block randomization with blocks of variable size will be prepared using a computer program and maintained confidentially from study staff by the Clinical Trials Pharmacist.

A chronological log of all enrolled patients will be maintained and the next available sequential study code will be assigned to each patient as they enroll. The assigned number will correspond to a coded,

sealed, package containing 50 ampoules of 15% Magnesium Sulphate or visually matched placebo. Drug appearance and administration schedules will be identical to maintain blinding amongst the attending physicians and nurses.

5.4. STUDY MEDICATION

Detailed information on the use of $MgSO_4$, the pharmacokinetics, side effect profile etc. is provided in Appendix 4.

Patients will be assigned to one of two treatment arms, and follow the same dosing schedule:

Group 1: MgSO4 15% solution in sterile water in 10ml vials (Fresenius Kabi) diluted to 10% solution by mixing with 5 ml Nacl 0.9%

Group 2: Placebo will be sterile water in 10 ml vials (Fresenius Krabi) diluted to 15ml by mixing with 5 ml Nacl 0.9% .

Schedule: Loading dose: 50mg/kg over 20 minutes (0.5ml/kg)

Maintainance: 30 – 50 mg/kg/hr (0.3 ml/kg/hr to 0.5 ml/kg/hr) for 72 hrs

Dispensing, storage and accountability:

$MgSO_4$ and placebo are available in 10 ml visually matched ampoules supplied by Fresenius Kabi. They will be prepared centrally by an unblinded study pharmacist and distributed to the site in a box as required. Drugs will be stored in accordance with the manufacturers' recommendations in a secure area. All movements of study medication will be recorded. Both individual subject and overall drug accountability records will be kept up to date by the study staff.

Dose adjustment:

After the initial loading dose the study infusion dose will be increased in 0.1 ml/kg/hr stages (10mg/kg/hr) every 15 minutes to a maximum dose of 0.5 ml/kg/hr (50 mg/kg/hr), with the following caveats:

- If the systolic BP decreases to < 90th percentile for age, gender and length the dose will be reduced by 1 stage every 15 mins
- If the systolic BP increases to the levels described below for treatment failure, action will be taken as indicated
- If the systolic BP decrease rapidly more than 25% over 15 minutes
- If the plasma Mg level > 2.5 mmol/l or < 1.8 mmol/l a 25% increase or decrease in the infusion rate will be implemented as appropriate.

To maintain blinding, plasma Mg will be measured in all patients every morning, and the results will be sent to a doctor to monitor Mg/Ca laboratory result. He/She will inform the PICU staff of any dose adjustments to be made to the $MgSO_4$ infusion but will not report the actual lab values to the staff. Similar (sham) dose adjustments will be made for the control infusions, according to a randomized list available only to the Mg/Ca monitoring doctor.

5.5. TREATMENT FAILURE CRITERIA

1. Criteria for discontinuation of study treatment

If any of the following occur the study treatment will be stopped immediately and rescue treatment given as appropriate (Appendix 5, 6).

- Serious cardiac arrhythmia (eg atrio-ventricular block, prolonged QT interval)
- Hypotension: SBP < 70 + (2 X age) mmHg for 15 minutes or more
- Urine output < 1ml/kg/hr for 4 hours or more
- Cardiac arrest or any other emergency situation where the treating physician feels there is a contra-indication to the study drug.

In addition, if a patient develops respiratory distress (as defined in Appendix 3) an urgent plasma Mg level will be performed. If intubation and ventilation is needed, or if the Mg level is > 3 mmol/L, the study treatment will be stopped and rescue treatment will be given as described in Appendix 6.

2. Criteria for addition of milrinone

The study drug will be continued for 72 hours as described in the protocol, unless one of the criteria for discontinuation indicated above occurs. Milrinone will be commenced in the following circumstances in accordance with the VN MOH guidelines.

- Hypertensive emergency (Appendix 2)
- SBP fails to decrease by 25% over the first 8 hours after enrolment despite maximum $MgSO_4$ maintenance infusion
- For children aged 1 year and over:
  - SBP increases to $\geq 99^{th}$ percentile plus between 5 -15 mm Hg consistently for 30 minutes
  - SBP increases to $\geq 99^{th}$ percentile plus 15 mm Hg consistently for 15 minutes
  - SBP increases to $\geq$ 40 mm Hg over baseline for 15 minutes, if this value is lower than either of the first two cutoffs. The baseline systolic blood pressure is defined as the lowest value measured at any time after admission to hospital before enrolment in the study.
- For children aged 6 months to 1 years:

- SBP increases to ≥110 mm Hg up to to 120 mmHg consistently for 30 minutes
- SBP increases to ≥ 120 mm Hg consistently for 15 minutes
- SBP increases to ≥ 40 mm Hg over baseline for 15 minutes, if this value is lower than either of the first two cutoffs. The baseline systolic blood pressure is defined as the lowest value measured at any time after admission to hospital before enrolment in the study.

Occasionally the clinical status of a patient may indicate that milrinone is not the best inotrope to use – in these cases the treating physician will make management decisions appropriate to the situation.

If the patient's clinical status remains unstable after starting milrinone additional measures including ventilation and/or haemofiltration will be considered in accordance with VN MOH guidelines for management of HFMD. If the blood pressure remains high (SBP > 99th percentile plus >15 mm Hg) despite maximal doses of milrinone and study drug infusion then additional antihypertensive agents may be added – e.g. nicardipine, captopril etc. depending on the clinical scenario.

5.6. UNBLINDING

The Clinical Trials Pharmacist will hold the unblinded randomization list with details of the contents of each individual treatment package. This list will be accessed only in the case of emergency unblinding, which will be authorized by an investigator following standard operating procedures. Emergency unblinding will be performed in the case of an adverse event when knowledge of the identity of the study treatment may contribute to the treating physician's ability to care for the patient.

In certain clinical scenarios when an urgent plasma Mg/Ca level is required to manage the patient effectively, the treating physician may be inadvertently unblinded to the treatment group (Appendix 6). In general in these cases the study treatment would already have been discontinued as per section 5.5.

5.7. CLINICAL ASSESSMENTS

Patients will be examined daily by trained HDU/PICU study doctors. A record of all significant events in the previous 24 hours plus detailed physical examination findings (in particular the nervous system, respiratory and cardiovascular systems) will be recorded each morning in the CRF.

Vital signs will be monitored continuously using devices with the facility for download of data into a readable database format using compatible software and/or accessory modules – at HTD suitable monitoring devices include models by Nihon Kohden, while at CH1 similar equipment from Spacelabs Healthcare would be appropriate. A standard ECG recording will be performed at the bedside by a trained nurse once daily and also if any abnormality is noted on the monitor by study staff (eg.

premature beats, atrial or ventricular arrhythmias). The treating physician will evaluate this recording and discuss action with respect to the study drug with the site PI before making any decisions, except in the event of an emergency.

Advanced haemodynamic parameters, including CO and SRV, can be monitored using a number of different devices. Established systems for continuous monitoring generally use some form of thermodilution methodology requiring catheterisation of the great vessels (pulmonary artery, central veins), and are therefore impractical for use in our situation. However, a number of relatively new systems rely on pulse waveform analysis from a peripheral arterial line. Unfortunately none of these devices have been validated for use in paediatric critical care although they are used frequently in children undergoing cardiothoracic surgery. We are exploring several different options at present, aiming to evaluate the most promising method to see if a simple system can provide useful additional information over and above basic clinical examination in assessing children with severe HFMD and ANS dysregulation. The LiDCOplus/LiDCOrapid techniques rely on pulse waveform analysis (calibrated using lithium dilution in the case of the LiDCOplus system) and have the advantage that a relatively cheap module is available that is consistent with the monitors in use at the two hospitals and that allows download of continuous haemodynamic data for up to 96 hours per patient, in an electronic database format. It is likely that we will elect to evaluate one of these systems for the study, but all analysis on CO and SRV will be regarded as exploratory.

Urine output will be monitored using specific collecting bags and calculated every 4 hours. If the urine output is less than 1ml/kg/hr over a 4 hour period, a bedside ultrasound scan will be done to confirm this situation, and if necessary urethral catheterization will be established to monitor the urine output closely.

Urine will be collected for catecholamine analysis each day – all urine will be stored in the appropriate collecting bottle (containing 10-15 ml of 6M HCl as preservative) by the bedside and the total volume will be recorded at the end of the 24 hour period. One aliquot (5 mls) will be sent to the OUCRU laboratory each day for storage at -20^{o}C.

5.8. LABORATORY ASSESSMENTS AND DIAGNOSTIC TESTING

See the tables in Appendix 7, 8 for a summary of the timing of all procedures and lab tests. All research samples will be stored at an appropriate temperature and analysed in batches.

- A combined nasal/throat swab and a rectal swab will be obtained at enrolment for enterovirus PCR / specific EV71 PCR. Enteroviruses will be detected from swabs at OUCRU using generic enterovirus and serotype specific EV71 and CVA16 real-time RT-PCR. Remaining enteroviruses will

be subtyped at OUCRU using VP1 nested RT-PCR and sequencing. Whole genome sequences will be determined in-house at OUCRU, at the J. Craig Venter Institute (JCVI)(US), the Wellcome Trust Sanger Institute (UK) and at Duke-NUS (Singapore).

- Blood glucose and arterial blood gases will be measured at least once daily in all study participants or more frequently according to the clinical situation
- Plasma magnesium and calcium concentrations will be measured at baseline and 12 hours after the start of the study infusion, then once daily for the remaining 72 hours. After data from the first 30 patients has been assessed, and if in the opinion of the DSMB the 12 hourly measurement does not add to patient safety, the measurements will only be performed once daily for the remaining patients. A doctor who monitors Mg/Ca laboratory result will review these results and inform the ward clinicians in charge of managing the patient if any dose adjustments to the study drug infusion are required (see section 5.4. above), or if the calcium level is low (<0.9 mml/l) and a calcium infusion is indicated.
- Plasma electrolytes, creatinine, CKMB and Troponin I will be measured at baseline and once daily thereafter.
- FBC and CRP are measured routinely when HFMD cases are admitted to PICU. If no results are available within the 24 hours prior to study enrolment these tests will be repeated at baseline and then subsequently according to clinical need.
- Plasma catecholamine (epinephrine and norepinephrine) concentrations will be measured at baseline and then once daily for 72 hours. Urine catecholamines will be measured every 24 hours using an aliquot obtained from the whole volume of urine collected during this period.
- Plasma cytokines (including IL1, IL6, IL13, TNF alpha) will be measured at baseline, 12 hours and 24 hours after starting $MgSO_4$/Placebo and on a final sample obtained on discharge.
- Serological testing will be done on plasma samples collected at enrolment and discharge. Titers against EV71 and related serotypes within the Human enterovirus A species will be assessed using micro-neutralization assays. This work will be done in collaboration with laboratories at Duke-NUS (Singapore), where these samples will be processed.
- Cells from these plasma samples will be separated and stored for assessment of host genetic factors associated with disease severity using exome sequencing. Exome sequencing is a strategy in which the coding regions of the genome are specifically targeted. It is an effective alternative to whole genome sequencing. Exons are the short, functionally important sequences of the human DNA, and represent the regions of the genes that are translated into protein. In the human genome there are about 180,000 exons that consist of 30 million base-pairs and these constitute about 1% of the human genome. Because of their functional relevance, it is estimated

that this fraction of the genome contains about 85% of the disease-causing mutations [33]. The Wellcome Trust Sanger Institute, UK, will perform this selective sequencing of the coding regions of the genome.

