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Benefits and Risks of Iron interventions in Children (BRISC): protocol for a three-arm parallel-group randomized controlled field trial in Bangladesh

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Benefits and Risks of Iron interventions in Children (BRISC): protocol for a three-arm parallel-group randomized controlled field trial in Bangladesh

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Abstract

Introduction: Anaemia is a major global health problem affecting about 43% of pre-school children globally and 60% of 6-24 months old children in rural Bangladesh, half of which is attributed to iron deficiency (ID). Although the World Health Organization (WHO) recommends universal supplementation with iron or home fortification with iron-containing multiple micronutrient powders (MMPs) to children under 2 years, evidence for benefits of these interventions on childhood development (a key rationale for these interventions) and harms (especially infection) remains limited. This study aims to evaluate the impact of iron or MMPs supplementation compared to placebo on a) children's development b) growth c) morbidity from infections, and d) haematologic and iron indices.

Methods and analysis: This study is a three-arm, blinded, double dummy, parallel-group, placebo controlled superiority trial using stratified individual block randomization. The trial will randomise 3300 children aged 8-9 months equally to Arm 1: iron syrup (12.5mg elemental iron), placebo MMPs; Arm 2: MMPs (including 12.5mg elemental iron), placebo syrup; and Arm 3: placebo syrup, placebo MNPs. Children will receive interventions for 3 months based on WHO recommendations and then be followed-up for 9 months post-intervention. The primary outcome is cognitive composite score measured by Bayley-III. Secondary outcomes include motor and language composite score by Bayley-III, behaviour rating using selected items from Wolke's rating scales and BSID-II behaviour ratings, temperament, growth, haemoglobin, anaemia and iron status, and infectious morbidity. Outcomes will be measured at baseline, at the end of 3-month intervention, and after 9 months post-intervention follow-up.

Ethics and dissemination: The trial has been approved by the Ethical Review Committee of icddr,b (Dhaka, Bangladesh) and the Melbourne Health Human Research Ethics Committee (Melbourne, Australia). Results of the study will be disseminated through scientific publications, presentations at international meetings, and policy briefs to key stakeholders.

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WHO Universal Trial Number U1111-1196-1125

Keywords Iron deficiency, anaemia, cognitive development, Bangladesh, randomized controlled trial

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Strengths and Limitations

- Trial design: double blind, double dummy design minimizing risk of bias in assessment of outcomes. The trial is designed to be able to compare the main interventions (iron drops and iron-containing micronutrient powders) used for anaemia control in young children against placebo.
- Outcome assessment: The tools we are using, including Bayley Scales, are the gold standard for directly measuring child development.
- Sample size: this is the largest trial to assess effects of iron interventions on child development, and as such the trial is powered to detect small but clinically relevant effect sizes.
- Trial setting: the trial is set in a low income South Asian setting where there is a high baseline prevalence of anaemia, and will exclude children at risk of high groundwater iron exposure.
- Biomarker assessment: measurement of anaemia and iron deficiency, along with growth, at baseline will facilitate subgroup analysis by baseline nutrition status.

BACKGROUND

Anaemia is highly prevalent in preschool children

Approximately 43% (up to 304 million) of under-5 children worldwide are anaemic. The number of children affected is greatest in South Asia, where the prevalence exceeds 55%.[1] The relative contribution of iron deficiency (ID) to the overall burden of anaemia varies by region. We have previously found that among rural Indian children aged 12-23 months, ID accounted for 72% of anaemia.[2] In rural Bangladesh, we found about 60% of children 6-24 months to be anaemic, with half of cases due to ID.[3] Conversely, in pre-schoolers in rural Gambia and Tanzania where malaria is endemic, ID accounted for only 20% of anaemia.[4]

Iron supplementation as a strategy for controlling anaemia in children in low-income settings

Iron supplementation involves administration of medicinal iron (usually ferrous salts).[5] Multiple micronutrient powders (MMPs) comprise single dose sachets of lipoencapsulated iron together with other micronutrients (usually at least vitamin A, zinc and folate) that can be sprinkled onto any semi-solid food, with the aim of providing a child with a recommended daily intake of micronutrients. The World Health Organization (WHO) recommends two different possible direct interventions for controlling anaemia in young children. Firstly, WHO recommends that all children aged 6-23 months, in settings where the prevalence of anaemia exceeds 40%, receive 3 months daily iron supplements.[6] Alternatively, where the prevalence of anaemia exceeds 20%, WHO recommends children 6-23 months receive 90 days home fortification with iron-containing multiple micronutrients powders (MMPs) every six months.[7] WHO does not recommend one approach over the other; their efficacy and safety have not been compared in a large head to head trial; earlier recommendations for MMPs proposed 2 months intervention every six months. Recent estimates indicate that in pre-school children, about 41% and 32% of cases of anaemia in South-East Asia and sub-Saharan Africa respectively, are responsive to iron.[8]

Adequate iron stores are important for neurological development

The prevalence of anaemia generally increases from 6 months of age and peaks in the second year of life,[9] especially if iron intake from complementary foods is inadequate to meet the demands of erythropoiesis and growth.[10 11] The peak in anaemia prevalence coincides with the critical period for neural development, sharing the same period of peak vulnerability: the ‘first 1000 days’ from conception to age 2 years.[12] Animal studies also indicate that iron is needed for

myelination and neurotransmitter synthesis, while ID alters neuronal metabolism.[13] [14]Observational studies have consistently linked anaemia in infancy to adverse short and longer term deficits in cognitive development.[15] Hence, animal data and observational studies in children suggest that ID impairs brain development.[16]

Evidence of beneficial effects of iron interventions in children at the population level

While iron interventions improve haemoglobin concentrations and iron indices and reduce the prevalence of anaemia, ID, and iron deficiency anaemia (IDA),[17-19] there are limited data from population clinical trials confirming that policies of universal iron interventions improve development, growth and health in young children.

Effects on development: Few RCTs have evaluated effects of iron supplements or MMPs on development in children under 2 years[18 20] and these trials were underpowered individually and collectively and most of the trials were in pre-selected patient groups (not populations) or were not blinded (i.e. high risk of bias) limiting the quality of evidence.[18] This paucity of available evidence has hampered systematic reviews and meta-analyses (RCTs) that have to date failed to find evidence of benefit from iron interventions (iron supplements, home fortification with MMPs, or other iron interventions) on development in young children.[21-24] Our systematic review of daily iron supplementation in children aged 4-23 months identified no significant difference in Bayley's mental development index (MDI) in children receiving iron compared with control (mean difference 1.65 [95% confidence interval -0.63, 3.94]); for psychomotor development index (PDI) the effect size was (mean difference 1.05 [-1.36, 3.46]).[18]
Systematic reviews evaluating the effects of MMPs on cognitive development did not identify RCTs that had reported effects on measures of cognitive development,[25 26] and only reported a single trial that found children receiving an intervention walked earlier than those from a parallel control group (i.e. children not included in the study at inception). More recently, a large randomized trial in Pakistan identified only transient benefits from MMPs on Bayley's cognitive, language and psychomotor development,[27] and motor development in the longer term.[28] This trial did not use placebo and was hence not adequately blinded; moreover, adherence to the supplements appeared limited and had no effect on haemoglobin concentration compared to control children.[27]

Effects on growth: Benefits on growth are often cited as a rationale for universal iron supplementation.[29] However previous systematic reviews have not found benefits on growth,

and indeed, have found that iron interventions can impair linear growth in iron-replete children.[30] Our systematic review suggested daily iron supplementation reduced length and weight gain in young children.[18] A systematic review of iron-containing MMPs found no increase in growth despite containing the growth-promoting micronutrient zinc.[20]

Evidence of harm from iron supplementation

In contrast to the lack of data on benefits, several large RCTs have reported adverse effects from iron interventions in low-income settings. This emerging data along with mechanistic studies in low-income settings are now providing convincing evidence that these interventions cause or exacerbate infection, including diarrhea, bloody diarrhea, and respiratory infections in endemic and non-endemic malaria settings.[27 31-33] For example, our meta-analysis of iron supplementation identified a 16% and 38% increased risk of fever and vomiting respectively.[18]

The need for a trial

Although immediate and long-term benefits from iron on functional outcomes such as cognitive development and growth have been assumed for decades, existing data from RCTs do not support this contention. In contrast, data for evidence of harm from iron interventions is accumulating. Furthermore, iron supplements have not been compared directly to MMPs in a large field trial. In this RCT, we aim to define the benefits and harms of daily iron supplementation and MMPs in young children, enabling evidence-based recommendations for implementation (or withdrawal) of iron interventions in this age group.

METHODS AND ANALYSIS

Benefits and risks of iron interventions in children (BRISC)

Trial objectives

The primary objective of this study is to determine if 3 months interventions with iron supplementation or home fortification with MMPs is superior to placebo on cognitive development in children aged 8 months.

The secondary objectives are to evaluate the impact of iron supplementation and home fortification with MMPs, compared with placebo, on:

- Developmental indices, i.e. cognitive (after 9 months post-intervention), and motor, language, behaviour and temperament (after 3 months intervention and 9 months post-intervention),
- Prevalence of anaemia and iron deficiency (after 3 months intervention and 9 months post-intervention), and
- Infection risks, especially diarrhoea and respiratory infection in these young children (after 3 months intervention and 9 months post-intervention).

