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The effect of once weekly folic acid supplementation on erythrocyte folate concentrations in women to determine potential to prevent neural tube defects: A randomised controlled dose-finding trial in Malaysia

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ABSTRACT

Introduction: Folic acid (0.4 mg) taken prior to and during early pregnancy reduces the risk of neural tube defects (NTD). Because these birth defects occur early in pregnancy, before women may know they are pregnant, many countries have mandated the addition of folic acid to food staples. In countries where fortification is not possible, and weekly iron folic acid programs exist to reduce anaemia, the World Health Organization (WHO) recommends that 2.8 mg (7 x 0.4 mg) folic acid be given instead of the current weekly practice of 0.4 mg. Currently, there is a lack of evidence to support if the 2.8 mg folic acid per week dose is sufficient to raise red cell folate concentrations to a level associated with a reduced risk of a NTD-affected pregnancy. We aim to conduct a three-arm randomised controlled trial to determine the effect of weekly folic acid with iron on erythrocyte folate, a biomarker of NTD risk.

Methods and analysis: We will recruit women (n=300; 18-45 y) from Selangor, Malaysia. Women will be randomised to receive either 2.8, 0.4, or 0 (placebo) mg folic acid with 60 mg iron weekly for 16 weeks, followed by a 4-week washout period. The primary outcome will be erythrocyte folate concentration at 16 weeks and the mean concentration will be compared between randomised treatment groups (intention-to-treat) using a linear regression model adjusting for the baseline measure.

Ethics and Dissemination: Ethical approval was obtained from the University of British Columbia (H18-00768) and Universiti Putra Malaysia (JKEUPM-2018-255). The results of this trial will be presented at scientific conferences and published in peer-reviewed journals.

Trial Registration Number: Australia New Zealand Clinical Trials Registry (ACTRN12619000818134).

KEY WORDS

Women, anaemia, folic acid, neural tube defects, periconception, policy, supplementation

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This is the first trial to investigate the effect of once-weekly iron folic acid supplements on erythrocyte folate concentrations, a biomarker of NTD risk.
- This study will assess whether the current WHO guidelines for the weekly supplementation of iron folic acid with 2.8 mg of folic acid are sufficient to raise red cell folate concentrations to a level associated with a reduced risk of a NTD-affected pregnancy.
- NTD incidence as a primary outcome would be unethical, but erythrocyte folate concentration is an accepted biomarker of NTD risk.

INTRODUCTION

In 2015, an estimated 260,000 infants were born globally with a NTD.[1] NTDs, such as anencephaly and spina bifida, are caused by the failure of the neural tube to close properly around 28 days post-conception and are a significant cause of mortality and morbidity. Evidence from controlled trials has shown that up to 80% of NTDs could be prevented if women were to take folic acid supplements prior to and during early pregnancy.[2–4] As such, the WHO recommends that women planning to become pregnant take 0.4 mg folic acid per day.[5] Because many pregnancies are unplanned and the neural tube closes early in pregnancy,[6,7] before women may know they are pregnant, many countries have mandated the addition of folic acid to wheat flour or other grain staples.[8] This has led to a reduction of NTDs in these countries.[9,10] In many countries where wheat flour is not the staple, or where milling of staple grains is at the village or household level, fortification is difficult.[11] Alternative strategies are needed to deliver folic acid to women of reproductive age.

In countries where the prevalence of anaemia is ≥20%, the WHO recommends blanket intermittent supplementation of all women of reproductive age with weekly iron (60 mg) and folic acid (2.8 mg). The dose of folic was chosen as it is seven times the 0.4 mg daily dose found to be effective in reducing NTDs in controlled trials.[4] However, there is a reluctance to change the dose of folic acid to 2.8 mg weekly for non-pregnant women due to the lack of evidence that this higher dose will reduce NTDs. Further, the global prevalence of anaemia due to folate deficiency is low and there have been calls to remove folic acid from these supplements altogether.[13]

Further randomized trials with NTDs as an outcome would be unethical. However, erythrocyte folate in women of reproductive age is accepted as a proxy indicator of NTD risk. In a case control study from Dublin, Ireland, erythrocyte folate measured early in pregnancy was inversely associated with NTD risk, with an erythrocyte folate >906 nmol/L considered desirable for maximal prevention.[14] There are no studies which compare the current effect of weekly 0.4 mg folic acid, the dose currently contained in most weekly supplements on the market, with the recommended weekly 2.8 mg folic acid on erythrocyte folate concentration in non-pregnant women of reproductive age.

The primary aim of this randomised placebo-controlled trial is to compare the effect of the WHO recommended weekly folic acid dose of 2.8 mg versus the current weekly 0.4 mg folic acid practice or no folic acid (placebo) on erythrocyte folate concentrations after 16 weeks of treatment. Weekly iron folic acid programs are often started when women are in secondary school.[12] Women in school have several periods of school break during the year. Thus, we will include a 4-week washout period to determine the effect that a school break would have on erythrocyte folate concentrations. This research is needed by policy makers across the globe and will inform WHO guidelines on the optimal weekly dose of folic acid, if any, needed for NTD prevention.