A summary table indicating the volumes of clinical and research blood tests that will be taken each day is presented below.

	D 1	D 2	D 3	D 4	Discharge or day 7 after discharge
Clinical blood tests	3 ml	2 ml	2 ml	2 ml	
Research blood tests	4 ml	3 ml	2 ml	2 ml	2 ml
Total blood volume	7 ml	5 ml	4 ml	4 ml	2 ml

5.9. FOLLOW UP

Patients will be assessed daily for the duration of the hospital stay. At discharge a full neurological and neurodevelopmental assessment will be performed and a final blood sample for diagnostic serology and cytokine measurements will be obtained. Patients who have not have recovered fully from the effects of sedation with phenobarbitone by the time of discharge but are otherwise considered fit to go home, will be asked to attend one week later for formal review and neurological assessment. All patients will be also asked to return at 6 months post-enrolment for a clinical and neurodevelopmental assessment. Any further follow-up will be according to clinical need and participants will be referred back to the standard hospital out-patient clinic system.

Neurodevelopmental assessments done at discharge (or 1 week later) and 6 months will use the Bayley and Movement ABC-2 tools. Children 36 months and under at enrolment will use the Bayley infant scales of development III. Children 48 months and above at enrolment will use the Movement ABC-2 tool for their assessments. The children aged between 37 and 47 months at enrolment will have both assessments done at both visits in which case total assessment time could be up to 2 hours.

6. DATA AND ANALYSIS

6.1. SOURCE DATA

Source documents are original documents, data, and records from which participants' study data are obtained. These include, but are not limited to, hospital records (from which medical history and

previous and concurrent medication may be summarized into the CRF), digital or printed laboratory and pharmacy records, MRI data files and digital or printed output from monitoring devices. CRF entries are considered source data if the CRF is the site of the original recording (i.e. there is no other written or electronic record of data). In this study the CRF will be used as the source document for some clinical data points. Whenever possible clinical laboratory data (haematology/biochemistry etc.) will be downloaded directly from the analysers into the study database.

All documents will be stored safely in secure, confidential locations. In order to maintain patient confidentiality, only the signed informed consent form, the patient master log and the trial drug documents will be labeled with the patient's name or identifying information. All other study documents will be identified by the subject number and initials only. Data recorded on paper will be entered on site into the database in accordance with standard operating procedures, and monitored for accuracy.

Attempts will be made to ensure that patients admitted to HDU/PICU who might potentially be eligible for the study are monitored on systems with the facility for subsequent automatic download of haemodynamic parameters. If the patient does enter the trial and gives appropriate consent, then these data for up to 2 hours prior to enrolment will be downloaded together with the information for the 72 hours after enrolment.

6.2. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

The study will be conducted in accordance with the current approved protocol, Viet Nam Guidelines for Good Clinical Practice and study standard operating procedures. The OUCRU Clinical Trials Unit will be engaged in assuring good governance, regulatory compliance and QA/QC of study execution.

6.3. DATA MONITORING

This trial will be monitored to ensure that data are generated, documented and reported in compliance with the protocol, standard operating procedures and the appropriate regulatory requirements. Monitoring will be performed according to procedures defined by the OUCRU Research Governance Team and the agreed monitoring plan.

6.4. STATISTICAL ANALYSIS

The primary analysis population will include all randomized patients who commenced the loading dose infusion of study drug, and analysis will be according to the randomized treatment arm (intention-to-treat). A per protocol analysis will also be performed , including all subjects who complete the full treatment schedule for 72 hours, or if death or one of the pre-specified criteria for

stopping the study drug occurs within 72 hours. An additional analysis will also be performed in the subgroup of patients with confirmed enterovirus 71 infection

The primary endpoint of disease progression will be compared between the two treatment arms based on logistic regression with the treatment assignment as the only covariate. As disease progression is evaluated in a short time frame, i.e. within 72 hours of study drug initiation, we expect to lose only a minimal number of patient to follow-up before that time point and will analyze these patients according to their last recorded disease status. We will also perfom regression analysis including a number of baseline covariates – age, gender, BMI, day of illness at study entry, study site, baseline severity assessed in terms of an internationally recognised score (e.g. PRISM III).

Pre-defined secondary endpoints and exploratory investigations will be compared between the two treatment arms based on linear regression for continuous endpoints, logistic regression for binary endpoints, Cox regression for time-to-event endpoints. For laboratory markers, analyses will be adjusted for the pre-dose value of the respective marker. For host genetics analyses in this sample set, putative severity alleles will be detected by filtering and comparison with reference data from control groups including the 1000 Genomes Project. For alleles with significant enrichment among severe case, validation will be attempted using samples from other cohorts with severe HFMD.

The values for CO, SVR, catecholamine and cytokine measurements will also be correlated with clinical information using descriptive statistical methods. Clinical information including temperature, skin perfusion, capillary refill time, respiratory rate and urine output will be summarised for defined time intervals (at least 6 hourly) and relationships assessed with the cardiovascular and biochemical markers measured during the same time interval.

All adverse events and serious adverse events (see next section) will be recorded on the patient CRF, and will be reported to the relevant authorities as indicated below. In addition, for analysis of the safety endpoints the intensity of clinical AEs will be recorded on a five-point scale adapted from the NCI guidelines (CTCAE version 4.03),[34] to ensure that the cutoffs used are appropriate to the age of the populations being studied.

Grade	Adverse Events	Description
0		No AE (or within normal limits).
1	Mild	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate	Minimal, local, or noninvasive intervention (e.g., packing, cautery) indicated; limiting age-appropriate instrumental activities of daily living (ADL).
3	Severe or medically	Hospitalization or prolongation of hospitalization

	significant but not immediately life-threatening	indicated; disabling; limiting self-care ADL.
4	Life-threatening consequences	Urgent intervention indicated.
5	Death	

The relationship of the AE to the study treatment will also be assessed and recorded as [35]:

Relationship	Attribution	Description
Unrelated to investigational agent/intervention	Unrelated	The AE is clearly not related to the intervention
	Unlikely	The AE is doubtfully related to the intervention
Related to investigational agent/intervention	Possible	The AE may be related to the intervention
	Probable	The AE is likely related to the intervention
	Definite	The AE is clearly related to the intervention

A formal statistical analysis plan which describes all planned analyses for this study will be written before the data is unblinded.

7. SAFETY CONSIDERATIONS/PATIENT SAFETY

7.1. DEFINITIONS

Adverse Event (AE) An unfavourable or unexpected sign, including an abnormal laboratory finding that is temporally associated with the use of an investigational product, whether or not considered related to the product. Events will be considered Adverse Events if they occur after the first infusion of study drug commences. Pre-existing conditions that worsen after the first infusion of study drug commences will also be reported as Adverse Events.

All clinical AEs will be recorded on an AE form.

All laboratory parameters will be included in the analysis, with cutoffs identifying significant abnormalities defined in the database (see section above). Significant laboratory abnormalities will be entered on an AE form only if they meet one or more of the following conditions:

- Accompanied by clinical symptoms
- Leading to a change in the study drug (dose modification or withdrawal)
- Requiring a change in concomitant therapy (e.g. dose adjustment or discontinuation)

Stopping of study drug for any clinical indication will be recorded as an adverse event.

Serious Adverse Event (SAE) A serious adverse event is an AE that:

- Results in death or
- Is life-threatening (i.e. the patient was at risk of death at the time of the AE) or
- Requires prolongation of existing hospitalization or
- Requires new inpatient hospitalization or
- Results in persistent or significant disability/incapacity

7.2. REPORTING PROCEDURES FOR SERIOUS ADVERSE EVENTS

All SAEs will be reported to the Sponsor (in this case OUCRU fulfills this role) and the site IRB at the relevant site (HTD or ND1) as soon as possible. A report of any SAE that is life threatening or results in death will also be sent to the office of the Vietnamese MoH Research Ethics Committee. This report will include details of the event and the recommendation from the site IRB. This report will be sent within 7 days of knowledge of the event. If all information is not available upon initial report, a complete report will be sent within 15 days of knowledge of the event. All other SAEs will be reported with a complete report sent to the Ministry of Health Research Ethics Committee, including the recommendation of the site ethical committee within 15 days of knowledge of the event.

7.3. DATA AND SAFETY MONITORING BOARD

An independent Data and Safety Monitoring Board will be set up consisting of expert Vietnamese and international researchers and doctors, with the necessary knowledge of pediatrics, clinical trials and statistics. The DSMB will review the protocol and agree to a data review schedule and reporting requirements before the study commences, with particular reference to SAE reporting. All data reviewed by the DSMB will be in the strictest confidence. A DSMB charter will outline its responsibilities and how it will operate.

The DSMB will perform a safety review of data for the first 30 patients enrolled to the trial. This review will include unblinded summary tables of SAEs, AEs or event reports submitted to the DSMB. An analysis of overall clinical outcome will be performed. The recommendations of the DSMB will be sent to the ethical committees of the Hospital for Tropical Diseases and Children's Hospital 1.

Children Hospital 1 will decide when to begin recruitment after receipt of the DSMB report. A second formal DSMB review will be performed after enrolment of 20 patients with Stage 2 hypertension or a total of 100 patients, whichever comes first. Recommendations of the DSMB will be sent to the Ethical Committees of both hospitals involved in the study. Additional safety reviews may be performed annually or at the discretion of the DSMB based on available data and ongoing reporting. All DSMB reports will be sent to the site ethical committees and the Oxford Tropical Research Ethics Committee for consideration. Recruitment will continue at any active site during the DSMB review period.