Study design

BRISC is a three-arm; parallel; researcher, caregiver, data collector, analysts, and participant-blinded-blind; individually randomised; double-dummy placebo controlled; superiority trial. It will compare the effects of 3 months of daily i) iron supplementation, or ii) MMPs, to iii) placebo in 8 months old Bangladeshi children, with a further 9 months follow up. The trial design is summarized in Figure 1.

Study settings and participants

The trial will be conducted in Rupganj, a rural sub-district/upazila of Narayanganj district about 50km from Dhaka, in Bangladesh. Three unions (regions) within the sub-district will be included, with each union covered by a dedicated study team. A recent national survey reported the prevalence of anaemia in 9-11 months old infants at 78.7%.[34] Diarrhoea and respiratory infections remain highly endemic in Bangladesh, with 4.6% and 5.8% of children <5 years experiencing these respectively in a 2-week period. The site is non malaria-endemic and drinking water consumed by the families does not contain high iron in most instances.[35 36] The trial will have global generalizability, especially to South Asia where the prevalence of anaemia in this age group approaches 90%.[12]

Eligibility Criteria

Children will be randomised only if they fulfill all the inclusion criteria and none of the exclusion criteria.

Inclusion criteria:

1. Aged 8 months (±14 days) at the time of randomization,
2. Not expected to leave the study location for more than one week over the next 3 months, or for more than one month over the next 12 months,
3. Has a legal guardian capable of providing informed consent.

Exclusion criteria:

Children meeting any of the following criteria will be excluded from the study:

1. Capillary haemoglobin (Hb) <8.0g/dL at the time of screening.
2. Drinking water iron concentration >1mg/L.
3. Diagnosed case of any clinical haemoglobinopathy (e.g. beta-thalassaemia major, HbE-beta thalassaemia).
4. Current infective illness (i.e. respiratory infection, diarrhoea) with fever; however, children may be rescreened again after recovery if otherwise eligible.
5. Received iron supplements or iron-containing MMP in the previous month.
6. Known congenital anomaly, developmental disorder or severe developmental delay.
7. Child of multiple birth e.g. twins, triplets.

Intervention

Participants will be randomised in a 1:1:1 ratio to each of the three arms. Infants in the two active intervention arms will receive 12.5 mg daily oral iron either in syrup form or as MMPs as recommended by WHO.[37 38] Each participant will receive both a syrup (to be dispensed via a syringe at a predefined volume) and a sachet (to be sprinkled on food), achieving double dummy blinding. Iron syrup and the corresponding placebo will be manufactured in Bangladesh by ACME Laboratories. Micronutrient powders and corresponding placebo will be manufactured by Renata Ltd. Mothers/caregivers will be instructed (with demonstrations) how to administer the supplements. Participants will be asked to take one dose of each formulation daily for 3 months.

Intervention arms:

Arm 1: (Iron syrup and placebo sachet): Daily oral supplementation of 12.5 mg elemental iron syrup and a placebo sachet containing powders in identical packaging to the MMP, but containing no micronutrients.

Arm 2 (MMP sachet and placebo syrup): Daily home-fortification with an MMP sachet containing 12.5 mg Iron, 0.3 mg Vitamin A, 30 mg Vitamin C, 0.16 mg Folic Acid, and 5 mg Zinc; placebo syrup containing no iron but identical in colour and flavour.

Arm 3: (Placebo syrup and placebo sachet): Control arm.

Each participant will receive a pouch every week containing a bottle of syrup and 7 sachets.

Randomisation

Participants will be randomly allocated to one of the three arms with 1:1:1 allocation using a computer-generated schedule of randomly permuted blocks of fixed size stratified by sex and union (each covered by a different field team) to achieve balance between the arms within each stratum. The randomisation list will be prepared by an independent statistician, who will not reveal the block size. The allocation will occur by the field team according to the list, within their assigned union, once eligibility criteria have been checked.

Allocation concealment and blinding of study agents

Blinding of the team visiting the site, the caregiver(s), and participants will be achieved through the use of identical packaging of sachets and syrup regardless of their contents (active or placebo), packaged in pouches that carry an allocation code. The independent statistician will hold the allocation codes until the data base is ready for unblinding. Researchers, caregivers, persons involved with data collection (i.e. field team) or analysis will be blinded to the allocation code until the database has been finalized for analysis. Breaking of the allocation code will occur only in the case of a severe adverse event or as requested by Data Safety Monitoring Board (DSMB), in which case the code will only be disclosed to the local study physician. Emergency unblinding will lead to discontinuation of the participant's involvement in the study.

Recruitment and visits

The schedule of visits is outline in Table 1. Trained Village Health Workers (VHWs) will identify all potentially eligible children by making household visits in their designated areas and collating these data centrally, enabling generation of a list of age-specific eligible participants in each village. Based on the list, VHWs and Senior Field Assistants (SFAs) will visit potentially eligible families. After providing preliminary information to the parents/guardians, the team will obtain

their consent for screening and determine their eligibility. During screening, drinking water iron level will be measured using "HACH" Iron (Ferrous) test Kit and the child's capillary Hb level will be measured by *HemoCue*-301. Children with Hb<8.0 gm/dl will be excluded and referred to nearby health centre for management. Mothers/guardians of eligible children will be briefed further about the trial, and be invited to a selected house/test centre for enrollment.

Enrolled families will attend a designated local study site on a proscribed day for enrolment and baseline data collection. The data collection team consisting of a psychological tester, a SFA, a phlebotomist and a VHW will undertake detailed data collection. At this visit, consent for participation in the study will be signed and we will collect baseline information, administer developmental tests and interviews, take anthropometric measurements and finally, a study phlebotomist will collect 3 mL of venous blood. The child will then receive the randomly allocated intervention. Testers will provide detailed instruction regarding medication to mothers or caregivers before they leave the test centre and they will give details of the enrolled child to the assigned VHW for prospective follow-up visits. The assigned VHW will then visit the child every week for the 3 month intervention period, and every month for the 9 month post intervention period. Morbidity data will be collected weekly and monthly during the intervention and post intervention period respectively. VHWs will also record and notify any unscheduled hospital or clinic admission experienced by the participant. The number of doses missed by participants will be recorded, empty bottles and sachets will be collected and new doses for the following week dispensed at routine weekly visits.

Table 1: Overview of study visits

ACTIVITIES	**STUDY PERIOD**					
	Screening	**Baseline/ enrolment**	**Post-allocation**			**Close-out**
Time point	$-t_1$	Day 0 Visit 1	Weekly visits Day 7,14,21,28,35, 42,49,56,63,70,77 Visit 2-12	Midline 3rd+ month Visit 13	Monthly visits (post- intervention) Month 4,5,6,7,8,9,10,11 Visit 14-20	Endline 12th +months Visit 21

Enrolment:	Age approx. 8±0.5 mo					
Eligibility screen	X					
Informed consent	X	X				
Allocation		X				
Interventions:		X	X			
Socio demographic information		X				
Family Care Indicators		X		X		X
Temperament questionnaire		X		X		X
Food security questionnaire		X		X		X
Adherence and morbidity questionnaire			X	X	X	X
Bayley-III		X		X		X
Wolke's Behaviour Rating Scale		X		X		X
Anthropometry		X		X		X
Adverse events reporting (AE,SAEs)			X	X	X	
Corneal lesions assessment				X		
Venous blood collection		X		X		X
Willingness to				X		

pay questionnaire						

Other visits

Withdrawal visit

Children who stop study drug may continue with assessments if their guardian wishes. If a participant withdraws early or investigator terminates participation, we will seek to undertake the following assessments:

- Reason for study withdrawal
- If within 2 weeks of visit 13 or 21, we will invite the participant to attend to undertake this visit unless the reason for withdrawal precludes this.

Recruitment is expected to commence in July 2017 and the trial will be open for 18 months. Expected participant flow is shown in Figure 2.

Study oversight and adherence

All staff will undergo specific training unique to their role in the study. Adherence will be monitored for all participants. VHWs will measure the amount of syrup and number of sachets unused, and it will be recorded on the case record forms of each child.

Outcomes

Primary outcome

Cognitive Composite Score (CogCS) measured by Bayley Scales of Infant and Toddler Development (Bayley-III) after 3 months of intervention is the primary outcome.[39] Bayley-III is a validated index of child development and the preferred field assessment tool. It is a standard series of measurements primarily to assess cognitive, motor (fine and gross) and language (receptive and expressive) development of infants and toddlers aged 0-3 ½ yrs. Total number of credited items is converted into scaled scores based on child's age, which are then converted to composite scores of each subscale. These scores are used to determine the child's performance compared with norms taken from typically developing children of their age in developed countries. Bayley-II has been adapted and extensively used on Bangladeshi children.[40-42] Bayley-III has now been adapted, with some components not familiar for the rural and urban children of this country changed according to the local context. It has been used in

several studies in this population.[43] Bayley testers will be certified and permitted to collect data only when their results agree >90% with the gold standard i.e. the trainer. About 5-10% of the tests by each tester will be observed by the trainer for inter-tester reliability over the course of the study.

