BMJ Open

Efficacy of adjunctive azithromycin versus single-dose cephalosporins prophylaxis for caesarean scar defect : study protocol for a randomized controlled trial

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-032379
Article Type:	Protocol
Date Submitted by the Author:	15-Jun-2019
Complete List of Authors:	CAI, Yanqing; International Peace Maternity& Child Health Hospital,School of Medicine,Shanghai Jiao Tong University, obstetric pan, hongjie Zhang, Jian CHENG, Weiwei shi, yiru Zeng, Min shi, liye Yu, Jin shen, ying Chen, Shan Zhu, Qian; International Peace Maternity and Child Health Hospital/School of Medicine, Shanghai Jiaotong University, Department of Obstetrics and Gynecology Mol, Ben; School of Medicine, Monash University, Melbourne, Australia, OB/GYN Huang, Ding
Keywords:	caesarean section, caesarean scar defect, azithromycin, RCT, antibiotic

Efficacy of adjunctive azithromycin versus single-dose cephalosporins prophylaxis for caesarean scar defect : study protocol for a randomized controlled trial

Yanqing Cai[1,*], Hongjie Pan[1,4*], Jian Zhang[1], Weiwei Chen[1], Yiru Shi[1], Min Zeng[2], Liye Shi[2], Jin Yu[1], Ying Shen[1], Sha Chen[1], Qian Zhu[1], Ben WJ Mol[3], Ding Huang[1]

[1]Department of Obstetrics and Gynecology, International Peace Maternity & Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

[2]Department of Ultrasound, International Peace Maternity & Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

[3]Department of Obstetrics and Gynaecology, Monash Medical Centre, Monash University, Melbourne, Victoria, Australia.

[4]Department of Obstetrics and Gynecology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Jianggan District, Hangzhou, China;

Correspondence author

Department of Obstetrics and Gynecology, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiaotong University, No. 910 Hengshan Road, Xuhui District, Shanghai, China, 200020

Email: dingding123hos@163.com; Tel : +86 13817771640 ; fax : +86 021-64078220

Word count: 2905

*These authors contributed equally to this work.

ABSTRACT

Introduction Perioperative infection should be considered predictors of caesarean scar defect (CSD), and multi-dose antibiotics have a protective effect. However, the effects of using adjunctive azithromycin combined cephalosporins to reduce the prevalence of CSD remain unclear. The planned study aims to clarify the protective effect of antibiotics on CSD and to assess the effectiveness of adjunctive azithromycin prophylaxis for CSD.

Methods and analysis This study is an open-label, double-blind, parallel control randomized clinical trial in the International Peace Maternity & Child Health Hospital.

Eligible patients will be randomised (1:1) to receive either adjunctive azithromycin or single-dose cephalosporin 30 minutes before the incision. The evaluation criteria are the prevalence and characteristics of CSD as assessed by transvaginal ultrasound (TVU) and saline infusion sonohysterography (SIS) at 42 days, 6 months and 12 months after delivery.

Ethics and dissemination This protocol received authorization from the Medical Research Ethics Committee of International Peace Maternity and Child Health Hospital on 25 April 2018(approval no. GKLW2017-84). Findings will be distributed through peer-reviewed publications and presentations at international scientific meetings.

Trial registration number ChiCTR-INR-17013272

Keywords: caesarean section, caesarean s**car** defect, azithromycin, RCT, antibiotic

ARTICLE SUMMARY

Strengths and limitations of this study:

1. This is the first interventional randomized controlled trial with the primary aims of assessing the protective effect of antibiotics for CSD and investigating the relationship of CSD occurrence and preoperative infection.
2. This study is based on a previous cohort study to improve the study effectiveness and quality of the study objectives[1].
3. This is the first time to set the initial CSD assessment timepoint as 6 weeks postpartum and follow patients for 1 year. We can find the optimal timepoint for the early detection of CSD.
4. The study is a randomized controlled trial with a relatively large sample size, long follow-up period, and an active control group to control for non-specific effects.
5. The trial is based in a single center and only Chinese individuals are included, which might limit the generalizability of the findings.

INTRODUCTION

The rate of caesarean section (CS) delivery is increasing worldwide, especially in China due to the prevalence of maternally requested caesarean deliveries and the 30-year national one-child policy[2 3]. According to the data from official figures of the China National Bureau of Statistics, there were approximately 17 million newborns in China in 2017, with caesarean delivery rate of approximately 40%, which means that there are approximately 7 million CS deliveries each year[4].