METHODS AND ANALYSIS

Trial design

This is a three-arm, parallel-group, randomised controlled trial with a 16-week intervention period followed by a 4-week washout period. Participants will attend three clinic visits: baseline (0 weeks), endline (16 weeks), and washout (20 weeks).

Location

This study will take place at Universiti Putra Malaysia in Selangor, Malaysia. Malaysia was chosen for the study location as there is significant technical infrastructure in place and the overall prevalence of anaemia in Malaysia in women of reproductive age is above 20%,[16,17] falling within the criteria of the current WHO guideline for intermittent iron folic acid supplementation.[12] Moreover, there is no folic acid fortification program in Malaysia and folic acid supplement use is not widespread.[18]

Study Population

A total of 300 women (18-45 y) from Selangor, Malaysia will be recruited**.**

Eligibility Criteria

Potential participants will be approached by research staff at Universiti Putra Malaysia.

Inclusion Criteria

Women must meet the following criteria to be enrolled in the study:

- Be between the ages of 18 to 45 years
- Plan on living, working, or studying near Universiti Putra Malaysia for 4 months following enrollment
- Be able to give informed consent

Exclusion criteria

Women must not have any of the following criteria to be enrolled in this study:

- Are pregnant (self-reported) or planning to become pregnant
- Taking folic acid containing supplements
- Participating in another nutritional intervention
- Taking any medication known to inhibit folate status (methotrexate, certain anti-convulsants, or sulphasalazine)

Study treatments

The supplements will be packaged in bottles containing 30 tablets. Each supplement will contain 60 mg of elemental iron as ferrous fumarate and either 0 mg, 0.4 mg, or 2.8 mg of folic acid. Supplements have been produced to United States Pharmacopeia standards. Supplements have been manufactured by Unison Nutraceutical Sdn Bhd in Ayer Keroh, Malacca, Malaysia and approved by the National Pharmaceutical Regulatory Agency in Malaysia. Supplements will be

dispensed in glass opaque bottles containing 30 tablets. The tablets, bottles, and labels on the bottle will be identical except for a coloured sticker which identifies the different treatments.

Monitoring adherence to study treatment

Study staff will be responsible for storage and dispensing of the supplements. Supplements will be administered by study staff at the baseline clinic visit . Participants will be asked to take their first supplement at the clinic and then asked to take the supplement at the same time each week. Participants will be reminded weekly by SMS to take their supplement to encourage adherence and asked to reply by SMS when they have taken the supplement. If they do not reply, research assisitants will contact them by phone. If the participant fails to take the supplement within the five days following their designated day, the supplement will not be taken, and the participant will be considered to have missed treatment that week. Adherence will be assessed by trained research staff by counting remaining tablets in the bottles at 16 weeks.

Outcome measures

The primary outcome will be erythrocyte folate concentration at 16 weeks.

Key secondary outcomes

- Erythrocyte folate concentration at 20 weeks
- Plasma folate concentration at 16 and 20 weeks

Participant timeline

Screening Period

Women who are interested in participating in the study will be given a participant's information sheet and research assistants will answer any questions they may have. Written informed consent will be obtained from each woman who meets the screening criteria and is willing to participate in the study. The assessment time points are summarized in **Table 1**.

Visit 1 (Baseline)

Participants will be asked to attend a clinic at Universiti Putra Malaysia following an overnight fast (since midnight). After reaffirming consent, blood samples will be collected by venipuncture into two evacuated tubes, containing EDTA as an anti-coagulant. Socio-demographic, health, and anthropometric data will be recorded by the researchers. Women with severe anaemia (defined as a haemoglobin concentration <80 g/L) will be contacted within 3 days and referred to a local health centre for follow-up, but will not be excluded from the study unless their medical practitioner recommends withdrawal.[19]

Visit 2 (~16 weeks)

Participants will attend a second clinic visit following an overnight fast. The second visit will be scheduled with a + 2 week allowance to accommodate participant schedules. Participants will be advised to take their last pill no less than 48 hours before the blood draw and up to one week

beforehand. The blood sample will be collected as described in the baseline visit. Adverse events will be recorded, and adherence determined via counting the remaining tablets.

Visit 3 (~20 weeks)
Following 4 weeks (+2 weeks) of not taking any supplements, women will return for a fasting blood sample. Adverse events will be recorded.

Sample size
To detect a clinically meaningful difference of 100 nmol/L in mean erythrocyte folate concentrations across treatment groups at the end of the intervention period (16 weeks), a sample size of 63 participants per group is required, assuming a standard deviation of 202 nmol/L with 80% power while adjusting for baseline erythrocyte folate concentration.[15] A two-sided α of 0.0167 will be used for pairwise comparisons between the three treatment groups (0 mg, 0.4 mg, and 2.8 mg) for an overall α of 0.05. The sample size assumes a correlation between erythrocyte folate concentrations at baseline and 16 weeks of 0.6 and allows for a dropout rate of 10%. We will recruit 100 participants per arm to allow for some uncertainty in the assumed values.