Secondary outcomes

Development: CogCS at the end of 9 months post intervention, motor and language composite scores by Bayley-III, behaviour rating on selected items from Wolke's rating scales and BSID-II behaviour ratings, temperament by using a modified version of Bates, quality of home stimulation by using family care indicators, and food insecurity by household food insecurity access scale will be measured immediately after intervention and post intervention follow-up.[44-47]

Physical growth: will be measured as length, weight, head circumference at end of intervention and post follow-up period. Length of the child will be measured to the nearest 0.1 cm by using the Shorr stadiometer (Shorr Products), which has been previously validated and used on local population. Weights will be obtained using a battery-powered digital scale (Tanita HD-318). Length and weight will be used to develop indicators of stunting and wasting compared to age-sex specific WHO international reference growth standards.[48] Measurements will be taken in duplicate with the average taken unless substantial discrepancy occurs. Presence of corneal lesions (caused by vitamin A deficiency) will be assessed by the field team at the midline visit.

Infectious morbidity: as rate and number of days affected by diarrhea/ bloody diarrhea (along with number of episodes per day), respiratory infection, vomiting, and fever during intervention and post intervention follow up period. Morbidity information will be collected by VHWs during routine weekly or monthly visits by interviewing caregivers.

Unplanned hospital or health-care facility attendance: as rate, will be measured by field workers along with morbidity questionnaires. Cause specific attendance will also be ascertained by checking health care records by study physician.

Adherence to study medication: measured by field workers' audit of packs or measuring the unused doses during weekly visits.

Economic data: Data for future health economic analyses will be collected along with unplanned clinic presentation and hospital admission. Willingness to pay (WTP) will be measured through contingent valuation method to predict the maximum price at or below which the participants will definitely buy one unit of the medicine at the end of intervention.

Blood samples: 3mL of venous blood will be collected. Anaemia (Hb<11gm/dL), Iron Deficiency (Ferritin<12ng/uL) and Iron Deficiency Anaemia (Anaemia + Iron Deficiency) will be measured at baseline, at the end of intervention and at post intervention follow-up periods. Hemoglobin will be assessed by HemoCue 301 and Ferritin will be assessed by cobas c 311 analyzer. Surplus serum and whole blood will be stored for related subsequent studies.

Sample size and power estimation

The sample size calculation is based on the primary objective which will be evaluated using the estimated mean difference and 95% confidence interval (CI) in the change from baseline to 3 months post-baseline of the Bayley III CogCS between the iron supplementation and placebo arm, and the MMPs and placebo arm. By construct, the Bayley III CogCS ranges between 55 and 150 (standardised mean 100; standard deviation [SD] 15) whereby a higher Bayley III CogCS indicates a better cognitive performance. Our systematic review estimated a difference of 1.65 points (n=1093 across six trials; random-effects 95% CI [-0.63, 3.94]) on Bayley Mental Development Index (MDI) (the cognitive scale reported on previous versions of the Bayley scales) in favor of daily iron supplementation compared to control in children aged 4-23 months. Among the six studies included in this systematic review, the highest quality (Cochrane risk of bias tool) study (in Indonesia) evaluating effects on development in a community setting found a 2-point difference of universal iron supplementation (n=136) compared to placebo (n=143) after 6 months' intervention (mean Bayley MDI: iron 101 versus placebo 99, p=0.76).[49] A more recent (but non-blinded) trial in Pakistan found a significant 2.5-point difference of MMPs (n=658) compared to control (n=699) at 12 months of age (mean Bayley III CogCS: MMPs 95.9 versus placebo 93.4, p=0.007).[27] The sample size required to detect a 2-point difference is 883 per arm to reach 80% power using a two-sided 2.5% level of significance for each comparison (Bonferroni correction), assuming a 15-point SD. Accounting for about 20% missing data in Bayley III CogCS at 3 months post-baseline, based on a randomised trial in Bangladesh which reported a 26% loss between birth and 6 months[41], the total sample size is 3300. This is currently the largest trial evaluating effects of iron compared to placebo on cognitive development ever to be conducted and will provide evidence for the overall, average effect of

these interventions when applied universally to a population with a high prevalence of anaemia, as presently recommended by the World Health Organization.

Statistical analysis plan

All randomised infants will be included in the analysis set according to the arm to which the infant was randomly allocated. Baseline characteristics will be examined across the arms to assess the randomization. Continuous data will be summarized using mean and SD or median and 25th-75th percentile if data are found to be skewed (e.g., ferritin). Categorical data will be presented as count and percentage. The primary outcome, Bayley-III CogCS scores at baseline, 3 month, and 12 month post-baseline, will be analysed using a constrained longitudinal data analysis method.[51] The model will incorporate time point as a categorical variable and assume a common baseline mean across the three arms. Furthermore, it will adjust for the stratification factors used in the randomisation (gender, union) as main factors and model the variance-covariance among the repeated measurements as unstructured. The estimate and 95% CI of the mean difference in change from baseline to each post-baseline time point between two arms will be obtained from this model. This model will yield unbiased results when the outcome data are missing at random. In addition, sensitivity analyses consisting of an adjusted analysis accounting for key prognostic baseline variables (e.g., socio-economic status) will be conducted. Secondary continuous outcomes (e.g., Bayley III domain scores, anthropometry, z-scores [growth], behavior rating scale) will be analysed similarly as the primary outcome. Appropriate transformations may be applied to the variables before fitting the model if considered skewed. Secondary binary outcomes (e.g., growth stunting, wasting, and underweight) will be analysed using generalized estimating equations with a logarithmic link function and unstructured correlation. A Poisson regression, or in case of over-dispersion negative binomial regression, will be used to analyse the rate of infections (e.g., fever) for the duration of the intervention period, the follow-up period, and 12-month study period. The number and percentage of infants with at least one infection, at least one AE, and at least one unplanned hospital or health-care facility attendance will be tabulated by arm for the duration of the intervention period, the follow-up period, and 12-month study period. A per-protocol analysis of efficacy outcomes, based on adherence, and as as-treated analysis of safety outcomes, in case of misrandomisation, will also be conducted. Exploratory subgroup analyses will be performed irrespective of the primary study findings by a) baseline anemia status (yes vs no anemia), b) baseline iron deficiency status (yes vs no iron deficient), c) baseline iron deficiency anemia status (yes vs no iron deficient anemia) d) baseline home stimulation (above

vs below median level as measured by family care indicators) e) wealth status (above or below median), f) growth (presence or absence of stunting), and g) infant's sex (male vs female) by adding subgroup as a main effect and its interaction with treatment arm to the model to evaluate if the treatment effect differs across subgroup categories. We postulate that infants with anemia, iron deficiency, iron deficiency anemia, or above median home stimulation will have a larger treatment effect compared to those whom are non-anaemic, non-iron deficient, non-iron deficient anaemic, or below median home stimulation respectively.

In addition, depending on the findings of the study, we will undertake subsequent health economics analysis of the data. For this purpose, we will collect and present all direct and indirect costs for the implementation of the project. The contingent valuation methods will be used to estimate weekly WTP and multiple regression analysis will be used to predict WTP by socioeconomic characters, past illness and type of medicine.

Data management

Data from questionnaires will be entered directly into electronic tablets in the field, along with GPS location data. Data will be checked in real time for quality by a dedicated data manager. Data for Bayley scales will be entered subsequently, with 10% undergoing double entry. Range checks will be applied automatically to all data. All aspects of the trial conduct (field work eg ethical recruitment and consent, randomisation, provision of interventions, outcome assessments, data collection and entry) will be audited at least annually by investigators from the University of Melbourne.

ETHICS AND DISSEMINATION:

The trial has been approved by the Melbourne Health Human Research Ethics Committee, Australia (2016.269); the Ethical Review Committee of icddr,b (PR-16063); and the Directorate General of Drug Administration, Ministry of Health and Family Welfare, Bangladesh. Informed written consent will be obtained from parents/guardians prior to both screening and enrollment procedures – either via signature or a thumbprint or mark for those who cannot signs. Written informed consent from the child's parent or legal guardian will be obtained by the SFA, the most senior member of the field data collection teams. Consent will encompass participation in the trial and its procedures, as well as storage and possible use of samples for related studies in the future; this includes non-diagnostic molecular and genetic studies. Children ineligible at recruitment due to illness will be referred for clinical care. Any information obtained in

connection with this research project or in any publication and/or presentation, will be provided in such a way that the individual cannot be identified. Only researchers on this project will have access to the data. Three years after the protocol completion date icddr,b research data in the repository will be made publicly available according to icddr,b data access policy.

Data Monitoring

An independent Data Safety Monitoring Board (DSMB) has been constituted and will provide oversight of the study. In cases of serious adverse events, the study physician will follow-up and document the course of events, will recommend for necessary suspension, refer if necessary and report to DSMB. As per best practice, the DSMB will define their meeting schedule and plan for interim analyses and define stopping rules in the DSMB charter. Amendments to the trial protocol will be updated in the trial protocol, the trial registration, informed by memo to all investigators as well as the ethical review committees, and if significant, will be explained in the final publications of the trial.