The recommendations of the DSMB will be based on the principle that, unless the benefit of MgSO4 is shown "beyond reasonable doubt" at an interim analysis, stopping for efficacy will not be considered. The Haybittle-Peto boundary, requiring $p<0.001$ at interim analysis to consider stopping for efficacy, will be used as a guide. However, the DSMB recommendation requires clinical judgment in addition to statistical tables. As the dissemination of preliminary summary data could influence the subsequent conduct of the trial and introduce bias, access to interim data and results will be confidential and strictly limited to the independent statistician and the monitoring board. No results (except for the recommendation) will be communicated to the outside and/or the clinical investigators involved in the trial.

7.4. SAFETY MONITORING FOR MAGNESIUM LEVELS

In order to maintain study blinding, data safety monitoring doctors (DSMDs) will be responsible for reviewing patient magnesium and calcium concentrations. These doctors will be responsible for implementing changes to the study drug dose as defined in the protocol and standard operating procedures. Decisions will be based on predefined and approved study procedures and communicated to the study doctor. Data safety monitoring doctors will form a sub-Committee of the DSMB and will be bound by the same rules of confidentiality; they will report any safety concerns to the Data Safety and Monitoring Board. Data safety monitoring doctors will not have access to unblinded analysed study results available to the DSMB.

7.5 SAFETY OVERSIGHT

Under the direction of the Ministry of Health Ethical Committee, the Hospital for Tropical Diseases and Children's Hospital 1 have the responsibility of overseeing the safety of all patients enrolled at their respective sites. In order to support this responsibility, the investigators will report the following to the ethical committees of these two hospitals: annual reports on progress of the trial and relevant events; all severe adverse events; recommendations of the Data Safety Monitoring Board; violations of the protocol which pose a risk to patient safety, patient welfare or the integrity

of the data. Annual reports and serious adverse event reports will also be reported to the Ministry of Health Ethical Committee. The ethical committees have the authority to stop or modify the trial at any time.

8. ETHICAL CONSIDERATIONS

8.1. REGULATIONS

The Investigator will ensure that this study is conducted in compliance with the current revision of the Declaration of Helsinki (Seoul 2008) and the Viet Nam Ministry of Health Guidelines of Good Clinical Practice.

8.2. ETHICAL REVIEW

The study protocol and its associated documents will be submitted to the ethical committees of the Hospital for Tropical Diseases, Ho Chi Minh City; Children's Hospital Number 1, Ho Chi Minh City; the Viet Nam Ministry of Health and the Oxford University Tropical Research Ethics Committee (OxTREC). The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

8.3. INFORMED CONSENT

The study staff will discuss the study with the accompanying parent/guardian. If both parents are dead or not actively involved in caring for the child, the main long-term carer for the child will be accepted as a guardian and considered able to give consent for the study. Study staff will describe the purpose of the study, the study procedures, possible risks/benefits, the rights and responsibilities of participants, and alternatives to enrolment. The parent/guardian will be invited to ask questions which will be answered by study staff, and they will be provided with appropriate numbers to contact if they have any questions subsequently. If the parent/guardian agrees for the child to participate, they will be asked to sign and date an informed consent form. A copy of the form will be given to them to keep. In addition to the procedures above, illiterate signatories will have the Informed Consent Form read to them in the presence of a witness who will sign to confirm this. All patient information sheets and consent forms will be written in the local language and will use terms that are easily understandable. Assent will not be requested as the children will be in severe medical condition at the time of consent. Additionally, hand, foot and mouth disease rarely presents in children over 7 years old.

Because of the circumstances in which the progression of HFMD may occur rapidly, the parents/guardians of all potentially eligible patients will be given information about the study as soon as possible after admission to the PICU/HDU. If later the child fulfills the necessary enrolment criteria the study staff will discuss the study again, allowing the family 1-2 hours to make a decision. Rarely children may present with established hypertension necessitating rapid intervention – in these cases the family will be given up to 30 minutes to make a decision. Screening and obtaining informed consent will not prolong the commencement of treatment, since insertion of the arterial and venous lines necessary for standard care takes a minimum of 30 minutes. The study doctor will also go though the PIS with the family at least once more the next day or any time the parents ask.

8.4. RISKS

This study will use a drug that has been studied thoroughly and its toxicities are well described (see Appendix 4). In general, the drug is well tolerated in children, including neonates. The maximum dose of $MgSO_4$ which has been used in neonates is 150 mg/kg/hour to keep the blood level of $MgSO_4$ up to 5.5 mmol/l [22]. In this study, the dose used is lower and patients will be closely monitored to ensure that the blood level of $MgSO_4$ is below 2.5 mmol/l, a level at which only relatively minor side-effects usually occur. Potential side effects of $MgSO_4$ at a level above 1.25 mmol/l (3mg/l) include reduced neuromuscular transmission and some degree of CNS depression, which in turn may have an advantageous effect in children with HFMD experiencing myoclonic jerks and generalised CNS irritability. Other side-effects that may occur with higher levels of $MgSO_4$ include respiratory depression, hypotension, and abdominal cramps [36]. Patients will be monitored very closely and $MgSO_4$ and other important electrolyte levels will be measured regularly to ensure that levels are appropriate; to maintain blinding only a team of independent clinicians who will be responsible for dose adjustments will see these blood results.

We do not anticipate any specific risks associated with the small volume of NaCl 0.9% that will be given in both arms (up to 0.17 ml/kg/hr). Patients in both treatment arms will have additional blood samples taken for research purposes, which poses a small risk of bruising and infection.

Parents/guardians of participants will be invited to give consent for sample storage, sample export and to have genetic studies performed on the participants' DNA. Each of these processes are optional, and the participant's inclusion in the study is independent of this. All stored and exported samples will be pseudonymized and linked to protected identifying information at the study site only. No identifying data will be exported or shared. Genetic studies will be performed at the Wellcome Trust Sanger Institute (UK). Any future studies which will use stored samples will be submitted for review by the appropriate Ethical Committee(s). Some people may consider it an

invasion of privacy to have genetic studies performed on their DNA. Although these genetic studies are exploratory and we do not know whether the results will be helpful, we hope that eventually they may provide useful information in treating patients with this disease in the future.

Stigmatization within a community is a major concern of genetic research. Genetic studies conducted at OUCRU within the patient population at HTD do not pose significant risk of stigmatization as the catchment population of the hospital is >90% Vietnamese Kinh, which is the ethnic majority of Viet Nam (86% of the estimated Vietnamese population of >91.5 million is Vietnamese Kinh). No studies or subgroup analyses will be done to target minority populations. Studies are conducted in urban and near urban populations that attend the hospital and are not focused on specific locations/regions/villages.

8.5. PATIENT BENEFITS & COMPENSATION

Trial patients will have the medical costs associated with diagnosis and treatment of HFMD covered from the time that the parent/guardian agrees to allow their child to enroll in the study until discharge, not including the medical costs related to previous treatment decisions made before enrolment. All trial related procedures will be covered by the study, and patients will be reimbursed the cost of local transport to attend for the follow up visit according to OUCRU compensation policy. This policy reflects the actual cost of transportation plus modest compensation for time lost from work.

8.6. ALTERNATIVES TO STUDY PARTICIPATION

Subjects are able to freely decline participation in this study. If so, they will receive standard care for their condition.

8.7. WITHDRAWAL OF PARTICIPANTS FROM THE STUDY

Parents/guardians of study participants may voluntarily withdraw from the study for any reason. If this occurs, the child will be managed in accordance with standard clinical care guidelines. The child's withdrawal from the study will not affect their access to the best standard of care within the local health system. With the agreement of the parent/guardian, a clinical assessment should be performed and recorded at the time of withdrawal. The reason for withdrawal should also be recorded in the CRF.

8.8. CONTEMPORANEOUS STUDIES

This study forms one part of a larger research program on HFMD that is taking place in HCMC. Where there is overlap between studies in the program the sampling/investigation protocol and case report forms have been aligned to minimise duplication and research fatigue.

Two other studies will be running contemporaneously at one or both of the study sites. First, a large observational study entitled "*An observational study of the epidemiology and virology of Enterovirus 71 and hand, foot and mouth disease at 3 referral hospitals in Ho Chi Minh City, Vietnam (OxTREC 1005-13, Study code 03EI)"* will be recruiting patients at all levels of severity (outpatient clinics, inpatient wards, ICUs) at both the HTD and CH1 sites. This study aims to monitor the virology of HFMD of all levels of severity, and to assess associations between clinical signs and symptoms, routine and virology laboratory data, and severity. The study will also assess the serological responses and cross-reactivity among all human enterovirus A species and explore the host genetics of severe illness. Initially, 03EI will only enroll at the outpatient clinic at HTD, to minimize interference with the magnesium trial and the prognostic indicators study described below. After the prognostic indicators study completes enrolment, the 03EI will also enroll on the wards at HTD, but not the ICUs while the magnesium trial is in progress. At CH1, patients will be recruited from the outpatient clinic and the in-patient ward throughout the trial. Patients already enrolled in the 03EI study at CH1 and HTD site who subsequently deteriorate to Grade 3 or 4 HFMD and fulfill the enrolment criteria for the magnesium study, will be invited to consider trial participation as per the consent and enrolment procedures described above. To minimize duplication, the daily clinical information and discharge sample for sero-diagnostics required for the 03EI study are also incorporated in the magnesium study protocol. Acute serum and cells for host genetics will have already been taken for these patients, and this will not be repeated. Data from patients who enroll directly into the magnesium sulphate trial, and have not been approached or enrolled for the 03EI observational study will be used to contribute to the analysis of 03EI endpoints. Details of this testing and laboratory analysis is found in the Laboratory Assessment and Diagnostic Testing (section 5.8) above.

Second, at the HTD site only, a study entitled "*A prospective cohort study evaluating prognostic indicators and sequelae following severe Hand foot and mouth disease (OxTREC 33-12, HTD CS/ND/12/24, Study Code 08RS)"* will simultaneously be recruiting patients with HFMD during the enrolment period of this trial. Participation in one study is not dependent on the other and parents will make the decisions independently without repercussion on enrolment to either study. This study uses the same sampling protocol for virology, immunology and host genetics as the 03EI study.

The prognostic indicators study enrolls two groups of patients with HFMD.