Recruitment
Potential participants will be approached by trained research staff and informed about the purpose of the study, the protocol, and potential risks and benefits of participation. Trained research staff will gather informed consent, voluntary and free from coercion, to ensure that information about the trial is understood.

Randomisation
Following confirmation of eligibility and enrollment, participants will be randomised after the first blood draw using a web-based randomisation service. Participants will be randomly assigned to receive 60 mg of iron as ferrous fumarate and either 0, 0.4, or 2.8 mg of folic acid. A computer-generated randomization schedule will be prepared using ralloc.ado in Stata by an independent statistician who is not involved with trial participants or data analysis. The randomization procedure will use randomly permuted blocks of size six to assign participants to one of six colour codes in the ratio 1:1:1:1:1:1. Two colour codes will be assigned to each treatment by an independent individual who will be responsible for labeling the study products but have no further involvement in the trial.

Blinding
Blinding will only be broken during the trial in the event of an emergency, when the investigator deems that a participant cannot be adequately treated without knowledge of the participant's treatment arm. The principal investigator will be contacted. In order to break the blinding for the affected participant, the investigator must contact the randomization personnel. All attempts to avoid study withdrawal will be made, although participants can cease treatment as appropriate.

Trial arms will only be unblinded once all data have been collected and entered in the study database and analysis of the primary and secondary outcomes has been completed.

Data collection, access, and storage

Up to seven research staff, as needed, will be on site to obtain informed consent, collect data, randomise and distribute tablets. All efforts to ensure data quality will be taken. Study data will be collected and managed using REDCap electronic data capture tools hosted at the South Australian Health and Medical Research Institute (SAHMRI).[20,21] Eleven investigators and research staff will have access to the data during the data collection period. All Co-Investigators will have access to the data at all stages of analyses and interpretation of data. Responsibilities concerning privacy and confidentiality will be reminded and discussed with all Co-Investigators and data entry staff.

Electronic data files will be stored on encrypted and password protected computers using secure servers. Any hard copies of data, consent forms, questionnaires or other papers containing data will be stored in locked filing cabinets in locked research rooms at Universiti Putra Malaysia in Selangor, Malaysia.

Blood collection and processing

A fasting venous blood sample will be collected at weeks 0, 16, and 20 of the trial into evacuated tubes containing EDTA.

Whole Blood for Haematocrit Determination

One EDTA tube will be sent to Clinipath Malaysia Sdn. Bhd. (Selangor, Malaysia) for a full blood count determination using an automated haematology analyzer.

Whole Blood for Plasma and Erythrocyte Folate, Buffy Coat, and Erythrocytes

The other tube will be inverted gently 8-10 times to ensure the blood is properly mixed. For erythrocyte folate, 100 µL of whole blood and 1000 µL of 1% ascorbic acid (~1 in 11), will be added to three separate tubes and then vortexed for five seconds. Samples will then be incubated at 38℃ for 30 minutes and subsequently placed on ice for storage at -80℃. Samples will be centrifuged at 3000 rpm for 10 minutes at 4°C. Plasma will be aliquoted into three labeled microtubes and stored at -80℃. Buffy coat will be aliquoted into a single labeled microtube and stored at -80℃.

Blood analyses

De-identified blood samples will be shipped on dry ice to SAHMRI, Adelaide, Australia where plasma folate (nmol/L) and erythrocyte folate (nmol/L) concentrations will be determined using techniques harmonized by the Centers for Disease Control and Prevention.[22] Plasma ferritin (µg/L), soluble transferrin receptor (sTfR, mg/L), α-1 acid glycoprotein (AGP, g/L), C-reactive

protein (CRP, mg/L) and retinol binding protein (RBP, μmol/L) will be analyzed at the VitMin lab in Germany (Table 2).

Statistical analysis

The primary analysis will be performed on an 'intention-to-treat' basis, according to treatment allocation at randomisation. A secondary 'per-protocol' analysis will also be performed including only women who complete the study and are >80% adherent to the treatment regime. Continuous outcomes, including the primary outcome erythrocyte folate concentration at 16 weeks, will be analysed using linear regression models and binary outcomes will be analysed using log binomial regression models. Independent variables will include randomised treatment group, time point (16 or 20 weeks) and a treatment group by time point interaction. Treatment effects (0.4 mg vs 0 mg, 2.8 mg vs 0 mg and 2.8 mg vs 0.4 mg) will be estimated for each time point separately (16 and 20 weeks) along with 95% confidence intervals and two-sided p-values. Clustering due to repeated measurements on the same individuals at different time points will be considered using generalised estimating equations. Adjustment will be made for the baseline measure of the analysed outcome. Missing data will be addressed using multiple imputation to create 100 complete datasets for analysis, with a sensitivity analysis performed on the available data. All analyses will follow a pre-specified statistical analysis plan.