Caesarean scar defect (CSD) is associated with abnormal uterine bleeding (75-

82%), postmenstrual spotting (29-34%) and caesarean scar ectopic pregnancies (1:1,800-1:2,216) and infertility (32/92[5] [6]. Uterine dehiscence, uterine rupture, caesarean scar pregnancy and morbidly adherent placenta are also associated with CSD[7-10]. There are thousands of fatalities or cases of near-death caused by uterine rupture or haemorrhage originated to CSD annually[1] [11].

In a random population of women with a history of CS, the prevalence of CSD ranged from 24% to 70% and 56% to 84% when assessed by TVU with and without contrast enhancement, respectively. saline infusion sonohysterography (SIS) is better than traditional TVU for characterizing CSD and has a higher sensitivity[12-14]. Moreover, sonohysterography can more precisely evaluate the size and shape of the defect and thus severity[2] [15-17]. We will record data to compare the difference in these two methods.

Our previously published data first introduced the CSD prevalence in Shanghai of 43.4% (95% confidence interval (CI)=39.1-47.7%, N=514), which is a cause for concern[1]. Our cohort study also revealed that infection might be an important risk factor for CSD and that multi-dose antibiotic administration has a significant protective effect compared with single-dose antibiotics (CSD prevalence 31.1%, 95% CI=23.8-38.3% versus 49.0%, 95% CI=43.8-54.3%; aOR=[1]0.4, 95% CI=0.3-0.7)[18-20]. Furthermore, the single-dose cefuroxime subgroup (n=190) was found to have a significantly lower CSD prevalence than the single-dose cefradine subgroup (n=162) (38.9%, 95% CI=34.7-48.6% vs 56.8%, 95% CI=51.3-66.7%; aOR=0.5, 95% CI=0.3-0.8, P = 0.006). These results strongly indicate that antibiotics may be a protective factor for CSD[21] [22].

According to the results of pathogen monitoring for nosocomial infections, ureaplasma urealyticum, escherichia coli, enterococcus and streptococcus are the most common infectious agents[23] [24]. Second-generation cephalosporins have a stronger antibacterial effect on *escherichia coli* and *enterobacteriaceae* than first-generation cephalosporins, while azithromycin has stronger antibacterial and bacteriostatic effects on atypical pathogens, such as *mycoplasma chlamydia* and anaerobic bacteria[25-27]. Current recommendations for antibiotic prophylaxis in cesarean delivery include standard use of a narrow-spectrum antibiotic administered before skin incision, most commonly a first-generation cephalosporin. Recent reports on adjunctive azithromycin prophylaxis for caesarean delivery have shown a significant protective effect for maternal endometritis (3.8% vs 6.1%, P=0.02), wound infection (2.4% vs 6.6%, P<0.001), and serious maternal adverse events (1.5% vs 2.9%, P=0.03), while there was no significant between group difference in secondary neonatal composite outcomes[28]. In a word, the addition of azithromycin to cesarean delivery prophylaxis is less costly and leads to better maternal outcomes in the index and subsequent deliveries[29] [30].

At present domestic and oversea has not been reported studies have confirmed that the prophylactic use of cephalosporins combined azithromycin before surgery can reduce the prevalence of the CSD. Therefore, we design the prospective, randomized, double-blind, parallel-group study to compare the prophylactic use of cephalosporins combined azithromycin is superior to single dose can reduce the prevalence of postpartum uterus diverticulum. Our study has a strong theoretical basis and foundation. This topic can provide evidence for the effective use of antibiotics in clinical practice and the reduction in the rate of CSD.