1. Patients with grade 2a and 2b HFMD will be enrolled at admission, have laboratory tests on day 1 and clinical follow-up throughout their illness. At discharge (or Day 8), these patients will have a convalescent blood sample taken and undergo an MRI scan and a neurodevelopmental assessment. The neurodevelopmental assessment will be repeated at 6 months and 18 month post-enrolment.

If a patient enrolled to this study progresses to grade 3 or 4 HFMD and becomes eligible for the magnesium trial, the parents will be invited to consider trial participation as per the consent and enrolment procedures described above. Acute samples for virology, immunology and host genetics will have already been collected and will not be repeated. Since the prognostic indicators study only requires collection of clinical information during participation in the in-patient phase of the magnesium trial, clinical data for the two studies will be amalgamated to reduce the burden of data collection. When the child is ready to discharge, the prognostic indicator study procedures will resume with a discharge blood sample, neurodevelopmental examination and MRI scan. As noted above, patients who have not have recovered fully from the effects of sedation with phenobarbitone by the time of discharge but are otherwise considered fit to go home, will be asked to attend one week later for formal review and neurological assessment. This appointment and the subsequent 6-month follow-up visit will be coordinated together to gather all information for both the 02EI and 08RS studies.

2. Patients admitted directly with grade 3 or 4 disease who subsequently recover will be recruited at discharge for the prognostic indicators study. Some of these patients may have participated in the magnesium trial. A separate consent form will be presented to parents to decide on participation. The children of those who agree will have an MRI and neurological assessment performed at discharge. Follow-up neurological assessments will be performed at 6 months and 18 months. The 6-month follow-up visits for both studies will be performed simultaneously.

8.9. CONFIDENTIALITY

Participants will be assured that all information generated in this study will remain confidential. All data (including clinical, laboratory and genetic data) will be stored in password-protected databases. Participants' names will be recorded at the time of enrolment to allow for their identification at follow-up visits. However identifiable information will be linked to stored data or samples only by a protected Master List. This list will not be shared outside the study staff at a unique hospital and no identifying information will be transferred between sites. All CRFs and samples will be labeled with a study identification number only and stored in suitable secure locations. Only persons who have signed the locally appropriate data protection training will have access to the password-protected

computer where entered data is stored. After conclusion of the project, data will be removed from the computers and stored in a safe place. Any scientific publications or reports will not identify any patient by name or initials. When the research team reviews their notes, they are also bound by professional confidentiality.

8.10. SAMPLE SHARING AND STORAGE

Samples collected will be used for the purpose of this study as stated in the protocol and stored for future use if agreed by the parent/guardian. Consent will be obtained from parents/guardians for sample storage and/or shipment of specific samples to collaborating institutions for investigations that cannot be performed locally. Any proposed plans to use samples other than for those investigations detailed in this protocol will be submitted to the relevant ethics committees prior to any testing.

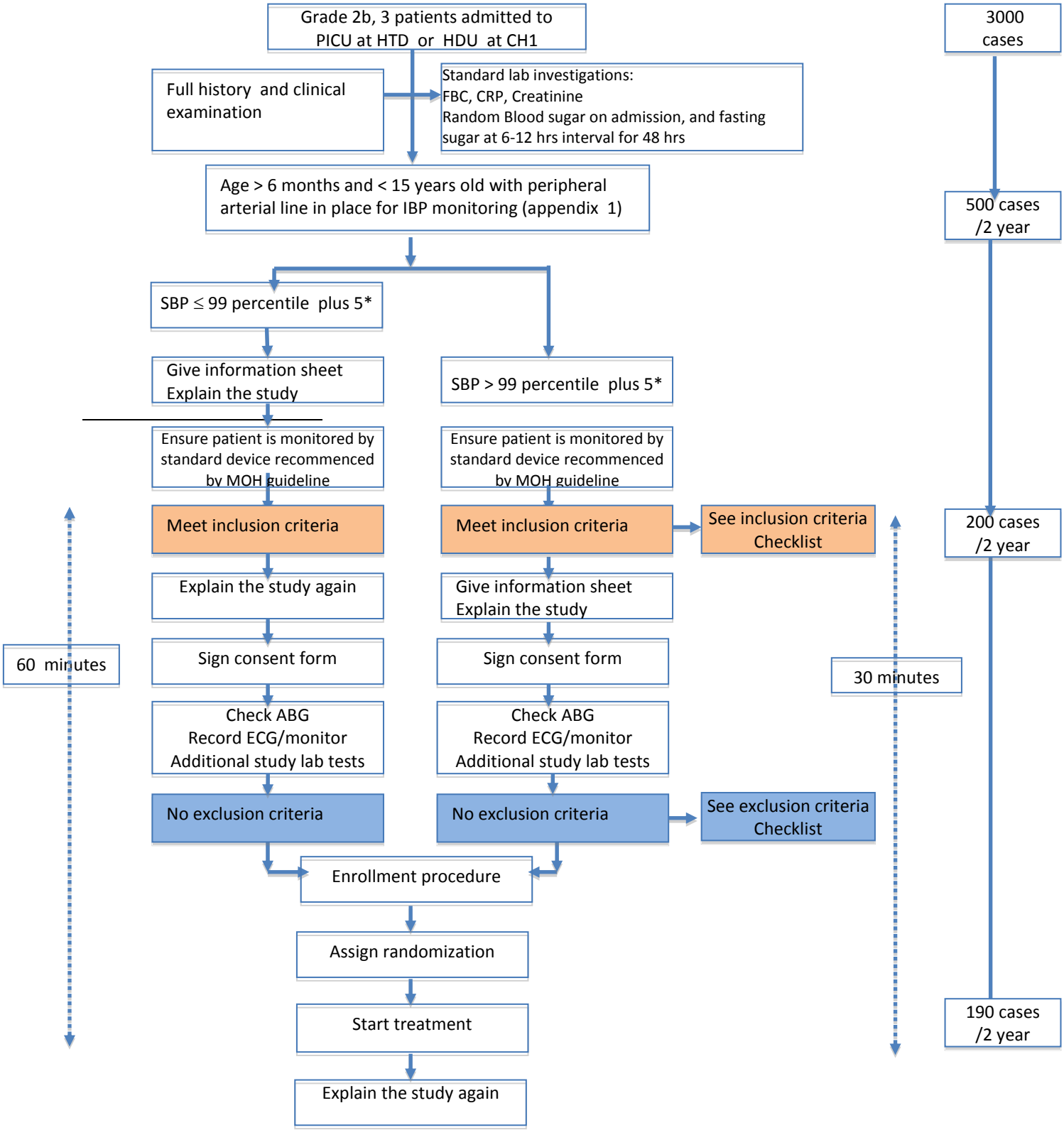
The participants will be identified only by a study specific participant number and/or code in any database. Data may be used alone or in combination with data from related studies in secondary analyses.

Parents/guardians of participants will also be invited to give consent to have genetic studies performed on the participants' DNA. These processes are optional, and the participant's inclusion in the study is independent of this. All samples will be pseudonymized and linked to protected identifying information at the study site only. No identifying data will be exported or shared. Genetic studies will be performed at the Wellcome Trust Sanger Institute (UK). These genetic studies are exploratory and we do not yet know whether the results will be helpful in treating patients with this disease.

8.11. SPONSORSHIP AND INSURANCE

This study is sponsored by the University of Oxford. Appropriate insurance-related arrangements are in place with respect to the University's role as research sponsor for this study.

Figure 1: **SCREENING FLOW CHART**



Note: *: if the indication for IBP monitoring is hypertension but there are no other signs or symptoms of autonomic dysfunction (see appendix 1), other causes for hypertension should be considered (obesity, underlying diseases) until the patient fulfills the inclusion criteria.