Discussion

Understanding the benefits and risks of universal iron interventions in young children at the population level is a public health priority. This pivotal trial will form the platform for global anaemia control policy in young children. It will define global guidelines, inform policymakers at the national and regional level, and provide the economic rationale for donors and governments to select and fund anaemia control interventions. The design (combining interventions with vaccination) will enable translation to the field. Results will be communicated to the academic community through publication in peer-reviewed journals. Criteria for authorship will reflect ICMJE guidelines. We will also communicate results to policy makers through policy-briefs and reports e.g. WHO, UNICEF, and major nutrition bodies (e.g. GAIN).

Author contributions: SP and BB conceived of the idea for the trial. SP, BB, MD, JF, SGM, JS, SA, JH prepared the initial funding submissions and proposals. MIH, SJH, SB, JH, SP and BB prepared the detailed trial protocol. SB and JAS developed the statistical analysis plan. MOH, SJH, FT and JH designed the field work. MIH, SJH and SP wrote the first draft of the manuscript, and all authors have reviewed and authorized it.

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Competing interest statement: The investigators have no financial or other conflicts of interest to declare.

Provenance and peer review: Not commissioned, externally peer reviewed.

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Figure 1: Trial Design

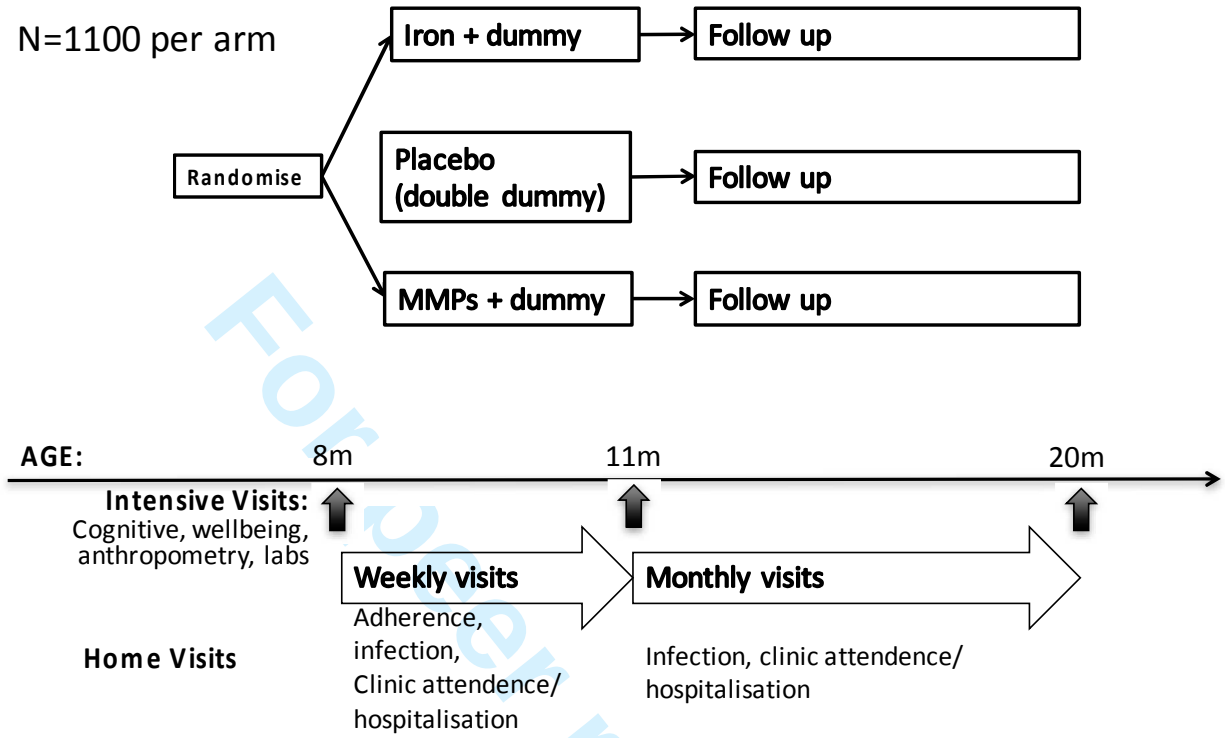
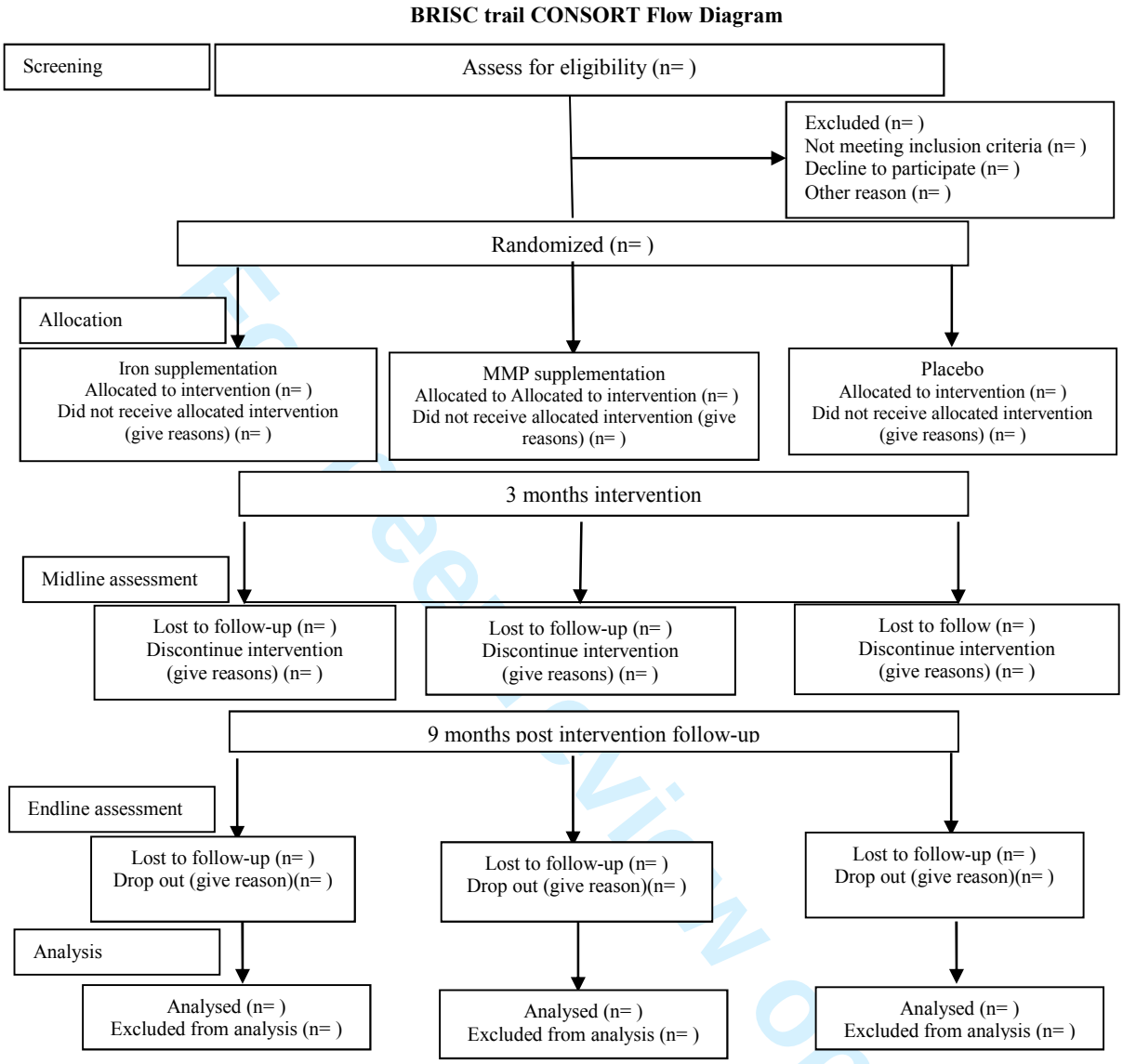


Figure 2: CONSORT Flow Diagram for the BRISC Trial



SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	__1_____
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	_3_____
	2b	All items from the World Health Organization Trial Registration Data Set	_3_____
Protocol version	3	Date and version identifier	_3_____
Funding	4	Sources and types of financial, material, and other support	_17_____
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	_1, 17_____
	5b	Name and contact information for the trial sponsor	_1, 17_____
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	_N/A_____ _
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	__N/A_____ _

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	___4_____
	6b	Explanation for choice of comparators	___4-6_____
Objectives	7	Specific objectives or hypotheses	____7_____
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	____7_____
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	___7_____
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	___8_____
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	__8_____
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	___NA_____
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	___8_____
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	___8_____
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	__12_____
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	___12_____

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	___14_____
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	__14_____
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	__9_____
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	__9_____
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	__9_____
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	__9_____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	__9_____
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	___9_____
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	___9_____

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	__16_____
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	_14_____
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	_14_____
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	_14_____
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	__16_____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	_16_____
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	_12_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_16_____
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	____16_____
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	____17_____

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	__17_____
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	__17_____
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	_____
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_____
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	_____
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	_____
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	__18_____
	31b	Authorship eligibility guidelines and any intended use of professional writers	__18_____
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_NA_____
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	_Supplementary materials_____ __
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	_14_____

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Benefits and Risks of Iron interventions in Children (BRISC): protocol for a three-arm parallel-group randomized controlled field trial in Bangladesh

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Primary Subject Heading:	Public health
Secondary Subject Heading:	Neurology, Paediatrics, Nutrition and metabolism, Health economics
Keywords:	Iron deficiency, cognitive development, Bangladesh, randomized controlled trial, children, Anaemia < HAEMATOLOGY

Benefits and Risks of Iron interventions in Children (BRISC): protocol for a three-arm parallel-group randomized controlled field trial in Bangladesh

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MIH and SJH contributed equally to this paper. JH, BB and SP contributed equally to this paper.