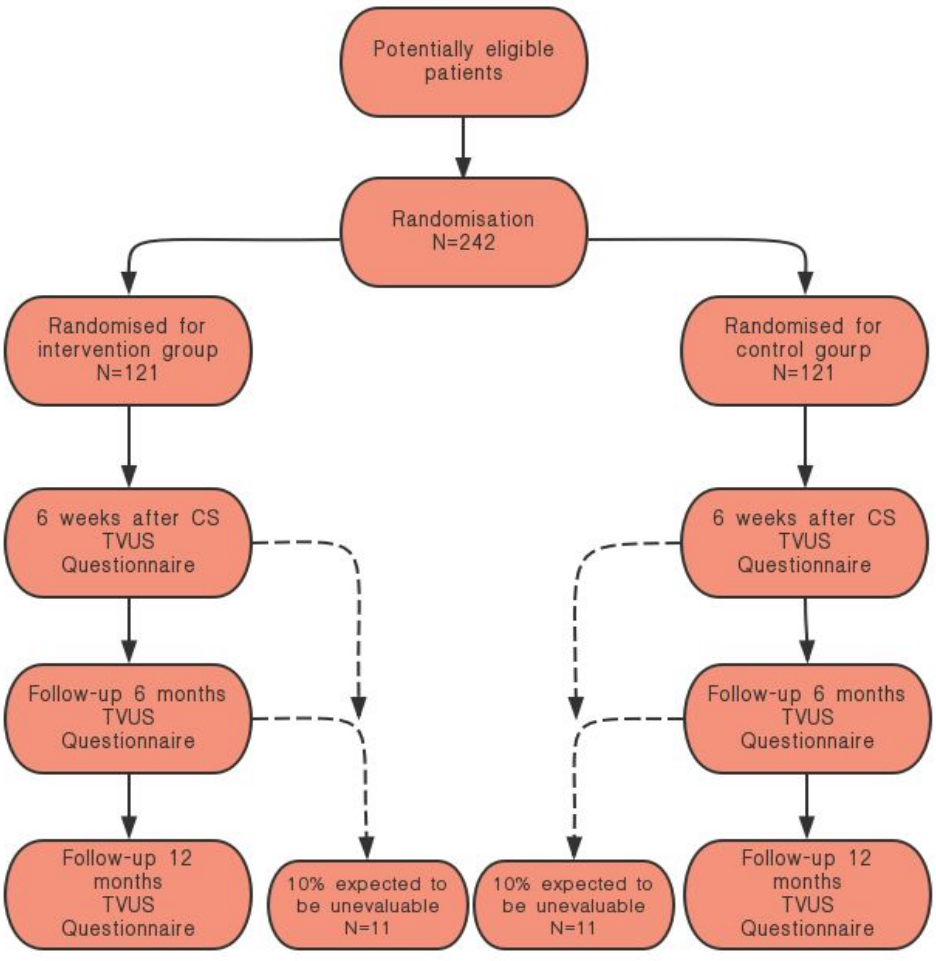

Figure 1 Flowchart of the study. CS, cesarean section; TVUS, transvaginal ultrasound

Objective and hypothesis

The objective of this trial is to clarify the protective effect of antibiotics on CSD and to assess the effectiveness of adjunctive azithromycin prophylaxis for CSD. Our hypothesis is that prophylactic use of cephalosporins combined azithromycin is superior to single dose can reduce the prevalence of CSD.

METHODS AND ANALYSIS

Trial design

The study design is an open-label, double-blind, parallel control design, randomized clinical trial. Pregnant women with singleton pregnancies in cephalic presentation who have ruptured membranes or signs of labour will be informed about the study and the need to undergo CS by the medical team. The subjects will be randomly divided into two groups. The trial group (group A) will be administered the regular single-dose cefuroxime sodium (1.5 g/50 ml 0.9% NaCl) plus azithromycin (0.5 g/250 ml 0.9% NaCl). The control group (group B) will be administered the regular single-dose cefuroxime sodium (1.5 g/50 ml 0.9% NaCl) plus an additional placebo (250 ml 0.9% NaCl) as an intravenous drip 30 min before surgery. The incidence and clinical symptoms of uterine incision malunion or incision diverticulum at 6 weeks, 6 months, and 12 months after delivery will be observed with TVU and SIS. We will include 220 women in total. The primary outcome measure will be the prevalence and characteristic of the CSD as assessed by means of TVU and SIS at 6 weeks after delivery. The secondary outcome measures will be infection indexes and labour results, as shown in Figure 1.

Participants

Patients are included in this study if they meet all the following criteria:

1. Ethnic Han Chinese with a maternal age of 18 years or older;
2. Singleton pregnancy with a gestational age ≥37 weeks;
3. Fibrinogen≥2 g/L, platelet count ≥$100*10^9$, and haemoglobin ≥90 g/L before surgery;
4. Nonelective caesarean delivery during labour or after membrane rupture.
5. Labour will be defined as regular contractions with cervical dilation of 3 cm or with documented cervical changes of at least 1 cm of dilation or at least 50% effacement. Women with membrane rupture for at least 4 hours will be eligible, regardless of whether labour has started.