Figure 2: ESTIMATED PARTICIPANT RECRUITMENT

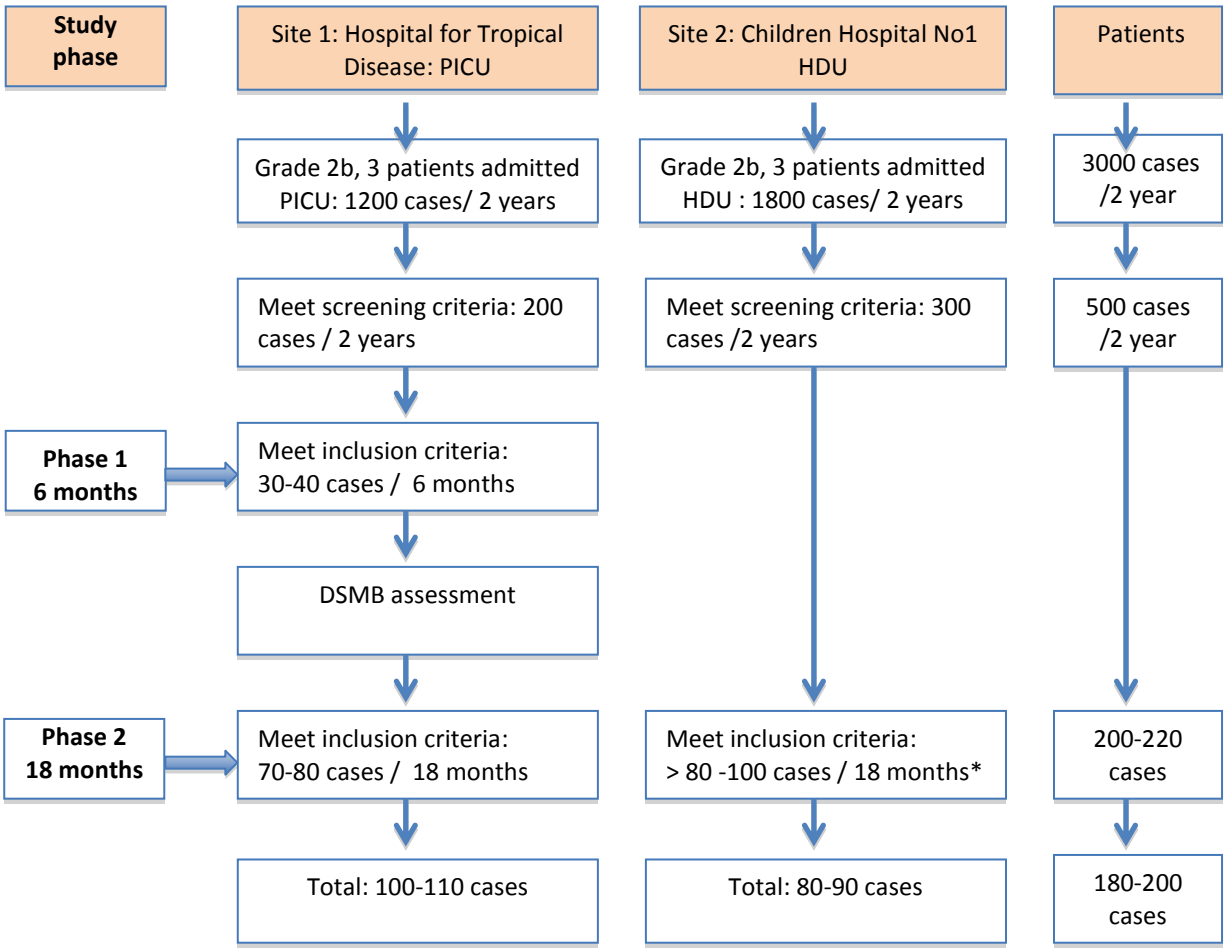


Figure 3: PARTICIPANT RECRUITMENT AND INDEPENDENT DSMB REVIEW PLAN

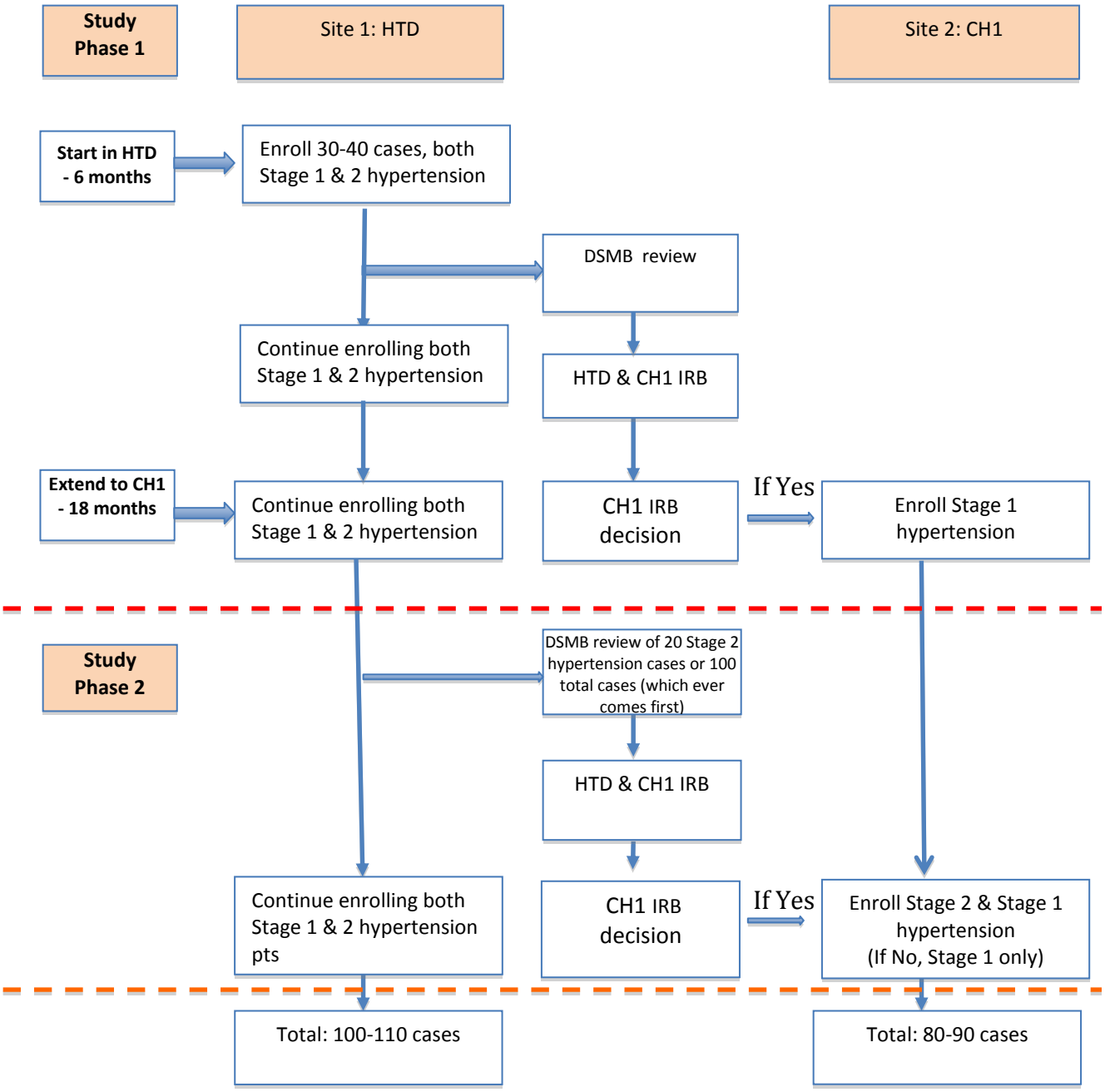
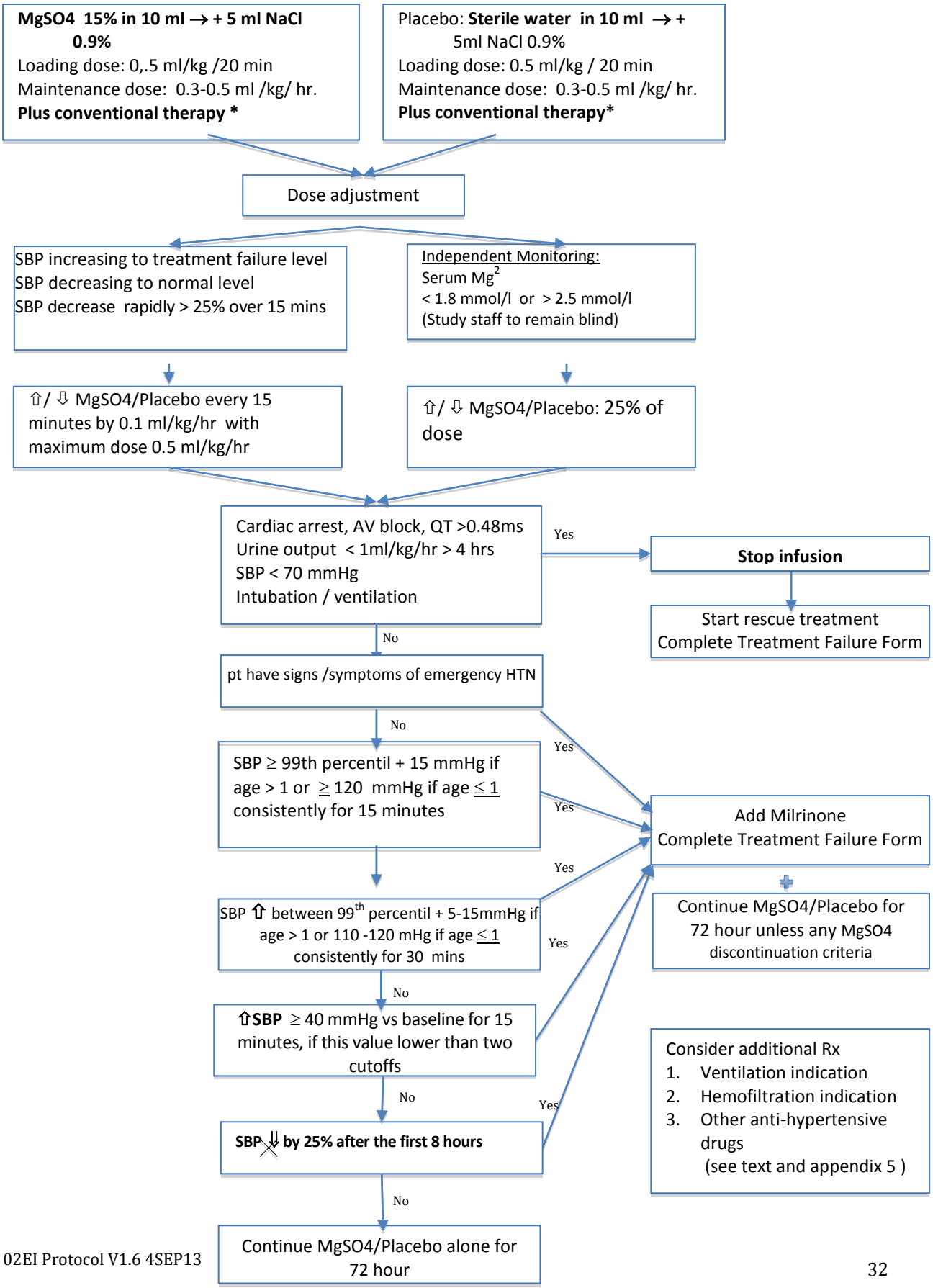


Figure 4: **TREATMENT FLOW CHART**



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APPENDIX 1: VIETNAMESE MOH HFMD CLASSIFICATION AND MANAGEMENT GUIDELINES

Classification	Signs/symptoms	Suggested Management
Grade 1	Oral ulcers and/or vesicular rash on the hands, feet, and/or the buttocks	Outpatient care, with advice sheet for family Careful observation for warning signs
Grade 2a	Grade 1 AND • Myoclonic jerk, but only observed by the family (not witnessed by medical staff) • Legarthy, agitation/irritability • Fever ≥ 39°C or ≥ 48 hours • Vomiting	Hospitalization Oral phenobarbitone
Grade 2b - Group 1	Grade 1 AND • myoclonic jerks witnessed by medical staff or by the family (≥ 2 jerks /30 minutes or 1 jerk and stupor) • Resting pulse rate > 130/min but <150/min (adjusted for fever*)	Admit to HDU/PCIU IV Phenobarbitone Antipyretics Vital Signs Monitoring: every 1-3 hours for ≥ 6 hrs
Grade 2b - Group2	Grade 1 AND myoclonic jerks accompanied by one of the following findings: • Continuous limb tremor, limb weakness or paralysis, or drowsiness (provided no hypoglycemia) • Resting pulse rate > 150 /min (adjusted for fever*) • Fever ≥ $39^0$5C (rectal T^0) and unresponsive to antipyretics over 4 hours	Give oxygen IV Phenobarbitone Antipyretics Start IVIG – 2g/kg in two divided doses Check: FBC, CRP, blood sugar, and consider lumbar puncture Vital Signs Monitoring: every 1-3 hours for ≥ 6hrs
Grade 3	Serious complications in CNS or cardiopulmonary systems: • Pulse >170 /min* • Profuse sweating • Hypertension (SBP > 95^{th} percentile for	Oxygenation Consider need for ventilation IV Phenobarbitone IVIG (as for Grade 2b) Milrinone if systolic blood pressure > 99^{th}

	age, approximately)
	• Respiratory abnormalities
	  ○ Tachypnea
	  ○ Labored breathing
	• Seizures
	• Coma (Glasgow coma score < 10)
		percentile for age + 5 mm Hg (approximately)
		Dobutamine if HR> 170 beats/minute
		Consider additional fever control measures
		Invasive blood pressure monitoring
		Check: FBC, CRP, blood sugar, and consider lumbar Puncture
		Vital signs monitoring: every 30-60 mins for ≥ 6 hrs