Abstract

Introduction: Anaemia is a major global health problem affecting about 43% of pre-school children globally and 60% of 6-24 months old children in rural Bangladesh, half of which is attributed to iron deficiency (ID). Although the World Health Organization (WHO) recommends universal supplementation with iron or home fortification with iron-containing multiple micronutrient powders (MMPs) to children under 2 years, evidence for benefits of these interventions on childhood development (a key rationale for these interventions) and harms (especially infection) remains limited. This study aims to evaluate the impact of iron or MMPs supplementation compared to placebo on a) children's development b) growth c) morbidity from infections, and d) haematologic and iron indices.

Methods and analysis: This study is a three-arm, blinded, double dummy, parallel-group, placebo controlled superiority trial using stratified individual block randomization. The trial will randomise 3300 children aged 8-9 months equally to Arm 1: iron syrup (12.5mg elemental iron), placebo MMPs; Arm 2: MMPs (including 12.5mg elemental iron), placebo syrup; and Arm 3: placebo syrup, placebo MNPs. Children will receive interventions for 3 months based on WHO recommendations and then be followed-up for 9 months post-intervention. The primary outcome is cognitive composite score measured by Bayley-III. Secondary outcomes include motor and language composite score by Bayley-III, behaviour rating using selected items from Wolke's rating scales and BSID-II behaviour ratings, temperament, growth, haemoglobin, anaemia and iron status, and infectious morbidity. Outcomes will be measured at baseline, at the end of 3-month intervention, and after 9 months post-intervention follow-up.

Ethics and dissemination: The trial has been approved by the Ethical Review Committee of icddr,b (Dhaka, Bangladesh) and the Melbourne Health Human Research Ethics Committee (Melbourne, Australia). Results of the study will be disseminated through scientific publications, presentations at international meetings, and policy briefs to key stakeholders.

Trial registration number ACTRN12617000660381

WHO Universal Trial Number U1111-1196-1125

Keywords Iron deficiency, anaemia, cognitive development, Bangladesh, randomized controlled trial

Version 1, June 19th 2017

Strengths and Limitations

- Trial design: double blind, double dummy design minimizing risk of bias in assessment of outcomes. The trial is designed to be able to compare the main interventions (iron drops and iron-containing micronutrient powders) used for anaemia control in young children against placebo.
- Outcome assessment: The tools we are using, including Bayley Scales, are the gold standard for directly measuring child development.
- Sample size: this is the largest trial to assess effects of iron interventions on child development, and as such the trial is powered to detect small but clinically relevant effect sizes.
- Trial setting: the trial is set in a low income South Asian setting where there is a high baseline prevalence of anaemia, and will exclude children at risk of high groundwater iron exposure.
- Biomarker assessment: measurement of anaemia and iron deficiency, along with growth, at baseline will facilitate subgroup analysis by baseline nutrition status.
- We will exclude children with Hb<8gm/dl to ensure they are referred for treatment, which means we will not have a chance to assess the effects of iron interventions on cognitive performance in this group at perhaps higher risk; similarly, children with severe malnutrition are also excluded. Our data may therefore not be able to generalized to children with severe anaemia or malnutrition.

BACKGROUND

Anaemia is highly prevalent in preschool children

Approximately 43% (up to 304 million) of under-5 children worldwide are anaemic. The number of children affected is greatest in South Asia, where the prevalence exceeds 55%.[1] The relative contribution of iron deficiency (ID) to the overall burden of anaemia varies by region. We have previously found that among rural Indian children aged 12-23 months, ID accounted for 72% of anaemia.[2] In rural Bangladesh, we found about 60% of children 6-24 months to be anaemic, with half of cases due to ID.[3] Conversely, in pre-schoolers in rural Gambia and Tanzania where malaria is endemic, ID accounted for only 20% of anaemia.[4]

Iron supplementation as a strategy for controlling anaemia in children in low-income settings

Iron supplementation involves administration of medicinal iron (usually ferrous salts).[5] Multiple micronutrient powders (MMPs) comprise single dose sachets of lipoencapsulated iron together with other micronutrients (usually at least vitamin A, zinc and folate) that can be sprinkled onto any semi-solid food, with the aim of providing a child with a recommended daily intake of micronutrients. The World Health Organization (WHO) recommends two different possible direct interventions for controlling anaemia in young children. Firstly, WHO recommends that all children aged 6-23 months, in settings where the prevalence of anaemia exceeds 40%, receive 3 months daily iron supplements.[6] Alternatively, where the prevalence of anaemia exceeds 20%, WHO recommends children 6-23 months receive 90 days home fortification with iron-containing multiple micronutrients powders (MMPs) every six months.[7] WHO does not recommend one approach over the other; their efficacy and safety have not been compared in a large head to head trial; earlier recommendations for MMPs proposed 2 months intervention every six months. Recent estimates indicate that in pre-school children, about 41% and 32% of cases of anaemia in South-East Asia and sub-Saharan Africa respectively, are responsive to iron.[8]

Adequate iron stores are important for neurological development

The prevalence of anaemia generally increases from 6 months of age and peaks in the second year of life,[9] especially if iron intake from complementary foods is inadequate to meet the demands of erythropoiesis and growth.[10,11] The peak in anaemia prevalence coincides with the critical period for neural development, sharing the same period of peak vulnerability: the ‘first 1000 days’ from conception to age 2 years.[12] Animal studies also indicate that iron is needed for

myelination and neurotransmitter synthesis, while ID alters neuronal metabolism.[13,14]Observational studies have consistently linked anaemia in infancy to adverse short and longer term deficits in cognitive development.[15] Hence, animal data and observational studies in children suggest that ID impairs brain development.[16]

Evidence of beneficial effects of iron interventions in children at the population level

While iron interventions improve haemoglobin concentrations and iron indices and reduce the prevalence of anaemia, ID, and iron deficiency anaemia (IDA),[17-19] there are limited data from population clinical trials confirming that policies of universal iron interventions improve development, growth and health in young children.

Effects on development: Few RCTs have evaluated effects of iron supplements or MMPs on development in children under 2 years[18,20] and these trials were underpowered individually and collectively and most of the trials were in pre-selected patient groups (not populations) or were not blinded (i.e. high risk of bias) limiting the quality of evidence.[18] This paucity of available evidence has hampered systematic reviews and meta-analyses (RCTs) that have to date failed to find evidence of benefit from iron interventions (iron supplements, home fortification with MMPs, or other iron interventions) on development in young children.[21-24] Our systematic review of daily iron supplementation in children aged 4-23 months identified no significant difference in Bayley's mental development index (MDI) in children receiving iron compared with control (mean difference 1.65 [95% confidence interval -0.63, 3.94]); for psychomotor development index (PDI) the effect size was (mean difference 1.05 [-1.36, 3.46]).[18]
Systematic reviews evaluating the effects of MMPs on cognitive development did not identify RCTs that had reported effects on measures of cognitive development,[25,26] and only reported a single trial that found children receiving an intervention walked earlier than those from a parallel control group (i.e. children not included in the study at inception). More recently, a large randomized trial in Pakistan identified only transient benefits from MMPs on Bayley's cognitive, language and psychomotor development,[27] and motor development in the longer term.[28] This trial did not use placebo and was hence not adequately blinded; moreover, adherence to the supplements appeared limited and had no effect on haemoglobin concentration compared to control children.[27]

Data regarding longer term effects of iron supplementation on children development are limited. A recent study in Thailand also documented no significant difference of IQ and school

performance at 9 years of age although the children were supplemented separately with iron and zinc for six months at age 4-6 months.[29] A study in Nepal also found no effect of infant iron supplementation on child's long term intelligence and executive functions.[30]

Effects on growth: Benefits on growth are often cited as a rationale for universal iron supplementation.[31] However previous systematic reviews have not found benefits on growth, and indeed, have found that iron interventions can impair linear growth in iron-replete children.[32] Our systematic review suggested daily iron supplementation reduced length and weight gain in young children.[18] A systematic review of iron-containing MMPs found no increase in growth despite containing the growth-promoting micronutrient zinc.[20]

Evidence of harm from iron supplementation

In contrast to the lack of data on benefits, several large RCTs have reported adverse effects from iron interventions in low-income settings. This emerging data along with mechanistic studies in low-income settings are now providing convincing evidence that these interventions cause or exacerbate infection, including diarrhea, bloody diarrhea, and respiratory infections in endemic and non-endemic malaria settings.[27,33-35] For example, our meta-analysis of iron supplementation identified a 16% and 38% increased risk of fever and vomiting respectively.[18]

The need for a trial

Although immediate and long-term benefits from iron on functional outcomes such as cognitive development and growth have been assumed for decades, existing data from RCTs do not support this contention. In contrast, data for evidence of harm from iron interventions is accumulating. Furthermore, iron supplements have not been compared directly to MMPs in a large field trial. In this RCT, we aim to define the benefits and harms of daily iron supplementation and MMPs in young children, enabling evidence-based recommendations for implementation (or withdrawal) of iron interventions in this age group.

