Title of the proposed research: Intravenous methylene blue for treating fluid-refractory, catecholamine-resistant, neonatal septic shock: a randomized, placebo-controlled, superiority trial

Summary

Rationale: Septic shock is associated with high mortality. Methylene blue (MB) inhibits guanylate cyclase and inducible nitric oxide synthase, promoting vasoconstriction. Thus, there may be a role of MB in treating septic shock, but there are very few studies in any age group on the same.

Gaps in existing knowledge: There are only a few randomized controlled trials (RCT) on MB among adults, none in post-neonatal children, and one small RCT among neonates (n=33). The trial on preterm neonates compared MB versus terlipressin in catecholamine-resistant septic shock. There are no RCTs evaluating MB versus no MB in neonatal shock, especially at relatively earlier stages of septic shock.

Novelty: Ours will be the first adequately-powered RCT comparing MB and no MB in catecholamine-resistant neonatal septic shock, with all-cause mortality as the primary outcome.

Objectives: To compare MB versus no MB among preterm neonates with septic shock with all-cause mortality as the primary outcome and other parameters of septic shock as secondary outcomes.

Methods: Preterm infants with definite or probable sepsis and fluid-refractory, catecholamine-resistant septic shock will be eligible for enrolment if they have no contraindication to receive MB. After obtaining parental consent, they will be randomly allocated to receive MB (bolus followed by infusion) or no MB for 24 hours. They will be observed for all-cause mortality (primary outcome), cause-specific mortality, time to achieve hemodynamic stability and adverse effects (secondary outcomes) over a 7-day period.

Expected Outcomes: MB is expected to decrease mortality associated with neonatal septic shock.

Hypothesis/ Research question

- Among preterm neonates with septic shock that is refractory to fluids and first-line catecholamines (P), intravenous MB (1 mg/kg bolus followed by an infusion of 0.15-0.5 mg/kg/hour over 24 hours) in addition to standard fluid, hydrocortisone and second-line catecholamines (I) compared to standard fluid, hydrocortisone and second-line catecholamines alone (C) reduces all-cause mortality (O) over 7 days (T) from 70% to 45% (E) in a randomized controlled trial (S).

Study Objectives

- **Primary objective**
- To determine whether treatment with intravenous MB therapy reduces all-cause mortality when compared to no MB treatment, among preterm neonates with catecholamine-resistant septic shock
- **Secondary objectives:**

1. To compare the time to achieve therapeutic endpoints among preterm neonates with catecholamine-resistant septic shock treated with intravenous MB versus no MB

2. To compare time to stoppage of all inotrope/vasopressor treatment among preterm neonates with catecholamine-resistant septic shock treated with intravenous MB versus no MB

3. To compare echocardiographic parameters (at 24 hours after randomization) among preterm neonates with catecholamine-resistant septic shock treated with intravenous MB versus no MB

Methodology:

Primary objective #1

a. Study design

Single-center, two-arm, randomized, controlled, blinded, superiority trial.

- **Screening Criteria:** preterm infants (<37 weeks) clinically diagnosed to have septic shock will be screened for inclusion
- **Inclusion criteria:** subjects must fulfill all the following:

1. Definite/probable sepsis: Clinical syndrome of sepsis for which bedside neonatologist starts intravenous antibiotics AND either a positive culture of otherwise sterile body fluid OR presence of any 2 or more of the following five markers of sepsis: (a) C-reactive protein >10 mg/dL; (b) procalcitonin as per age-appropriate cut-off (c) total leukocyte count and absolute neutrophilic count beyond acceptable range 25 chest X-ray adjudged as pneumonia by two independent Neonatologists.

2. Shock: adapted from the definition given by Davis et al

(a) Either SBP < age and gestation appropriate cut-off OR

(b) Presence of any 2 of the following 6 parameters

i. HR >205/min

ii. Central pulses either week OR bounding

iii. CRT ≥3 sec OR flash refill (<1 sec)

iv. Skin mottled/cool OR flushed

v. urine output <0.5 ml/kg/h in the preceding 6 hours

vi. DBP < age and gestation appropriate cut-off

3. Fluid and catecholamine-resistant shock: received fluid boluses up to a maximum of 40 ml/kg followed by catecholamine infusion titrated up to the maximum dose. The catecholamine infusion could be either dopamine (maximum dose 20 µg/kg/min) or

epinephrine (maximum dose 0.4 µg/kg/min) or norepinephrine (maximum dose 0.4 µg/kg/min).

- **Exclusion criteria:** excluded if ≥1 criteria positive
  - G6PD deficient or family history of G6PD deficiency
  - potentially lethal malformation
  - congenital heart disease
  - severe acute kidney injury
  - family history of allergy to methylene blue or food dyes
- **Informed consent and enrolment:** Neonates will enrolled after written informed parental consent.

b. Study site: Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh**.** It has a catchment area that includes Chandigarh, Punjab, Haryana, Himachal Pradesh, Jammu and Kashmir, Western Uttar Pradesh, and parts of Rajasthan.

c. Methods:

- The research question is whether, among preterm neonates with septic shock refractory to fluids and first-line catecholamines (P), intravenous MB with standard fluid, hydrocortisone, and second-line catecholamine therapy (I) compared to standard fluid, hydrocortisone, and second-line catecholamine therapy alone (C) reduces all-cause mortality (O).
- **Baseline variables:** identification and demographic variables, sepsis- and shock-related clinical and laboratory variables
- **Randomization:** Enrolled subjects will be randomly assigned in 1:1 ratio to receive MB or no MB using a stratified block-randomized design. Stratification will be by the type of first-line catecholamine infusion. Permuted, randomly varying, even-numbered blocks will be used. Randomization sequence will be generated from a website.
- **Allocation concealment**: Serially-numbered, opaque, sealed envelopes will be used.
- **Blinding:** After opening the envelope, a research nurse will administer a bolus of the study drug or placebo behind a screen, shielded from other staff. The infusion will be drawn up in an opaque syringe and infused through an intravenous tubing covered completely with aluminum foil.
- **Intervention:** Subjects in the intervention arm will receive a 1 mg/kg bolus of MB over 30 minutes, followed by an infusion of 0.15 mg/kg/h. The infusion rate may be increased in steps of 0.15 mg/kg/h every 30 minutes until a maximum of 0.5 mg/kg/h. Subjects in the control arm will receive a placebo infusion (normal saline) at the same volumetric rate.
- **Co-interventions:** Standard fluid, hydrocortisone and second-line catecholamine treatment will be provided to both arms. Hydrocortisone will be administered at a dose of 1 mg/kg/dose every 8 hours for 48 hours followed by 1 mg/kg/dose every 12 hours for 72 hours. Subjects who had received dopamine as the 1st line catecholamine will be started on norepinephrine at 0.2 µg/kg/min. Subjects who had received either epinephrine or norepinephrine as the 1st line

catecholamine will be started on dopamine at 10 µg/kg/min. The 2nd line vasoactive drug will be hiked every 15 minutes if the therapeutic endpoints of shock are not met.

If the subject is normotensive (blood pressure > 5th percentile) but has CFT > 3 seconds with other signs of peripheral circulatory failure, dobutamine will be added at 10 µg/kg/min and will be increased by 5 µg/kg/min to a maximum dose of 20 µg/kg/min. If the subject develops signs of persistent pulmonary artery hypertension, vasopressin will be added at a dose of 0.01 U/kg/h and hiked to a maximum of 0.04 U/kg/h.

Treatment for other conditions will continue as per the unit protocols. Serum electrolytes (sodium, potassium and calcium), blood glucose and hematocrit will be maintained at normal levels.

- **Monitoring:** Vital parameters, urine output, sensorium will be monitored hourly until 7 days after randomization; echocardiographic parameters will be measured at 24 and 48 h; and neonatal Sequential Organ Failure Assessment (nSOFA) score will be assessed. Subjects will be followed until discharge/death/LAMA/transfer for recording death and duration of hospital stay.
- **Outcome of primary objective:** All-cause mortality.

- **Secondary outcomes**

  3 key secondary outcomes are mentioned under the work plan for the respective secondary objectives.

  Other secondary outcomes not covered specifically under any objective include:

  - Time taken to stop inotrope/vasopressor treatment
  - Echocardiographic parameters (at 24 and 48 h after randomization)
  - Septic shock-related mortality within 7 days after randomization
  - Duration of hospital stay
  - Mortality during hospital stay
  - Serious adverse effects, with special reference to oliguria, gastrointestinal bleeds, abdominal distension, and bluish discoloration of skin and urine.

d. Sample size: The incidence of all-cause mortalityin dopamine-resistant septic shockin our unit in 70%. 65 subjects will be required per group to reduce the incidence by 25%, assuming 1:1 allocation ratio, 80% power, 5% alpha error and 10% loss to follow-up.

e. Implementation strategy:

The research staff and the study team will be extensively trained and supervised, so as to ensure proper collection and maintenance of good quality data. The research coordinator will screen the patients as per inclusion criteria and will enroll the subjects after obtaining informed parental consent. Once the subject is enrolled, the research coordinator will inform the trained research nurse to collect the baseline data from the patient's record. Research coordinator will have daily

follow ups and will prospectively collect all relevant data from patients records, and wherever required, by interviewing the clinical staff. Any serious adverse event will be immediately communicated to all the investigators. Hard copies of Case Record Form will be kept securely by the principal investigator.

Statistical strategy: Categorical variables will be described as frequencies and percentages, normally distributed numerical variables as mean (standard deviation) and variables with skewed distributions as median (1st, 3rd quartile). Dichotomous variables will be compared between the 2 groups by chi-square test and Fisher's exact test as appropriate. Normally distributed numerical variables will be compared by unpaired student's t-test. Variables with skewed distributions will be compared by Mann-Whitney U test. The magnitude of the effect size will be expressed as unadjusted odds ratios (95% confidence interval). Multi-variable logistic regression analysis will be performed with the primary outcome as the outcome variable and the allocation group, stratum, gestational age, birth weight, and nSOFA score as the predictor variables.

f. **Ethical issue:** Intravenous MB has already been used in the neonatal age group to treat methemoglobinemia in doses higher than that proposed in the current study (for methemoglobinemia in neonates 0.3-1 mg/kg stat, repeated after 1 hour and 4 hourly thereafter, maximum cumulative dose 7 mg/kg). MB will be used only after maximal doses of dopamine ± dobutamine has been tried and failed. Standard treatment with fluids, inotropes and vasopressors will not be withheld. The trial will be started after obtaining ethical approval from the ethics committee. All amendments to the protocol will be approved by the ethics committees. Parents will be provided a trilingual information sheet with the details about the study. Neonates will be enrolled only after obtaining written informed parental consent. No extra blood samples will be drawn, and no extra invasive procedure will be performed as part of the study. Confidentiality of the data will be maintained.