Grade 4	Severe complications: • Acute pulmonary edema • Cardiac collapse • SpO2 < 92% with cannula oxygen 6 litres/min) • Respiratory arrest or gasp	Intubation and ventilation Dobutamine IV Phenobarbitone Fluid challenge Antipyretics Access CVP Invasive blood pressure monitoring Vital signs monitoring: every 15-30 mins for ≥6 hrs

Note: *: heart rate is adjusted down by 10 beats/min for each 1 degree Celsius above 37^{o}C

APPENDIX 2: DEFINITIONS FOR HYPERTENSION IN THE STUDY POPULATION

Hypertension in Children

The following definitions for hypertension in children are taken from the 2004 (US) national high blood pressure education program working group (NHBPEP) [37]. All percentiles refer to the relevant value for age, gender and length.

- Normal BP — both systolic and diastolic BP <90th percentile
- Prehypertension — systolic and/or diastolic BP ≥ 90th percentile but <95th percentile or if BP exceeds 120/80 mmHg (even if <90th percentile).
- **Hypertension:**
    - Stage 1 HTN — systolic and/or diastolic BP between the 95th percentile and 5 mmHg above the 99th percentile.
    - Stage 2 HTN — systolic and/or diastolic BP **>** 99th percentile plus 5 mmHg.
    - Hypertensive emergency: A severe symptomatic elevation in BP (> 30% compared to baseline blood pressure) WITH evidence of acute target organ damage defines a hypertensive emergency
        - Brain (seizures, increased intracranial pressure)
        - Kidneys (renal insufficiency)
        - Eyes (papilledema, retinal hemorrhages, exudates)
        - Heart (heart failure)

Hypertension in infants (6-12 months) [38]:

Because there are no normative data that describe 95th percentile BP values for infants less than one year of age, and the blood pressure is almost unchanged in infants from 6 months to 12 months, the following thresholds will be used to identify hypertension in infants:

- An invasive blood pressure measurement of >100/60 will be taken as indicating Stage 1 hypertension. (An oscillometric awake BP of > 100/60 is accepted as the level at which follow-up BP monitoring and an evaluation for an underlying cause for elevated BP is usually recommended).
- An invasive blood pressure measurement that is persistently **≥** 110/65 will be considered as Stage 2 hypertension. (Treatment is generally initiated for BP persistently **≥** 110/65, or sooner if left ventricular hypertrophy is present).

BP Levels for Girls by Age and Height Percentile

Age, y	BP Percentile	SBP, mm Hg							DBP, mm Hg						
			Percentile of Height						Percentile of Height						
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
1	50th	83	84	85	86	88	89	90	38	39	39	40	41	41	42
	90th	97	97	98	100	101	102	103	52	53	53	54	55	55	56
	95th	100	101	102	104	105	106	107	56	57	57	58	59	59	60
	99th	108	108	109	111	112	113	114	64	64	65	65	66	67	67
2	50th	85	85	87	88	89	91	91	43	44	44	45	46	46	47
	90th	98	99	100	101	103	104	105	57	58	58	59	60	61	61
	95th	102	103	104	105	107	108	109	61	62	62	63	64	65	65
	99th	109	110	111	112	114	115	116	69	69	70	70	71	72	72
3	50th	86	87	88	89	91	92	93	47	48	48	49	50	50	51
	90th	100	100	102	103	104	106	106	61	62	62	63	64	64	65
	95th	104	104	105	107	108	109	110	65	66	66	67	68	68	69
	99th	111	111	113	114	115	116	117	73	73	74	74	75	76	76
4	50th	88	88	90	91	92	94	94	50	50	51	52	52	53	54
	90th	101	102	103	104	106	107	108	64	64	65	66	67	67	68
	95th	105	106	107	108	110	111	112	68	68	69	70	71	71	72
	99th	112	113	114	115	117	118	119	76	76	76	77	78	79	79
5	50th	89	90	91	93	94	95	96	52	53	53	54	55	55	56
	90th	103	103	105	106	107	109	109	66	67	67	68	69	69	70
	95th	107	107	108	110	111	112	113	70	71	71	72	73	73	74
	99th	114	114	116	117	118	120	120	78	78	79	79	80	81	81
6	50th	91	92	93	94	96	97	98	54	54	55	56	56	57	58
	90th	104	105	106	108	109	110	111	68	68	69	70	70	71	72
	95th	108	109	110	111	113	114	115	72	72	73	74	74	75	76
	99th	115	116	117	119	120	121	122	80	80	80	81	82	83	83
7	50th	93	93	95	96	97	99	99	55	56	56	57	58	58	59
	90th	106	107	108	109	111	112	113	69	70	70	71	72	72	73
	95th	110	111	112	113	115	116	116	73	74	74	75	76	76	77
	99th	117	118	119	120	122	123	124	81	81	82	82	83	84	84
8	50th	95	95	96	98	99	100	101	57	57	57	58	59	60	60
	90th	108	109	110	111	113	114	114	71	71	71	72	73	74	74
	95th	112	112	114	115	116	118	118	75	75	75	76	77	78	78
	99th	119	120	121	122	123	125	125	82	82	83	83	84	85	86
9	50th	96	97	98	100	101	102	103	58	58	58	59	60	61	61
	90th	110	110	112	113	114	116	116	72	72	72	73	74	75	75
	95th	114	114	115	117	118	119	120	76	76	76	77	78	79	79
	99th	121	121	123	124	125	127	127	83	83	84	84	85	86	87
10	50th	98	99	100	102	103	104	105	59	59	59	60	61	62	62
	90th	112	112	114	115	116	118	118	73	73	73	74	75	76	76
	95th	116	116	117	119	120	121	122	77	77	77	78	79	80	80
	99th	123	123	125	126	127	129	129	84	84	85	86	86	87	88
11	50th	100	101	102	103	105	106	107	60	60	60	61	62	63	63
	90th	114	114	116	117	118	119	120	74	74	74	75	76	77	77
	95th	118	118	119	121	122	123	124	78	78	78	79	80	81	81
	99th	125	125	126	128	129	130	131	85	85	86	87	87	88	89
12	50th	102	103	104	105	107	108	109	61	61	61	62	63	64	64
	90th	116	116	117	119	120	121	122	75	75	75	76	77	78	78
	95th	119	120	121	123	124	125	126	79	79	79	80	81	82	82
	99th	127	127	128	130	131	132	133	86	86	87	88	88	89	90
13	50th	104	105	106	107	109	110	110	62	62	62	63	64	65	65
	90th	117	118	119	121	122	123	124	76	76	76	77	78	79	79
	95th	121	122	123	124	126	127	128	80	80	80	81	82	83	83
	99th	128	129	130	132	133	134	135	87	87	88	89	89	90	91
14	50th	106	106	107	109	110	111	112	63	63	63	64	65	66	66
	90th	119	120	121	122	124	125	125	77	77	77	78	79	80	80
	95th	123	123	125	126	127	129	129	81	81	81	82	83	84	84
	99th	130	131	132	133	135	136	136	88	88	89	90	90	91	92
15	50th	107	108	109	110	111	113	113	64	64	64	65	66	67	67
	90th	120	121	122	123	125	126	127	78	78	78	79	80	81	81
	95th	124	125	126	127	129	130	131	82	82	82	83	84	85	85
	99th	131	132	133	134	136	137	138	89	89	90	91	91	92	93
16	50th	108	108	110	111	112	114	114	64	64	65	66	66	67	68
	90th	121	122	123	124	126	127	128	78	78	79	80	81	81	82
	95th	125	126	127	128	130	131	132	82	82	83	84	85	85	86
	99th	132	133	134	135	137	138	139	90	90	90	91	92	93	93
17	50th	108	109	110	111	113	114	115	64	65	65	66	67	67	68
	90th	122	122	123	125	126	127	128	78	79	79	80	81	81	82
	95th	125	126	127	129	130	131	132	82	83	83	84	85	85	86
	99th	133	133	134	136	137	138	139	90	90	91	91	92	93	93