METHODS AND ANALYSIS

Benefits and risks of iron interventions in children (BRISC)

Trial objectives

The primary objective of this study is to determine if 3 months interventions with iron supplementation or home fortification with MMPs is superior to placebo on cognitive development in children aged 8 months ± 14 days.

The secondary objectives are to evaluate the impact of iron supplementation and home fortification with MMPs, compared with placebo, on:

- Developmental indices, i.e. cognitive (after 9 months post-intervention), and motor, language, behaviour and temperament (after 3 months intervention and 9 months post-intervention),
- Prevalence of anaemia and iron deficiency (after 3 months intervention and 9 months post-intervention), and
- Infection risks, especially diarrhoea and respiratory infection in these young children (after 3 months intervention and 9 months post-intervention).

Study design

BRISC is a three-arm; parallel; researcher, caregiver, data collector, analysts, and participant-blinded-blind; individually randomised; double-dummy placebo controlled; superiority trial. It will compare the effects of 3 months of daily i) iron supplementation, or ii) MMPs, to iii) placebo in 8 months old Bangladeshi children, with a further 9 months follow up. The trial design is summarized in Figure 1.

Study settings and participants

The trial will be conducted in Rupganj, a rural sub-district/upazila of Narayanganj district about 50km from Dhaka, in Bangladesh. Three unions (regions) within the sub-district will be included, with each union covered by a dedicated study team. A recent national survey reported the prevalence of anaemia in 9-11 months old infants at 78.7%.[36] Diarrhoea and respiratory infections remain highly endemic in Bangladesh, with 4.6% and 5.8% of children <5 years experiencing these respectively in a 2-week period.

Like many other developing countries, anaemia is highly prevalent in Bangladesh, and iron deficiency is expected to contribute to half the total burden of anaemia[3,12] Our study site,

Rupganj, is a non-malaria endemic setting in rural Bangladesh and has low ground water iron level. [37,38] Furthermore, we will exclude any child from a household with elevated groundwater iron. We therefore expect that results from this trial will have generalizability to other low and middle income countries where the prevalence of anaemia is high. This may include malaria endemic countries; however, the proportion of anaemia attributable to iron deficiency in such settings is lower, and the iron-infection interactions may be different. As such, iron trials in malaria-endemic countries should incorporate specific malaria prevention measures which our study does not require. Our study team is also proposing a similar study in Malawi where malaria is endemic, incorporating the requirements for malaria treatment or prevention.

Eligibility Criteria

Children will be randomised only if they fulfill all the inclusion criteria and none of the exclusion criteria.

Inclusion criteria:

1. Aged 8 months (±14 days) at the time of randomization,
2. Not expected to leave the study location for more than one week over the next 3 months, or for more than one month over the next 12 months,
3. Has a legal guardian capable of providing informed consent.

Exclusion criteria:

Children meeting any of the following criteria will be excluded from the study:

1. Capillary haemoglobin (Hb) <8.0g/dL at the time of screening.
2. Drinking water iron concentration >1mg/L.
3. Diagnosed case of any clinical haemoglobinopathy (e.g. beta-thalassaemia major, HbE-beta thalassaemia).
4. Current infective illness (i.e. respiratory infection, diarrhoea) with fever; however, children may be rescreened again after recovery if otherwise eligible.
5. Received iron supplements or iron-containing MMP in the previous month.
6. Known congenital anomaly, developmental disorder or severe developmental delay.
7. Child of multiple birth e.g. twins, triplets.

Intervention

Participants will be randomised in a 1:1:1 ratio to each of the three arms. Infants in the two active intervention arms will receive 12.5 mg daily oral iron either in syrup form or as MMPs as

recommended by WHO.[39,40] Each participant will receive both a syrup (to be dispensed via a syringe at a predefined volume) and a sachet (to be sprinkled on food), achieving double dummy blinding. Iron syrup and the corresponding placebo will be manufactured in Bangladesh by ACME Laboratories. Micronutrient powders and corresponding placebo will be manufactured by Renata Ltd. Mothers/caregivers will be instructed (with demonstrations) how to administer the supplements. Participants will be asked to take one dose of each formulation daily for 3 months.

Intervention arms:

Arm 1: (Iron syrup and placebo sachet): Daily oral supplementation of 12.5 mg elemental iron syrup and a placebo sachet containing powders in identical packaging to the MMP, but containing no micronutrients.

Arm 2 (MMP sachet and placebo syrup): Daily home-fortification with an MMP sachet containing 12.5 mg Iron, 0.3 mg Vitamin A, 30 mg Vitamin C, 0.16 mg Folic Acid, and 5 mg Zinc; placebo syrup containing no iron but identical in colour and flavour.

Arm 3: (Placebo syrup and placebo sachet): Control arm.

Each participant will receive a pouch every week containing a bottle of syrup and 7 sachets.

Randomisation

Participants will be randomly allocated to one of the three arms with 1:1:1 allocation using a computer-generated schedule of randomly permuted blocks of fixed size stratified by sex and union (each covered by a different field team) to achieve balance between the arms within each stratum. The randomisation list will be prepared by an independent statistician, who will not reveal the block size. The allocation will occur by the field team according to the list, within their assigned union, once eligibility criteria have been checked.

Allocation concealment and blinding of study agents

Blinding of the team visiting the site, the caregiver(s), and participants will be achieved through the use of identical packaging of sachets and syrup regardless of their contents (active or placebo), packaged in pouches that carry an allocation code. The independent statistician will hold the allocation codes until the data base is ready for unblinding. Researchers, caregivers, persons involved with data collection (i.e. field team) or analysis will be blinded to the allocation code until the database has been finalized for analysis. Breaking of the allocation code will occur only in the case of a severe adverse event or as requested by Data Safety Monitoring

Board (DSMB), in which case the code will only be disclosed to the local study physician. Emergency unblinding will lead to discontinuation of the participant's involvement in the study.

Recruitment and visits

The schedule of visits is outline in Table 1. Trained Village Health Workers (VHWs) will identify all potentially eligible children by making household visits in their designated areas and collating these data centrally, enabling generation of a list of age-specific eligible participants in each village. Based on the list, VHWs and Senior Field Assistants (SFAs) will visit potentially eligible families. After providing preliminary information to the parents/guardians, the team will obtain their consent for screening and determine their eligibility. During screening, drinking water iron level will be measured using "HACH" Iron (Ferrous) test Kit and the child's capillary Hb level will be measured by *HemoCue*-301. Children with Hb<8.0 gm/dl will be excluded and referred to nearby health centre for management. Mothers/guardians of eligible children will be briefed further about the trial, and be invited to a selected house/test centre for enrollment.

Enrolled families will attend a designated local study site on a prescribed day for enrolment and baseline data collection. The data collection team consisting of a psychological tester, a SFA, a phlebotomist and a VHW will undertake detailed data collection. SFAs will screen children for eligibility, motivate the mothers/ guardians for participation; collect socio-economic data, household food security and willingness to pay information. At this visit, consent for participation in the study will be signed by Bayley testers .They will also collect baseline information on child's temperament, family care indicator questionnaire, administer Bayley scale of infant and toddler development (3rd edition), rate the child's behavior by using Wolke's behavior rating scale, take anthropometric measurements and finally, a study phlebotomist will collect 3 mL of venous blood. The child will then receive the randomly allocated intervention. Testers will provide detailed instruction regarding medication to mothers or caregivers before they leave the test centre and they will give details of the enrolled child to the assigned VHW for prospective follow-up visits. The assigned VHW will then visit the child every week for the 3 month intervention period, and every month for the 9 month post intervention period. Morbidity data will be collected weekly and monthly during the intervention and post intervention period respectively. VHWs will also record and notify any unscheduled hospital or clinic admission experienced by the participant. The number of doses missed by participants will be recorded, empty bottles and sachets will be collected and new doses for the following week dispensed at

routine weekly visits. A total of 23 VHWs, 05 SFAs and 10 Psychological testers are expected to be recruited and trained for this trial.