ETHICS AND DISSEMINATION

Ethics

The Ethics Committee for Research Involving Human Subjects of Universiti Putra Malaysia (JKEUPM-2018-255) and The University of British Columbia Clinical Research Ethics Board (H18-00768) approved the research. This trial is also registered with the Malaysian National Medical Register (NMRR-19-119-45736).

Confidentiality

Participant confidentiality will be maintained throughout the trial. Confidentiality will extend to the biological testing of samples and additional medical information. The protocol and all study documents will be held in strict confidence. No information about the study or its data will be released to unauthorized third parties. Any medical information of the participants will not be released without the permission of the participant.

Use of data and publication policy

Publication of information regarding this protocol or its data in formats including, but not limited to, conference abstracts, posters or presentation, seminars, journal articles, public reports and internet postings. Approval of these activities must have the permission of all Co-Investigators before the event. The results of this trial will be published in peer-reviewed scientific journals and presented at conferences. Additionally, all results will be relayed to the relevant stakeholders, including the Ministry of Health, and the WHO.

Patient and Public Involvement

The development of the research question and outcome measures were not informed by the participants' priorities, experience, and preferences. Participants were not involved in the design of this study. Participants will be provided with a summary of the trial findings and their personal results. Results will also be disseminated to policy makers and The Ministry of Health through briefing papers containing summaries of the main findings.

Authors' contributions: LSP, KGL, MLR, ZMBS, LMDR, TJG, and CDK conceived the trial and proposed the trial design; LNY, SL and JAH advised on sample size calculations and trial design and analysis; KLIS, TJG, and CDK drafted the protocol, all authors reviewed the protocol and approved the final submission.

Funding statement: This work was supported by Nutrition International grant number (10-1798-SOUAUS-02). LNY is supported by an Australian National Health and Medical Research Council Early Career Fellowship (ID 1052388).

Competing interest statement: The authors declare no conflict of interest.

Data sharing statement: Once the primary trial is published, the data will be available for data sharing to appropriately qualified investigators upon submission of a protocol and approval of the study steering committee. Please send requests to Tim Green (Tim.Green@sahmri.com).

TABLES

Table 1. Assessment time points

	Assessment Time Points			
Visit (V)	**Screening**	**V1**	**V2**	**V3**
Time per study site session				
Week		Week 0	Week 16	Week 20
Enrollment and Randomization				
Eligibility assessment	**X**			
Randomization		**X**		
Implementation				
Questionnaire		**X**		
Blood collection		**X**	**X**	**X**
Adverse event reporting			**X**	**X**
Supplementation				
Tablet distribution		**X**		
Tablet count			**X**	

Table 2. A summary of study blood analytes and methods

Analyte	Methods
Plasma folate	Microtiter technique with chloramphenicol-resistant Lactobacillus casei as the test microorganism [23]
Erythrocyte folate	Calculated from whole blood folate by subtracting plasma folate and correcting for haematocrit
Plasma vitamin B_{12}	Elecsys® 2010 (Roche Diagnostics, Switzerland) automated electrochemiluminescence immunoassay
Plasma ferritin, sTfR, AGP, CRP, and RBP	Single sandwich-enzyme linked immunosorbent assay (s-ELISA)[24]

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SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

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	2
	2b	All items from the World Health Organization Trial Registration Data Set *This trial is registered in the Australia New Zealand Clinical Trials Registry*	N/A
Protocol version	3	Date and version identifier *This information is not applicable to the manuscript paper*	N/A
Funding	4	Sources and types of financial, material, and other support	9
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	1, 9
	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	1,9
	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)	9

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	3
	6b	Explanation for choice of comparators	3-4
Objectives	7	Specific objectives or hypotheses	5
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)	4
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	4-5
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)	4
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	4-5
	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)	7
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	5
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	4
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	3,5
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)	5-6,9

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	6
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	6
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	6
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	6
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	7
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	7
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	7-8
	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	5

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	7
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	8
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	8
	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)	8
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 *This information is not included in the manuscript.*	N/A
	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 *This information is not included in the manuscript.*	N/A
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	6
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor *This information is not included in the manuscript.*	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	8

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) *This information is not included in the manuscript.*	N/A
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	5
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable *This is not written in the consent or protocol as we are not requesting this.*	N/A
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	7-8
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	9
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	N/A
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	5
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	8-9
	31b	Authorship eligibility guidelines and any intended use of professional writers	No
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	No
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates *This journal does not require submission of a consent form along with the protocol paper.*	N/A

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	_____7-8_____

*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

The effect of once weekly folic acid supplementation on erythrocyte folate concentrations in women to determine potential to prevent neural tube defects: A randomised controlled dose-finding trial in Malaysia