BP Levels for Boys by Age and Height Percentile

Age, y	BP Percentile	SBP, mm Hg							DBP, mm Hg						
		Percentile of Height							Percentile of Height						
		5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
1	50th	80	81	83	85	87	88	89	34	35	36	37	38	39	39
	90th	94	95	97	99	100	102	103	49	50	51	52	53	53	54
	95th	98	99	101	103	104	106	106	54	54	55	56	57	58	58
	99th	105	106	108	110	112	113	114	61	62	63	64	65	66	66
2	50th	84	85	87	88	90	92	92	39	40	41	42	43	44	44
	90th	97	99	100	102	104	105	106	54	55	56	57	58	58	59
	95th	101	102	104	106	108	109	110	59	59	60	61	62	63	63
	99th	109	110	111	113	115	117	117	66	67	68	69	70	71	71
3	50th	86	87	89	91	93	94	95	44	44	45	46	47	48	48
	90th	100	101	103	105	107	108	109	59	59	60	61	62	63	63
	95th	104	105	107	109	110	112	113	63	63	64	65	66	67	67
	99th	111	112	114	116	118	119	120	71	71	72	73	74	75	75
4	50th	88	89	91	93	95	96	97	47	48	49	50	51	51	52
	90th	102	103	105	107	109	110	111	62	63	64	65	66	66	67
	95th	106	107	109	111	112	114	115	66	67	68	69	70	71	71
	99th	113	114	116	118	120	121	122	74	75	76	77	78	78	79
5	50th	90	91	93	95	96	98	98	50	51	52	53	54	55	55
	90th	104	105	106	108	110	111	112	65	66	67	68	69	69	70
	95th	108	109	110	112	114	115	116	69	70	71	72	73	74	74
	99th	115	116	118	120	121	123	123	77	78	79	80	81	81	82
6	50th	91	92	94	96	98	99	100	53	53	54	55	56	57	57
	90th	105	106	108	110	111	113	113	68	68	69	70	71	72	72
	95th	109	110	112	114	115	117	117	72	72	73	74	75	76	76
	99th	116	117	119	121	123	124	125	80	80	81	82	83	84	84
7	50th	92	94	95	97	99	100	101	55	55	56	57	58	59	59
	90th	106	107	109	111	113	114	115	70	70	71	72	73	74	74
	95th	110	111	113	115	117	118	119	74	74	75	76	77	78	78
	99th	117	118	120	122	124	125	126	82	82	83	84	85	86	86
8	50th	94	95	97	99	100	102	102	56	57	58	59	60	60	61
	90th	107	109	110	112	114	115	116	71	72	72	73	74	75	76
	95th	111	112	114	116	118	119	120	75	76	77	78	79	79	80
	99th	119	120	122	123	125	127	127	83	84	85	86	87	87	88
9	50th	95	96	98	100	102	103	104	57	58	59	60	61	61	62
	90th	109	110	112	114	115	117	118	72	73	74	75	76	76	77
	95th	113	114	116	118	119	121	121	76	77	78	79	80	81	81
	99th	120	121	123	125	127	128	129	84	85	86	87	88	88	89
10	50th	97	98	100	102	103	105	106	58	59	60	61	61	62	63
	90th	111	112	114	115	117	119	119	73	73	74	75	76	77	78
	95th	115	116	117	119	121	122	123	77	78	79	80	81	81	82
	99th	122	123	125	127	128	130	130	85	86	86	88	88	89	90
11	50th	99	100	102	104	105	107	107	59	59	60	61	62	63	63
	90th	113	114	115	117	119	120	121	74	74	75	76	77	78	78
	95th	117	118	119	121	123	124	125	78	78	79	80	81	82	82
	99th	124	125	127	129	130	132	132	86	86	87	88	89	90	90
12	50th	101	102	104	106	108	109	110	59	60	61	62	63	63	64
	90th	115	116	118	120	121	123	123	74	75	75	76	77	78	79
	95th	119	120	122	123	125	127	127	78	79	80	81	82	82	83
	99th	126	127	129	131	133	134	135	86	87	88	89	90	90	91
13	50th	104	105	106	108	110	111	112	60	60	61	62	63	64	64
	90th	117	118	120	122	124	125	126	75	75	76	77	78	79	79
	95th	121	122	124	126	128	129	130	79	79	80	81	82	83	83
	99th	128	130	131	133	135	136	137	87	87	88	89	90	91	91
14	50th	106	107	109	111	113	114	115	60	61	62	63	64	65	65
	90th	120	121	123	125	126	128	128	75	76	77	78	79	79	80
	95th	124	125	127	128	130	132	132	80	80	81	82	83	84	84
	99th	131	132	134	136	138	139	140	87	88	89	90	91	92	92
15	50th	109	110	112	113	115	117	117	61	62	63	64	65	66	66
	90th	122	124	125	127	129	130	131	76	77	78	79	80	80	81
	95th	126	127	129	131	133	134	135	81	81	82	83	84	85	85
	99th	134	135	136	138	140	142	142	88	89	90	91	92	93	93
16	50th	111	112	114	116	118	119	120	63	63	64	65	66	67	67
	90th	125	126	128	130	131	133	134	78	78	79	80	81	82	82
	95th	129	130	132	134	135	137	137	82	83	83	84	85	86	87
	99th	136	137	139	141	143	144	145	90	90	91	92	93	94	94
17	50th	114	115	116	118	120	121	122	65	66	66	67	68	69	70
	90th	127	128	130	132	134	135	136	80	80	81	82	83	84	84
	95th	131	132	134	136	138	139	140	84	85	86	87	87	88	89
	99th	139	140	141	143	145	146	147	92	93	93	94	95	96	97

APPENDIX 3: ADDITIONAL STUDY DEFINITIONS

Autonomic dysfunction is defined by WHO as a combination of the following features: heart rate of 150-170 beats/min, systolic blood pressure variability with absolute values higher than the 95th percentile for age, gender and height, profuse sweating, mottled skin, respiratory abnormalities, and hyperglycemia.

For this study we will use the following definitions for the inclusion/exclusion criteria:-

Tachypnea: respiratory rate

- 6 – 12 months $\geq$ 50
- 13 – 72 months $\geq$ 40
- 6 – 12 years $\geq$ 30
- 13 – 15 years $\geq$ 25

Refractory Fever: Core temperature > 40^{o}C for at least 4 hours despite antipyretics

Hyperglycemia: >150 mg/dl (8.3 mmol/l) on a random test [39], or >126 mg/dl (7 mmol/l) on a fasting test (at least 4 hours after feeding).

Respiratory distress: if the patient has any one of the following findings:-

- Tachypnea
- Irregular breathing
- Wheeze
- Stridor
- Cheyne-Stokes breathing
- Gasp
- Apnoeic episodes

Cardiac arrhythmia:

- Sinus tachycardia is not an exclusion criterion, but any other cardiac arrhythmia is, including AV block (Grades I-III) or QT prolonged >0.48 ms

Acute Renal failure:

- Serum creatinine > 2mg/dl (176 μ mol/l) or urine output < 1ml/kg/hr for 4 hours or more

Prolongation of existing hospitalization: duration of hospitalization > 14 days

APPENDIX 4: MAGNESIUM SULFATE BACKGROUND INFORMATION

PHARMACOLOGY:

Magnesium is important as a cofactor in many enzymatic reactions in the body. There are at least 300 enzymes that are dependent upon magnesium for normal functioning. Actions on lipoprotein lipase have been found to be important in reducing serum cholesterol. Magnesium is necessary for the maintaining of serum potassium and calcium levels due to its effect on the renal tubule. In the heart, magnesium acts as a calcium channel blocker. It also activates sodium potassium ATPase in the cell membrane to promote resting polarization and produce arrhythmias. Magnesium prevents premature labor by inhibiting myometrium contractions. In the CNS, magnesium prevents or controls seizures by blocking neuromuscular transmission and decreasing the amount of acetylcholine liberated at the end-plate by the motor nerve impulse. It also has a depressant effect on the CNS.

SAFETY IN CLINICAL PRACTICE

Although there are few formal research studies in young children magnesium sulphate is generally considered to be safe, even in neonates. A randomised trial that compared magnesium sulphate with placebo for women with pre-eclampsia found that exposure to MgSO4 during labor did not effect long term morbidity or mortality among the 827 children involved in the study; neonatal outcomes were similar in the MgSO4 and control patients[25]. In a Cochrane review of four studies using $MgSO_4$ for persistent pulmonary hypertension of the newborn (loading dose of 200 mg/kg MgSO4, followed by a continuous infusion of 20 to 150 mg/kg/hour lasting for 72 hours), among 40 term infants treated, no adverse events were reported except for transient bradycardia responsive to dobutamine in one of the studies. [40]. Finally, in a meta-analysis of 5 randomised controlled trials assessing use of intravenous $MgSO_4$ for treating acute asthma (182 children, doses ranging from 25 mg/kg to 75 mg/kg), the treatment was well tolerated and only minor side effects were reported, such as epigastric or facial warmth, flushing, pain and numbness at infusion site, dry mouth, and malaise [41].

DOSAGE:

In a randomized trial in neonates focused on management of pulmonary hypertension the following doses were used: A loading dose of 200 mg/kg MgSO4 diluted to 10% in sterile water was given intravenously over 20 minutes, followed by a continuous infusion of 20 to 150 mg/kg/hour lasting 72 hours, aiming to obtain a concentration of serum magnesium from 3.5 to 5.5 mmol/l [22]

In a randomized trial in adults with severe tetanus: A loading dose of 2g/hour MgSO4 diluted to 10% in sterile water was given intravenously over 30 minutes, followed by a continuous infusion of 40 mg/kg/hour, lasting up to 7 days, aiming for serum magnesium levels of 2 to 4 mmol/l [18]

In severe exacerbations of asthma the following regimen is recommended by the Royal Children's Hospital in Melbourne: A loading dose of 50 mg/kg MgSO4 diluted to 10% in sterile water given intravenously over 20 minutes, followed by a continuous infusion of 30 mg/kg/hour lasting 24 -48 hours [21]

SIDE EFFECTS [36, 42]:

Adverse effects with magnesium therapy are primarily related to the serum magnesium level. The approximate relation between clinical manifestations and the degree of hypermagnesemia can be summarized as follows:

- Plasma Mg concentration > 1.25 mmol/l – impaired peripheral neuromuscular transmission leading to anticonvulsant effects
- Plasma Mg concentration 2 to 3 mmol/L – nausea, flushing, headache, lethargy, drowsiness, and diminished deep tendon reflexes.
- Plasma Mg concentration 3 to 5 mmol/L – somnolence, hypocalcemia, absent deep tendon reflexes, hypotension, bradycardia, and ECG changes.
- Plasma Mg concentration above 5 mmol/L – muscle paralysis, respiratory paralysis, complete heart block, and cardiac arrest. In most cases, respiratory failure precedes cardiac collapse.

Other recognised effects include:

1. Cardiovascular: Although a Mg level > 5 mmol/l may precipitate cardiac arrhythmias, magnesium is also used as a treatment for certain rhythm disturbances – such as irregular/polymorphic VT with normal baseline QT interval
2. Gastrointestinal: Abdominal cramps, diarrhea, gas formation

DOSING ADJUSTMENT IN RENAL IMPAIRMENT:

Patients in severe renal failure (creatinine clearance below 10ml/minute) should not receive magnesium due to toxicity as a result of accumulation. Patients with a creatinine clearance of <25 mL/minute receiving magnesium should have serum magnesium levels carefully monitored.