Table 1: Overview of study visits

ACTIVITIES	**STUDY PERIOD**					
	Screening	**Baseline/ enrolment**	**Post-allocation**			**Close-out**
Time point (expected duration of visit)	$-t_1$	Day 0 Visit 1	Weekly visits Day 7,14,21,28,35, 42,49,56,63,70,77 Visit 2-12	Midline 3rd+ month Visit 13	Monthly visits (post-intervention) Month 4,5,6,7,8,9,10,11 Visit 14-20	Endline 12^{th} $^{+}$months Visit 21
Enrolment:	Age approx. 8±0.5 mo					
Eligibility screen (20 min)	X					
Informed consent (15 min)	X	X				
Allocation		X				
Interventions:		X	X			
Socio demographic information (15 min)		X				
Family Care Indicators (15 min)		X		X		X
Temperament questionnaire		X		X		X

(20 min)						
Food security questionnaire (15 min)		X		X		X
Adherence and morbidity questionnaire (15 min)			X	X	X	X
Bayley-III (90-120 min)		X		X		X
Wolke's Behaviour Rating Scale (10 min)		X		X		X
Anthropometry (5 min)		X		X		X
Adverse events reporting (AE,SAEs)			X	X	X	
Corneal lesions assessment				X		
Venous blood collection (10 min)		X		X		X
Willingness to pay questionnaire (10 min)				X		

Other visits

Withdrawal visit

Children who stop study drug may continue with assessments if their guardian wishes. If a participant withdraws early or investigator terminates participation, we will seek to undertake the following assessments:

- Reason for study withdrawal
- If within 2 weeks of visit 13 or 21, we will invite the participant to attend to undertake this visit unless the reason for withdrawal precludes this.

Recruitment is expected to commence in July 2017 and the trial will be open for 18 months. Expected participant flow is shown in Figure 2.

Study oversight and adherence

All staff will undergo specific training unique to their role in the study. Adherence will be monitored for all participants. VHWs will measure the amount of syrup and number of sachets unused, and it will be recorded on the case record forms of each child.

Outcomes

Primary outcome

Cognitive Composite Score (CogCS) measured by Bayley Scales of Infant and Toddler Development (Bayley-III) after 3 months of intervention is the primary outcome.[41] Bayley-III is a validated index of child development and the preferred field assessment tool. It is a standard series of measurements primarily to assess cognitive, motor (fine and gross) and language (receptive and expressive) development of infants and toddlers aged 0-3 ½ yrs. Total number of credited items is converted into scaled scores based on child's age, which are then converted to composite scores of each subscale. These scores are used to determine the child's performance compared with norms taken from typically developing children of their age in developed countries. Bayley-II has been adapted and extensively used on Bangladeshi children.[42-44] Bayley-III has now been adapted, with some components not familiar for the rural and urban children of this country changed according to the local context. It has been used in several studies in this population.[45] Each tester will receive month long training for Bayley assessments after employment. Training will cover administration of the testing instruments across all age groups from 1 to 42 months. Refresher training will be provided every three months to maintain the consistency and agreements between testers. New testers hired during the course of the study will undergo the same training process. Bayley testers will be certified

and permitted to collect data only when their results agree >90% with the gold standard i.e. the trainer. About 5-10% of the tests by each tester will be observed by the trainer for inter-tester reliability over the course of the study.

Secondary outcomes

Development: CogCS at the end of 9 months post intervention, motor and language composite scores by Bayley-III, behaviour rating on selected items from Wolke's rating scales and BSID-II behaviour ratings, temperament by using a modified version of Bates, quality of home stimulation by using family care indicators, and food insecurity by household food insecurity access scale will be measured immediately after intervention and post intervention follow-up.[46-49] All the secondary outcomes will be assessed at 3-months (end of intervention) and 9-months post intervention.

Physical growth: will be measured as length, weight, head circumference at end of intervention and post follow-up period. Length of the child will be measured to the nearest 0.1 cm by using the Shorr stadiometer (Shorr Products), which has been previously validated and used on local population. Weights will be obtained using a battery-powered digital scale (Tanita HD-318). Length and weight will be used to develop indicators of stunting and wasting compared to age-sex specific WHO international reference growth standards.[50] Measurements will be taken in duplicate with the average taken unless substantial discrepancy occurs. Physical growth is a secondary outcome of interest, but it can be a confounding factor because malnutrition is correlated to development. We will therefore treat physical growth as both an outcome and a confounder in our analysis. Testers will be certified to take anthropometric measurements only when they achieve a high inter-rater reliability with the trainer i.e. the gold standard. About 5-10% measures will be checked by the quality assurance team for inter-observer reliability throughout the study period. Presence of corneal lesions (caused by vitamin A deficiency) will be assessed by the field team at the midline visit.

Infectious morbidity: as rate and number of days affected by diarrhea/ bloody diarrhea (along with number of episodes per day), respiratory infection, vomiting, and fever. Infectious morbidity data will be based on previous 7 days recall by caregivers during the 3 months of active intervention. During the subsequent 9 months post intervention follow-up visit morbidity data will be based on recall for the previous 2 weeks, except for hospitalization, which will cover previous

month's recall. Morbidity information will be collected by VHWs during routine weekly or monthly visits by interviewing caregivers

Unplanned hospital or health-care facility attendance: as rate, will be measured by field workers along with morbidity questionnaires. Cause specific attendance will also be ascertained by checking health care records by study physician.

Adherence to study medication: measured by field workers' audit of packs or measuring the unused doses during weekly visits.

Economic data: We will collect Willingness to Pay (WTP) data to predict the participant's interest and affordability to pay for the price of the intervention. Parent's perception of the benefits (or lack thereof) can also play an important role for uptake of the supplementation. Willingness to pay (WTP) will be measured through the contingent valuation method to predict the maximum price at or below which the participants will definitely buy one unit of the medicine at the end of intervention. Data for future health economic analyses will be collected along with unplanned clinic presentation and hospital admission.

Blood samples: 3mL of venous blood will be collected. Anaemia (Hb<11gm/dL), Iron Deficiency (Ferritin<12ng/uL) and Iron Deficiency Anaemia (Anaemia + Iron Deficiency) will be measured at baseline, at the end of intervention and at post intervention follow-up periods. Hemoglobin will be assessed by HemoCue 301 and Ferritin will be assessed by cobas c 311 analyzer. Surplus serum and whole blood will be stored for related subsequent studies.

Sample size and power estimation

The sample size calculation is based on the primary objective which will be evaluated using the estimated mean difference and 95% confidence interval (CI) in the change from baseline to 3 months post-baseline of the Bayley III CogCS between the iron supplementation and placebo arm, and the MMPs and placebo arm. By construct, the Bayley III CogCS ranges between 55 and 150 (standardised mean 100; standard deviation [SD] 15) whereby a higher Bayley III CogCS indicates a better cognitive performance. Our systematic review estimated a difference of 1.65 points (n=1093 across six trials; random-effects 95% CI [-0.63, 3.94]) on Bayley Mental Development Index (MDI) (the cognitive scale reported on previous versions of the Bayley scales) in favor of daily iron supplementation compared to control in children aged 4-23 months.

Among the six studies included in this systematic review, the highest quality (Cochrane risk of bias tool) study (in Indonesia) evaluating effects on development in a community setting found a 2-point difference of universal iron supplementation (n=136) compared to placebo (n=143) after 6 months' intervention (mean Bayley MDI: iron 101 versus placebo 99, p=0.76).[51] A more recent (but non-blinded) trial in Pakistan found a significant 2.5-point difference of MMPs (n=658) compared to control (n=699) at 12 months of age (mean Bayley III CogCS: MMPs 95.9 versus placebo 93.4, p=0.007).[27] The sample size required to detect a 2-point difference is 883 per arm to reach 80% power using a two-sided 2.5% level of significance for each comparison (Bonferroni correction), assuming a 15-point SD. Accounting for about 20% missing data in Bayley III CogCS at 3 months post-baseline, based on a randomised trial in Bangladesh which reported a 26% loss between birth and 6 months[43], the total sample size is 3300. This is currently the largest trial evaluating effects of iron compared to placebo on cognitive development ever to be conducted and will provide evidence for the overall, average effect of these interventions when applied universally to a population with a high prevalence of anaemia, as presently recommended by the World Health Organization.