Patients are excluded from study participation if one of the following criteria is met:

1. Patients who decline to participate in the trial;
2. Patients who are known to be allergic to cefuroxime sodium or azithromycin (including an allergy to any kind of macrolides or cephalosporins);
3. Patients who are administered azithromycin within 7 days before randomization;
4. Patients who are positive for Group B Streptococcus haemolyticus (GBS) on screening at approximately 36 weeks;
5. Patients with diagnosed non-reproductive tract infections (appendicitis, upper respiratory infections, urinary tract infections) who need additional antibiotic

treatment;

6. Patients with severe maternal diseases (pregnant women with severe liver and renal dysfunction, pneumonoedema, cardiac structural abnormalities or taking antiarrhythmic drugs, systematic lupus erythaematosus, or inadequately controlled diabetes);
7. Stillbirths;
8. Patients with a preoperative diagnosis of uterine abnormalities (such as uterine malformation, adenomyosis, or myoma of uterus); and
9. Patients who have undergone a previous CS.

Table 1 Schedule of enrolment, interventions and assessement in the trial

	Days								
Data collection	BL	0	1	2	3	4	42	6 months	12 months
Age	×								
BMI	×								
Gestational age	×								

Randomization

Randomization will be performed by accessing a computer-generated randomization sequence in a 1:1 ratio. Demographical, medical and obstetrical information will be collected at baseline. Antibiotic prophylaxis will be administered within 30 min before incision.

Withdrawal of individual subjects

Subjects will be able leave the study at any time for any reason if they wish to do so without any consequences. The investigator will be able to decide to withdraw a subject from the study for urgent medical reasons. After randomization, if a woman wishes to change her assigned protocol, she will be considered a crossover subject. All subjects will remain in the study for analysis based on the intention-to-treat principle.

Participant timeline

The schedule for enrolment, interventions and assessments are summarised in Table 1.

Day 0 is the day the caesarean section is performed, and D1 is the day antibiotic treatment, which is randomly assigned, is started.

All included patients will undergo randomization and treatment. We will conduct preoperative and postoperative routine blood tests and detection of C-reactive protein (CRP) levels, procalcitonin (PCT) levels, and blood coagulation function. We will also culture vaginal secretions before the surgery and at 42 days postpartum.

Screen	×								
Informed consent	×								
Randomization	×								
TVU							×	×	×
SIS							×	×	×
Antibiotics		×							
Blood infection index	×		×						
Blood coagulation function	×		×						
Secretion culture	×	×					×		
Temperature	×	×	×	×	×	×			
The skin infection of incision		×	×	×	×	×			
Endometritis		×	×	×	×	×			
Puerperal fever		×	×	×	×	×			
Other postoperative complications		×							

BL base line;BMI, body mass index;TVU,transvaginal ultrasound;SIS,saline infusion sonohysterography

Clinical evaluation of the efficacy and safety of anti-infection prophylaxis will be performed during hospitalization, including evaluation of the incidence of puerperal fever, skin infections at the incision site, endometritis and other postoperative complications.

The clinical symptoms will be recorded as well as the prevalence and characteristic of CSD at 6 weeks, 6 months, 12 months after caesarean delivery.

Primary outcome measure

The prevalence and characteristic of CSD will be assessed by TVU and SIS 42 days after delivery.

Secondary outcome measure

1. The changes in infection indexes, such as routine blood routine examinations including C-reactive protein and procalcitonin before and after surgery;
2. Vaginal secretion culture results before surgery and intraoperative uterine cavity culture results;
3. Body temperature 6 hours before and 72 hours after CS;
4. Postoperative morbidity, endometritis and incisional infection after surgery and severe infection in 42 days after delivery;
5. Type and dosage of the all antibiotics administered postoperatively according to standard processes;
6. Appearance of the niche and its location, the thickness of the anterior wall muscle layer at the scar of the lower part of the uterus as detected by TVS and SIS at approximately 6 weeks (which is the primary outcome measure in our RCT), 6

months and 12 months after delivery; and Postpartum questionnaire at approximately 6 weeks, 6 months and 12 months.