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The effect of once weekly folic acid supplementation on erythrocyte folate concentrations in women to determine potential to prevent neural tube defects: A randomised controlled dose-finding trial in Malaysia

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ABSTRACT

Introduction: Folic acid (0.4 mg) taken prior to and during early pregnancy reduces the risk of neural tube defects (NTD). Because these birth defects occur early in pregnancy, before women may know they are pregnant, many countries have mandated the addition of folic acid to food staples. In countries where fortification is not possible, and weekly iron folic acid programs exist to reduce anaemia, the World Health Organization (WHO) recommends that 2.8 mg (7 x 0.4 mg) folic acid be given instead of the current weekly practice of 0.4 mg. Currently, there is a lack of evidence to support if the 2.8 mg folic acid per week dose is sufficient to raise erythrocyte folate concentrations to a level associated with a reduced risk of a NTD-affected pregnancy. We aim to conduct a three-arm randomised controlled trial to determine the effect of weekly folic acid with iron on erythrocyte folate, a biomarker of NTD risk.

Methods and analysis: We will recruit non-pregnant women (n=300; 18-45 y) from Selangor, Malaysia. Women will be randomised to receive either 2.8, 0.4, or 0 (placebo) mg folic acid with 60 mg iron weekly for 16 weeks, followed by a 4-week washout period. The primary outcome will be erythrocyte folate concentration at 16 weeks and the mean concentration will be compared between randomised treatment groups (intention-to-treat) using a linear regression model adjusting for the baseline measure.

Ethics and Dissemination: Ethical approval was obtained from the University of British Columbia (H18-00768) and Universiti Putra Malaysia (JKEUPM-2018-255). The results of this trial will be presented at scientific conferences and published in peer-reviewed journals.

Trial Registration Number: Australia New Zealand Clinical Trials Registry (ACTRN12619000818134).

KEY WORDS

Women, anaemia, folic acid, neural tube defects, periconception, policy, supplementation

STRENGTHS AND LIMITATIONS OF THIS STUDY

- This is the first study to determine the optimal dose of folic acid to be included in weekly iron folic acid supplements to reduce NTD risk.
- Women of reproductive age will be randomised to a weekly iron (60 mg) supplement with either 0 mg, 0.4 mg (current practice), or 2.8 mg (WHO recommendation) folic acid.
- The primary outcome is erythrocyte folate, a biomarker of NTD risk, concentration after 16 weeks by treatment group.
- Erythrocyte folate is inversely associated with NTD risk.
- NTD as an outcome would not be ethical or feasible.

INTRODUCTION

In 2015, an estimated 260,000 infants were born globally with a NTD.[1] NTDs, such as anencephaly and spina bifida, are caused by the failure of the neural tube to close properly around 28 days post-conception and are a significant cause of mortality and morbidity. Evidence from controlled trials has shown that up to 80% of NTDs could be prevented if women were to take folic acid supplements prior to and during early pregnancy.[2–4] As such, the WHO recommends that women planning to become pregnant take 0.4 mg folic acid per day.[5] Because many pregnancies are unplanned and the neural tube closes early in pregnancy,[6,7] before women may know they are pregnant, many countries have mandated the addition of folic acid to wheat flour or other grain staples.[8] This has led to a reduction of NTDs in these countries.[9,10] In many countries where wheat flour is not the staple, or where milling of staple grains is at the village or household level, fortification is difficult.[11] Alternative strategies are needed to deliver folic acid to women of reproductive age.

In countries where the prevalence of anaemia is ≥20%, the WHO recommends blanket intermittent supplementation of all women of reproductive age with weekly iron (60 mg) and folic acid (2.8 mg). The dose of folic was chosen as it is seven times the 0.4 mg daily dose found to be effective in reducing NTDs in controlled trials.[4] However, there is a reluctance to change the dose of folic acid to 2.8 mg weekly for non-pregnant women due to the lack of evidence that this higher dose will reduce NTDs. Further, the global prevalence of anaemia due to folate deficiency is low and there have been calls to remove folic acid from these supplements altogether.[12]

Further randomised trials with NTDs as an outcome would be unethical. However, erythrocyte folate in women of reproductive age is accepted as a proxy indicator of NTD risk. In a case control study from Dublin, Ireland, erythrocyte folate measured early in pregnancy was inversely associated with NTD risk, with an erythrocyte folate >906 nmol/L considered desirable for maximal prevention.[13] There are no studies which compare the current effect of weekly 0.4 mg folic acid, the dose currently contained in most weekly supplements on the market, with the recommended weekly 2.8 mg folic acid on erythrocyte folate concentration in non-pregnant women of reproductive age.