Appendix 5: SUGGESTED INDICATIONS FOR SPECIFIC INTERVENTIONS FOLLOWING VIETNAMESE MOH GUIDELINES:

Ventilation criteria:

- If a patient continues to display any of the following criteria despite oxygenation via nasal cannula and cardiac support with inotropic drugs for more than 60 minutes.
    - Labored breathing
    - Tachypnea with resting respiratory rate > 70 / minute without fever
    - Hypoxemia and/or fluctuating SpO2
    - Poor tissue perfusion and persistent resting HR > 180 beats/minute without fever
- Or
    - Decorticate or decerebrate rigidity
    - Coma (GCS < 10)

Hemofiltration indication criteria:

- Acute renal failure and one of the following:
    - Severe respiratory distress: Ventilation with FiO_2 > 60%, inspiratory pressure > 25 cmH_2O and PEEP > 10 cm H_2O
    - Unstable hemodynamic status despite intensive resuscitation for 3 hours
    - Coagulopathy (INR> 1,5)
    - Acute hepatic failure
    - GCS < 10

Or

- Ventilated patients with one or more of the following
    - Coma and refractory fever
    - Coma and refractory shock (shock status not improved after two hours of intensive resuscitation)
    - Heart failure or a positive Troponin I

APPENDIX 6: RESCUE TREATMENT GUIDELINES

Cardiac arrhythmia and/or prolonged QT interval:

When plasma Mg level is high, usually above 5 mmol/l, this may result in prolongation of the QT interval and this could precipitate an arrhythmia. An ECG will be done at baseline and then daily and the QT interval will be measured as a routine. In addition if any arrhythmia is noted on the cardiac monitor the following actions will be taken.

- Assess the patient clinically, perform a full ECG, check plasma Mg and Ca level and discuss the clinical situation with the site PI.
- If the QT interval is above 480 mms, the study drug will be stopped immediately.
- Any other serious cardiac arrhythmia will be managed according to APLS guidelines
- The results of the Mg/Ca levels will go to the Mg/Ca resultmonitoring doctor for review. He/she will discuss the situation with the treating doctor. If the clinician has any clinical concerns the Mg/Ca monitoring doctor will release the blood results so that the treating doctor can take any necessary action immediately. However, if the treating doctor and the site PI consider that the arrhythmia is minor (e.g occasional SVE's) and there are no other clinical issues of concern, then the blood results will not be released. The Mg/Ca monitoring doctor may recommend adjustments to the study drug infusion according to the blood test results, but sham adjustments will also be made to the placebo arm to maintain blinding.
- However, if the calcium level < 0.9 mmol/l, the Mg/Ca monitoring doctor will inform the treating doctor of this result specifically so that a rescue dose of 0.5ml/kg of calcium gluconate 10% can be given.

Hypotension*:*

Development of hypotension could be the natural progression of severe disease or a side effect of MgS04, especially if the plasma Mg level is above 3 mmol/l. If hypotension occurs, defined as a drop in systolic blood pressure to below 70mmHg + 2 X the age in years lasting for 15 minutes, the follow actions will be taken:

- Study drug will be stopped immediately
- Assess the patient clinically, check plasma Mg/Ca levels and creatinine, and discuss the clinical situation with the site PI
- Start with a fluid challenge of 5ml/kg of Lactate Ringers or NaCl 0.9% over 15 mins

- Access the central venous pressure (CVP) and titrate the rate of fluid infusion based on CVP measurements and the clinical response.
- Start inotropes or vasopressors (such as dobutamine, noradrenaline or adrenaline), and continue with interventions following the Vietnamese MOH guideline.
- The results of the Mg/Ca levels will go directly to the ward clinicians. If the calcium level is < 0.9 mmol/l, regardless of the plasma Mg level, a rescue dose of 0.5ml/kg of calcium gluconate 10% will be given.
- The plasma Mg/Ca results may unblind the clinical team to the randomization arm and the fact that an urgent level has been reported to the ward doctors will be recorded in the CRF.
- If the creatinine level is increasing and the treating doctor suspects a diagnosis of acute renal failure, there may be a risk of toxicity due to Mg accumulation. Hemofiltration will be commenced according to MOH guidelines regardless of the plasma Mg level.

Urine output < 1ml/kg/hr:

If the urine output is < 1ml/kg/ hr over 4 hours the following actions will be taken:

- A bedside ultrasound will be performed to check whether there is any urine in the bladder
- A urethral catheter will be inserted to monitor the urine output closely, and the plasma creatinine will be checked urgently
- If the creatinine has increased to twice the baseline value, the study drug will be stopped and hemofiltration/dialysis will be performed according to the MOH guidelines regardless of the plasma Mg level.
- If the creatinine is in the normal range but the bladder is empty, then the urine output will be monitored closely for 4 hours more. If the urine output is increasing during this time the study drug will be continued, but if there is no improvement the study drug will be stopped and hemofiltration/dialysis will be performed according to the MOH guidelines regardless of the plasma Mg level.
- If the creatinine is in the normal range and there is urine in bladder, the urine output will be monitoring hourly for at least the next 24 hours

Cardiac arrest:

In the event of a cardiac arrest the following actions will be taken:

- Stop study drug infusion immediately

- Emergency resuscitation will be started immediately according to APLS guidelines
- Plasma Mg and Ca levels will be checked urgently and the results will be returned directly to the ward clinicians.
- A rescue dose of 0.5ml/kg of calcium gluconate 10% will be given while awaiting the results if other attempts including CPR and conventional resuscitation drugs fail to restore sinus rhythm and effective cardiac output.
- The plasma Mg/Ca results may unblind the clinical team to the randomization arm and the fact that an urgent level has been reported to the ward doctors will be recorded in the CRF.
- Further intensive intervention will be done according to the Vietnamese MOH guidelines for HFMD management and APLS resuscitation guidelines.

Respiratory muscle weakness:

If the patient develops new or worsening signs and symptoms of respiratory distress the follow actions will be taken:

- Assess the patient clinically including deep tendon reflexes, check the plasma Mg and Ca levels and an arterial blood gas and discuss the clinical situation with the site PI.
- If the patient meets the MOH ventilation criteria as indicated above, (or the treating doctor thinks that the patient needs to be intubated for any reason) the study drug will be stopped immediately,
    - The results of the Mg/Ca levels will go directly to the ward clinicians. If the calcium level is < 0.9 mmol/l, regardless of the plasma Mg level, a rescue dose of 0.5ml/kg of calcium gluconate 10% will be given.
    - The plasma Mg/Ca results may unblind the clinical team to the randomization arm and the fact that an urgent level has been reported to the ward doctors will be recorded in the CRF.
- If the patient does not meet the MOH ventilation criteria and the treating doctor and site PI agree that immediate intervention/respiratory support is not needed the patient will be observed closely for at least 60 minutes.
    - The results of the Mg/Ca levels will go to the Mg/Ca monitoring doctor for review as soon as possible. If the Mg level is above 2.5 mmol/l but below 3 mmol/l, the treating doctor will be informed to reduce the infusion dose as described in the protocol. Similar sham adjustments can also be made to the placebo arm to maintain blinding.

- If the Mg level is 3 mmol/l or above, the Mg/Ca monitoring doctor will release the result and inform the study doctor to stop the study drug infusion.
- If the Ca level is < 0.9 mmol/l the treating doctor will be informed so that a rescue dose of 0.5ml/kg of calcium gluconate 10% can be given if appropriate.
- If the patient subsequently meets the criteria for intubation, or the $PaCO_2$ rises >45 mmHg, the intubation steps described above will be acted upon.

- Any further interventions will be performed according to the Vietnamese MOH guidelines for HFMD management

Appendix 7: STUDY SCHEDULE FOR ALL PLANNED INVESTIGATION

	Screening	Enrolment[a] / D 1				D 2	D 3	D 4	D 5+	Disccharge	Disch	M6
Hour from admission	T-x	T-1	T(-1/2)	T0	T12	T24	T48	T72	T96+			
Clinical activities												
Screening assessment												
Informed consent												
History and physical assessment												
Randomization assign and study drug												
Study drug administration												
Patient assessment									b	c	(c)	c
Recording/ downloading hemodynamic data		d	d	d/e	d/e	d/e	d/e	d/e				
Research blood test												
Chemistries		0.5 ml		1.5 ml	1 ml	2 ml	2 ml	2 ml				
Specialized additional research blood test												
Serum catecholamine				2 ml		2 ml	2ml	2 ml				
Cytokine profile				1 ml	1 ml	1 ml				1 ml		
Serology	1 ml									1 ml		
Research procedures												
ECG recording[f]												
Arterial catheterization												
Blood draw												
Diagnosis swabs												
Additional procedure												
Brain MRI									Just in stable			
Urine-Research Laboratory												
Urine Catecholamine												
Total amount of blood	1 ml	7 ml				5 ml	4 ml	4 ml		2 ml		

Note that additional clinical investigation will be performed as necessary as indication

Note: a – Enrollment stage should be done as fast as possible: 30 munite to take informed consent and check exclusion criteria, 30 minutes for drug administration.

b- Clinical assessment still be carry out daily until discharge

c- Neurological outcome and development will be assessed in this stage. Patients unable to be assessed at discharge will be invited to attend for assessment after 7 day.

d- Standard hemodynamic data will be recording/downloading and stored in PC

e- Advance hemodynamic data will be downloading from LiDCOrapid and stored in PC

f: Standard ECG recording will be performed daily and whenever an abnormality is noted on the monitor

g: indicated for 08RS study

Appendix 8: LABORATORY SCHEDULE (CLINICAL AND RESEARCH INVESTIGATIONS)

	D1		**D2**	**D 3**	**D4**	**Discharge or D+7 days**
(Hour)	T0	T 12	T 24	T 48	T 72	
Na, K, Mg, Ca, Cl	✓		✓	✓	✓	
Mg, Ca		✓				
Blood Gases	✓		✓	✓	✓	
Creatinine	✓		✓	✓	✓	
CK MB	✓		✓	✓	✓	
Troponin I	✓		✓	✓	✓	
Plasma Catecholamines	✓		✓	✓	✓	
Urine Catecholamines	✓		✓	✓	✓	
Cytokines	✓	✓	✓			✓
Serology	✓					✓
Amount of blood (ml)	5	2	5	4	4	2
Total blood volume	7 ml		5 ml	4 ml	4 ml	2 ml

The amount of blood taken for the various tests is as follows:

Electrolytes (including Mg, Ca), Creatinine, CKMB, Troponin I, and Glucose: 1,5 mls

Plasma Mg/Ca alone: 1 ml

ABG: 0.5 ml

Plasma catecholamines: 2 mls

Cytokine, serology – 1-2 ml

Blood sugar will also be checked on the ward 6 hourly on a drop of blood from the arterial line

Appendix 9: Viet Nam Ministry of Health Treatment Guidelines for Hand, Foot and Mouth Disease

Full guidelines available in Vietnamese only at www.kcb.vn.
Summary in English is in Appendix 1 and 5.