Statistical analysis plan

All randomised infants will be included in the analysis set according to the arm to which the infant was randomly allocated. Baseline characteristics will be examined across the arms to assess the randomization. Continuous data will be summarized using mean and SD or median and 25^{th}-75^{th} percentile if data are found to be skewed (e.g., ferritin). Categorical data will be presented as count and percentage. The primary outcome, Bayley-III CogCS scores at baseline, 3 month, and 12 month post-baseline, will be analysed using a constrained longitudinal data analysis method.[52] The model will incorporate time point as a categorical variable and assume a common baseline mean across the three arms. Furthermore, it will adjust for the stratification factors used in the randomisation (gender, union) as main factors and model the variance-covariance among the repeated measurements as unstructured. The estimate and 95% CI of the mean difference in change from baseline to each post-baseline time point between two arms will be obtained from this model. This model will yield unbiased results when the outcome data are missing at random. In addition, sensitivity analyses consisting of an adjusted analysis accounting for key prognostic baseline variables (e.g., socio-economic status) will be conducted. Secondary continuous outcomes (e.g., Bayley III domain scores, anthropometry, z-scores [growth], behavior rating scale) will be analysed similarly as the primary outcome. Appropriate transformations may be applied to the variables before fitting the

model if considered skewed. Secondary binary outcomes (e.g., growth stunting, wasting, and underweight) will be analysed using generalized estimating equations with a logarithmic link function and unstructured correlation. A Poisson regression, or in case of over-dispersion negative binomial regression, will be used to analyse the rate of infections (e.g., fever) for the duration of the intervention period, the follow-up period, and 12-month study period. The number and percentage of infants with at least one infection, at least one AE, and at least one unplanned hospital or health-care facility attendance will be tabulated by arm for the duration of the intervention period, the follow-up period, and 12-month study period. A per-protocol analysis of efficacy outcomes, based on adherence, and as as-treated analysis of safety outcomes, in case of misrandomisation, will also be conducted. Exploratory subgroup analyses will be performed irrespective of the primary study findings by a) baseline anemia status (yes vs no anemia), b) baseline iron deficiency status (yes vs no iron deficient), c) baseline iron deficiency anemia status (yes vs no iron deficient anemia) d) baseline home stimulation (above vs below median level as measured by family care indicators) e) wealth status (above or below median), f) growth (presence or absence of stunting), g) infant's sex (male vs female), and h) food security status, by adding subgroup as a main effect and its interaction with treatment arm to the model to evaluate if the treatment effect differs across subgroup categories. We postulate that infants with anemia, iron deficiency, iron deficiency anemia, or above median home stimulation will have a larger treatment effect compared to those whom are non-anaemic, non-iron deficient, non-iron deficient anaemic, or below median home stimulation respectively.

In addition, depending on the findings of the study, we will undertake subsequent health economics analysis of the data. For this purpose, we will collect and present all direct and indirect costs for the implementation of the project. The contingent valuation methods will be used to estimate weekly WTP and multiple regression analysis will be used to predict WTP by socioeconomic characters, past illness and type of medicine.

Data management

Data from questionnaires will be entered directly into electronic tablets in the field, along with GPS location data. Data will be checked in real time for quality by a dedicated data manager. Data for Bayley scales will be entered subsequently, with 10% undergoing double entry. Range checks will be applied automatically to all data. All aspects of the trial conduct (field work eg ethical recruitment and consent, randomisation, provision of interventions, outcome

assessments, data collection and entry) will be audited at least annually by investigators from the University of Melbourne.

ETHICS AND DISSEMINATION:

A placebo controlled trial is essential to establish the efficacy and adverse effects of iron on children's health and development, and is considered ethically justifiable because: 1) there is uncertainty regarding the benefits of iron supplementation on cognitive function, 2) all families will be educated about iron nutrition, 3) children with anaemia at the final measurement (+12m) will be referred to health centres, 4) there is previous experience of use of placebo arm in large iron/MMP RCTs e.g. in Tanzania, Ghana, Nepal and many other countries, and 5) mild-moderate iron deficiency is not yet known to cause, and iron interventions to alleviate, moderate or severe, permanent cognitive delay. Even though universal supplementation is recommended by WHO, it is not yet practiced in Bangladesh.

The trial has been approved by the Melbourne Health Human Research Ethics Committee, Australia (2016.269); the Ethical Review Committee of icddr,b (PR-16063); and the Directorate General of Drug Administration, Ministry of Health and Family Welfare, Bangladesh. Informed written consent will be obtained from parents/guardians prior to both screening and enrollment procedures – either via signature or a thumbprint or mark for those who cannot signs. Written informed consent from the child's parent or legal guardian will be obtained by the SFA, the most senior member of the field data collection teams. Consent will encompass participation in the trial and its procedures, as well as storage and possible use of samples for related studies in the future; this includes non-diagnostic molecular and genetic studies. Children ineligible at recruitment due to illness will be referred for clinical care. Any information obtained in connection with this research project or in any publication and/or presentation, will be provided in such a way that the individual cannot be identified. Only researchers on this project will have access to the data. Three years after the protocol completion date icddr,b research data in the repository will be made publicly available according to icddr,b data access policy.

Data Monitoring

An independent Data Safety Monitoring Board (DSMB) has been constituted and will provide oversight of the study. In cases of serious adverse events, the study physician will follow-up and document the course of events, will recommend for necessary suspension, refer if necessary and report to DSMB. As per best practice, the DSMB will define their meeting schedule and plan

for interim analyses and define stopping rules in the DSMB charter. Amendments to the trial protocol will be updated in the trial protocol, the trial registration, informed by memo to all investigators as well as the ethical review committees, and if significant, will be explained in the final publications of the trial.

Discussion

Understanding the benefits and risks of universal iron interventions in young children at the population level is a public health priority. This pivotal trial will form the platform for global anaemia control policy in young children. It will define global guidelines, inform policymakers at the national and regional level, and provide the economic rationale for donors and governments to select and fund anaemia control interventions. The design (combining interventions with vaccination) will enable translation to the field. Results will be communicated to the academic community through publication in peer-reviewed journals. Criteria for authorship will reflect ICMJE guidelines. We will also communicate results to policy makers through policy-briefs and reports e.g. WHO, UNICEF, and major nutrition bodies (e.g. GAIN).

Author contributions: SP and BB conceived of the idea for the trial. SP, BB, MD, JF, SGM, JS, SA, JH prepared the initial funding submissions and proposals. MIH, SJH, SB, JH, SP and BB prepared the detailed trial protocol. SB and JAS developed the statistical analysis plan. MOH, SJH, FT and JH designed the field work. MIH, SJH and SP wrote the first draft of the manuscript, and all authors have reviewed and authorized it.

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Figure 1: Trial Design

Figure 2: CONSORT Flow Diagram for the BRISC Trial

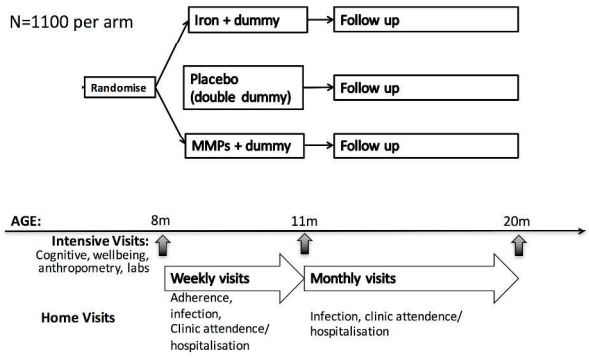

Figure 1: Trial Design

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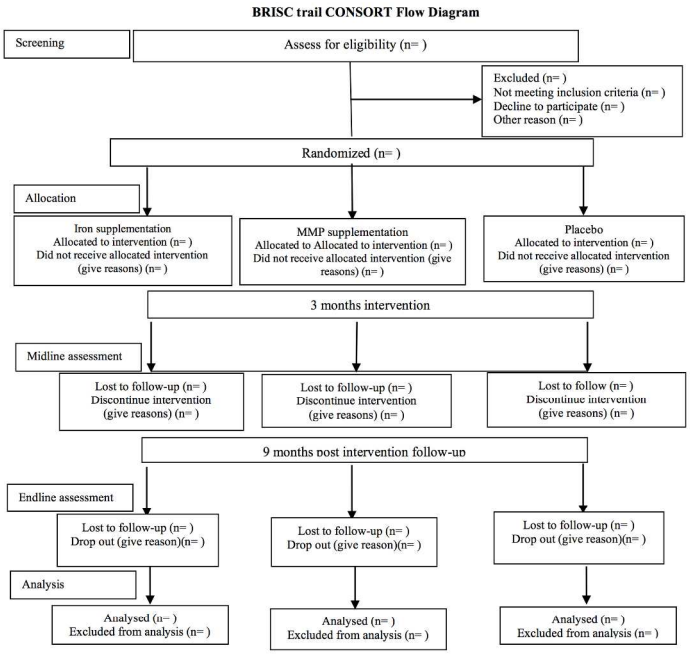

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Figure 2: CONSORT Flow Diagram for the BRISC Trial

215x279mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	__1_____
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	_3_____
	2b	All items from the World Health Organization Trial Registration Data Set	_3_____
Protocol version	3	Date and version identifier	_3_____
Funding	4	Sources and types of financial, material, and other support	_17_____
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	_1, 17_____
	5b	Name and contact information for the trial sponsor	_1, 17_____
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	_N/A_____ _
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	__N/A_____ _

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	___4_____
	6b	Explanation for choice of comparators	___4-6_____
Objectives	7	Specific objectives or hypotheses	____7_____
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	____7_____
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	___7_____
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	___8_____
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	__8_____
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	___NA_____
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	___8_____
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	___8_____
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	__12_____
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	___12_____

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	___14_____
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	__14_____
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	__9_____
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	__9_____
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	__9_____
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	__9_____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	__9_____
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	___9_____
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	___9_____

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	__16_____
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	_14_____
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	_14_____
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	_14_____
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	__16_____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	_16_____
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	_12_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_16_____
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	____16_____
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	____17_____

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	__17_____
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	__17_____
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	_____
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	_____
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	_____
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	_____
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	__18_____
	31b	Authorship eligibility guidelines and any intended use of professional writers	__18_____
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	_NA_____
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	_Supplementary materials_____ __
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	_14_____

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.