The primary aim of this randomised placebo-controlled trial is to compare the effect of the WHO recommended weekly folic acid dose of 2.8 mg versus the current weekly 0.4 mg folic acid practice or no folic acid (placebo) on erythrocyte folate concentrations after 16 weeks of treatment. Weekly iron folic acid programs are often started when women are in secondary school.[14] Women in school have several periods of school break during the year. Thus, we will include a 4-week washout period to determine the effect that a school break would have on erythrocyte folate concentrations. This research is needed by policy makers across the globe and will inform WHO guidelines on the optimal weekly dose of folic acid, if any, needed for NTD prevention.

METHODS AND ANALYSIS

Trial design

This is a three-arm, parallel-group, randomised controlled trial with a 16-week intervention period followed by a 4-week washout period. Participants will attend three clinic visits: baseline (0 weeks), endline (16 weeks), and washout (20 weeks). This trial started in September 2019 and is projected to run until February 2020.

Location

This study will take place at Universiti Putra Malaysia in Selangor, Malaysia. Malaysia was chosen for the study location as there is significant technical infrastructure in place and the overall prevalence of anaemia in Malaysia in women of reproductive age is above 20%,[15,16] falling within the criteria of the current WHO guideline for intermittent iron folic acid supplementation.[14] Moreover, there is no folic acid fortification program in Malaysia and folic acid supplement use is not widespread.[17–19]

Study Population

A total of 300 women (18-45 y) from Selangor, Malaysia will be recruited**.**

Eligibility Criteria

Potential participants will be approached by research staff at Universiti Putra Malaysia.

Inclusion Criteria

Women must meet the following criteria to be enrolled in the study:

- Be between the ages of 18 to 45 years
- Not be pregnant
- Plan on living, working, or studying near Universiti Putra Malaysia for 4 months following enrollment
- Be able to give informed consent

Exclusion criteria

Women must not have any of the following criteria to be enrolled in this study:

- Are pregnant (self-reported) or planning to become pregnant
- Taking folic acid containing supplements
- Participating in another nutritional intervention
- Taking any medication known to inhibit folate status (methotrexate, certain anti-convulsants, or sulphasalazine)

Study treatments

The supplements will be packaged in bottles containing 30 tablets. Each supplement will contain 60 mg of elemental iron as ferrous fumarate and either 0 mg, 0.4 mg, or 2.8 mg of folic acid to be taken weekly. Supplements have been produced to United States Pharmacopeia standards.

Supplements have been manufactured by Unison Nutraceutical Sdn Bhd in Ayer Keroh, Malacca, Malaysia and approved by the National Pharmaceutical Regulatory Agency in Malaysia. Supplements will be dispensed in glass opaque bottles containing 30 tablets. The tablets, bottles, and labels on the bottle will be identical except for a coloured sticker which identifies the different treatments.

Monitoring adherence to study treatment

Study staff will be responsible for storage and dispensing of the supplements. Supplements will be administered by study staff at the baseline clinic visit . Participants will be asked to take their first supplement at the clinic and then asked to take the supplement at the same time each week. Participants will be reminded weekly by SMS to take their supplement to encourage adherence and asked to reply by SMS when they have taken the supplement. If they do not reply, research assistants will contact them by phone. If the participant fails to take the supplement within the five days following their designated day, the supplement will not be taken, and the participant will be considered to have missed treatment that week. Adherence will be assessed by trained research staff by counting remaining tablets in the bottles at 16 weeks.

Outcome measures

The primary outcome will be erythrocyte folate concentration at 16 weeks.

Key secondary outcomes

- Erythrocyte folate concentration at 20 weeks
- Plasma folate concentration at 16 and 20 weeks

Participant timeline

Screening Period

Women who are interested in participating in the study will be given a participant's information sheet and research assistants will answer any questions they may have. Written informed consent will be obtained from each woman who meets the screening criteria and is willing to participate in the study. The assessment time points are summarized in **Table 1**.

Visit 1 (Baseline)

Participants will be asked to attend a clinic at Universiti Putra Malaysia following an overnight fast (since midnight). After reaffirming consent, blood samples will be collected by venipuncture into two evacuated tubes, containing EDTA as an anti-coagulant. Socio-demographic, health, and anthropometric data will be recorded by the researchers. Women with severe anaemia (defined as a haemoglobin concentration <80 g/L) will be contacted within 3 days and referred to a local health centre for follow-up, but will not be excluded from the study unless their medical practitioner recommends withdrawal.[20]

Visit 2 (~16 weeks)
Participants will attend a second clinic visit following an overnight fast. The second visit will be scheduled with a + 2 week allowance to accommodate participant schedules. Participants will be advised to take their last pill no less than 48 hours before the blood draw and up to one week beforehand. The blood sample will be collected as described in the baseline visit. Adverse events will be recorded, and adherence determined via counting the remaining tablets.

Visit 3 (~20 weeks)
Following 4 weeks (+2 weeks) of not taking any supplements, women will return for a fasting blood sample. Adverse events will be recorded.

Sample size

To detect a clinically meaningful difference of 100 nmol/L in mean erythrocyte folate concentrations across treatment groups at the end of the intervention period (16 weeks), a sample size of 63 participants per group is required, assuming a standard deviation of 202 nmol/L with 80% power while adjusting for baseline erythrocyte folate concentration.[21] A two-sided α of 0.0167 will be used for pairwise comparisons between the three treatment groups (0 mg, 0.4 mg, and 2.8 mg) for an overall α of 0.05. The sample size assumes a correlation between erythrocyte folate concentrations at baseline and 16 weeks of 0.6 and allows for a dropout rate of 10%. We will recruit 100 participants per arm to allow for some uncertainty in the assumed values.

Recruitment

Potential participants will be approached by trained research staff and informed about the purpose of the study, the protocol, and potential risks and benefits of participation. Trained research staff will gather informed consent, voluntary and free from coercion, to ensure that information about the trial is understood.

Randomisation

Following confirmation of eligibility and enrollment, participants will be randomised after the first blood draw using a web-based randomisation service. Participants will be randomly assigned to receive 60 mg of iron as ferrous fumarate and either 0, 0.4, or 2.8 mg of folic acid weekly. A computer-generated randomization schedule will be prepared using ralloc.ado in Stata by an independent statistician who is not involved with trial participants or data analysis. The randomization procedure will use randomly permuted blocks of size six to assign participants to one of six colour codes in the ratio 1:1:1:1:1:1. Two colour codes will be assigned to each treatment by an independent individual who will be responsible for labeling the study products but have no further involvement in the trial.

Blinding

Blinding will only be broken during the trial in the event of an emergency, when the investigator deems that a participant cannot be adequately treated without knowledge of the participant's treatment arm. The principal investigator will be contacted. In order to break the blinding for the affected participant, the investigator must contact the randomization personnel. All attempts to avoid study withdrawal will be made, although participants can cease treatment as appropriate. Trial arms will only be unblinded once all data have been collected and entered in the study database and analysis of the primary and secondary outcomes has been completed.

Data collection, access, and storage

Up to seven research staff, as needed, will be on site to obtain informed consent, collect data, randomise and distribute tablets. All efforts to ensure data quality will be taken. Study data will be collected and managed using REDCap electronic data capture tools hosted at the South Australian Health and Medical Research Institute (SAHMRI).[22,23] Eleven investigators and research staff will have access to the data during the data collection period. All Co-Investigators will have access to the data at all stages of analyses and interpretation of data. Responsibilities concerning privacy and confidentiality will be reminded and discussed with all Co-Investigators and data entry staff.

Electronic data files will be stored on encrypted and password protected computers using secure servers. Any hard copies of data, consent forms, questionnaires or other papers containing data will be stored in locked filing cabinets in locked research rooms at Universiti Putra Malaysia in Selangor, Malaysia.

Blood collection and processing

A fasting venous blood sample will be collected at weeks 0, 16, and 20 of the trial into evacuated tubes containing EDTA.

Whole Blood for Haematocrit Determination

One EDTA tube will be sent to Clinipath Malaysia Sdn. Bhd. (Selangor, Malaysia) for a full blood count determination using an automated haematology analyzer.

Whole Blood for Plasma and Erythrocyte Folate, Buffy Coat, and Erythrocytes

The other tube will be inverted gently 8-10 times to ensure the blood is properly mixed. For erythrocyte folate, 100 µL of whole blood and 1000 µL of 1% ascorbic acid (~1 in 11), will be added to three separate tubes and then vortexed for five seconds. Samples will then be incubated at 38℃ for 30 minutes and subsequently placed on ice for storage at -80℃. Samples will be centrifuged at 3000 rpm for 10 minutes at 4°C. Plasma will be aliquoted into three labeled microtubes and stored at -80℃. Buffy coat will be aliquoted into a single labeled microtube and stored at -80℃.

Blood analyses

De-identified blood samples will be shipped on dry ice to SAHMRI, Adelaide, Australia where plasma folate (nmol/L) and erythrocyte folate (nmol/L) concentrations will be determined using the folate microbiological assay harmonized by the Centers for Disease Control and Prevention.[24] Plasma ferritin (μg/L), soluble transferrin receptor (sTfR, mg/L), α-1 acid glycoprotein (AGP, g/L), C-reactive protein (CRP, mg/L) and retinol binding protein (RBP, μmol/L) will be analyzed at the VitMin lab in Germany (Table 2).

Statistical analysis

The primary analysis will be performed on an 'intention-to-treat' basis, according to treatment allocation at randomisation. A secondary 'per-protocol' analysis will also be performed including only women who complete the study and are >80% adherent to the treatment regime. Continuous outcomes, including the primary outcome erythrocyte folate concentration at 16 weeks, will be analysed using linear regression models and binary outcomes will be analysed using log binomial regression models. Independent variables will include randomised treatment group, time point (16 or 20 weeks) and a treatment group by time point interaction. Treatment effects (0.4 mg vs 0 mg, 2.8 mg vs 0 mg and 2.8 mg vs 0.4 mg) will be estimated for each time point separately (16 and 20 weeks) along with 95% confidence intervals and two-sided p-values. Clustering due to repeated measurements on the same individuals at different time points will be considered using generalised estimating equations. Adjustment will be made for the baseline measure of the analysed outcome as this is expected to be strongly related to the outcome; no adjustment for other baseline variables or subgroup analyses are planned. Missing data will be addressed using multiple imputation to create 100 complete datasets for analysis, with a sensitivity analysis performed on the available data. All analyses will follow a pre-specified statistical analysis plan.

ETHICS AND DISSEMINATION

Ethics

The Ethics Committee for Research Involving Human Subjects of Universiti Putra Malaysia (JKEUPM-2018-255) and The University of British Columbia Clinical Research Ethics Board (H18-00768) approved the research. This trial is also registered with the Malaysian National Medical Register (NMRR-19-119-45736).

Confidentiality

Participant confidentiality will be maintained throughout the trial. Confidentiality will extend to the biological testing of samples and additional medical information. The protocol and all study documents will be held in strict confidence. No information about the study or its data will be released to unauthorized third parties. Any medical information of the participants will not be released without the permission of the participant.

Use of data and publication policy

Publication of information regarding this protocol or its data in formats including, but not limited to, conference abstracts, posters or presentation, seminars, journal articles, public reports and internet postings. Approval of these activities must have the permission of all Co-Investigators before the event. The results of this trial will be published in peer-reviewed scientific journals and presented at conferences. Additionally, all results will be relayed to the relevant stakeholders, including the Ministry of Health and the WHO.

Patient and Public Involvement

The development of the research question and outcome measures were not informed by the participants' priorities, experience, and preferences. Participants were not involved in the design of this study. Participants will be provided with a summary of the trial findings and their personal results. Results will also be disseminated to policy makers and the Ministry of Health through briefing papers containing summaries of the main findings.

Authors' contributions: SPL, GLK, MLR, ZBMS, MM, LMDR, TJG, and CDK conceived the trial and proposed the trial design; LNY, SL and JAH advised on sample size calculations, trial design, and analysis; DCS advised on analytical methodology; KLIS, JJY, TJG, and CDK drafted the protocol, all authors reviewed the protocol and approved the final submission.

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Competing interest statement: The authors declare no conflict of interest.

Data sharing statement: Once the primary trial is published, the data will be available for data sharing to appropriately qualified investigators upon submission of a protocol and approval of the study steering committee. Please send requests to Tim Green (Tim.Green@sahmri.com).

TABLES

Table 1. Assessment time points

	Assessment Time Points			
Visit (V)	**Screening**	**V1**	**V2**	**V3**
Time per study site session				
Week		Week 0	Week 16	Week 20
Enrollment and Randomization				
Eligibility assessment	**X**			
Randomization		**X**		
Implementation				
Questionnaire		**X**		
Blood collection		**X**	**X**	**X**
Adverse event reporting			**X**	**X**
Supplementation				
Tablet distribution		**X**		
Tablet count			**X**	

Table 2. A summary of study blood analytes and methods

Analyte	**Methods**
Plasma folate	Microtiter technique with chloramphenicol-resistant Lactobacillus casei as the test microorganism [25]
Erythrocyte folate	Calculated from whole blood folate by subtracting plasma folate and correcting for haematocrit
Plasma vitamin B_{12}	Elecsys® 2010 (Roche Diagnostics, Switzerland) automated electrochemiluminescence immunoassay
Plasma ferritin, sTfR, AGP, CRP, and RBP	Single sandwich-enzyme linked immunosorbent assay (s-ELISA)[26]

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SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

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	2
	2b	All items from the World Health Organization Trial Registration Data Set *This trial is registered in the Australia New Zealand Clinical Trials Registry*	N/A
Protocol version	3	Date and version identifier *This information is not applicable to the manuscript paper*	N/A
Funding	4	Sources and types of financial, material, and other support	9
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	1, 9
	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	1,9
	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)	9

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	3
	6b	Explanation for choice of comparators	3-4
Objectives	7	Specific objectives or hypotheses	5
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)	4
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	4-5
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)	4
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	4-5
	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)	7
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	5
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	4
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	3,5
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)	5-6,9

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	6
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	6
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	6
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	6
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	7
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	7
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	7-8
	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	5

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	7
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	8
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	8
	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)	8
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 *This information is not included in the manuscript.*	N/A
	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 *This information is not included in the manuscript.*	N/A
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	6
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor *This information is not included in the manuscript.*	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	8

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) *This information is not included in the manuscript.*	N/A
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	5
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable *This is not written in the consent or protocol as we are not requesting this.*	N/A
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	7-8
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	9
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	N/A
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	5
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	8-9
	31b	Authorship eligibility guidelines and any intended use of professional writers	No
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	No
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates *This journal does not require submission of a consent form along with the protocol paper.*	N/A

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	7-8

*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.