STATISTICAL CONSIDERATIONS

Sample size calculation

Based on findings of previous studies, we concluded that the occurrence of CSD decreases from 47.9% to 29% after administration of multi-dose antibiotics during emergency caesarean delivery. We will predict the occurrence of CSD after using two prophylactic antibiotics with PASS sample prediction software version 11.0. We will need to include 220 women in total (two groups of 110 women), with an alpha error of .05. Assuming a 10% drop-out rate, we will need to randomize a total of 242 participants (121 participants per group).

Statistical analysis

For analysis, the adopted EDC system will be used to collect data uniformly and record all relevant personal information for data collection points. The method of automatic computer verification will be adopted, including data that clearly do not conform to the conventional standards and missing values. Researchers will be required to check the original data, and all changes in the data will be managed with traces. Query tables will be checked if necessary.

The t-test, analysis of variance, chi-square tests and other differential tests will be carried out on the data with SPSS software.

Descriptive statistics will be used to analyse demographic data and laboratory indicators, such as the basic information from preoperative period, postoperative hospitalization period, and period 6 weeks postoperatively. Preoperative and postoperative indicators will be used for baseline data, and the normality of the distribution of continuous variables from the baseline data will be tested. Normally distributed data will be reported as the mean and standard deviation, and the two groups will be assessed by the independent samples t-test; if the data are nonnormally distributed, a non-parametric analysis will be performed, and the data will be reported as the median and interquartile range. We will use the chi-square test or Fisher's exact test to analyse categorical variables.

We will apply the chi-square test to assess the primary outcome to compare the occurrence rate of the CSD in two groups.

We will apply the T test, analysis of variance and chi-square test to evaluate the secondary outcome according to the characteristics of the endpoint.

Patient and public involvement

Patients or the public will not be not involved in the study design. They also will not be involved in the recruitment process or conduction of the study. The results will be disseminated to patients via an open access publication and our local trials teams.

DISCUSSION

Since the mechanism of wound healing of uterine caesarean incisions is not fully known and the natural development of CSD over time is still unclear, uterine scars and CSDs may change over time. One prospective observational study showed that the incidence of CSD occurred from 6 weeks to 6 months and even longer[29]. Another prospective study showed some women with or without CSD may change to the opposite status from 6 weeks to 6 months[30]. Another cross-sectional study showed that the CSD prevalence varied based on time (3-12 months, 1-5 years, and 5-10 years) after CS. We will set the initial CSD assessment timepoint as 6 weeks postpartum and follow patients for 1 year, aiming to find the optimal timepoint for the early detection of CSD. The data from our previous cohort were based on the measurement of CSD with TVU and may have resulted in an underestimation. Our sample size calculation was also based on those data. Since we believe that the difference between the two groups will be more significant when CSD is measured with SIS, the sample size could be large enough to observe a difference.

ETHICS AND DISSEMINATION

The study has been approved by the Medical Research Ethics Committee of International Peace Maternity and Child Health Hospital (GKLW2017-84). Findings will be distributed through peer-reviewed publications and presentations at international scientific meetings.

All women eligible for participation in the study will be invited for additional counselling by a research doctor to ensure that they are fully informed regarding the nature of the study by means of both oral and written information. Women who agree to participate will be asked to sign written informed consent, of which they will receive a copy.

The protocol of the trial is registered in Chinese Clinical Trials Register(ChiCTR) as ChiCTR-INR-17013272.

Author's contributions:

Yanqing Cai, Hongjie Pan and Ben WJ Mol are responsible for the overall logistical aspects of the trial and drafted the paper. Ding Huang, Ben WJ Mol, Weiwei Chen and Jian Zhang designed the trial and were responsible for the development of the protocol.. The senior doctors Ding Huang and Yiru Shi are the main operators for the RCT. Min Zeng and Liye Shi, senior ultrasound doctors, are responsible for the ultrasonographic measurements of the uterus diverticulum. Jinyu, Ying Shen, Sha Chen, Qian Zhu contribute to the protocol including literature retrieval, specimen collection, data analysis, and follow-up visits. Ding Huang is responsible for the project and contributes to the organization and operation of the trial.

Competing interests: None declared

Patient consent: Obtained

Ethics approval: The study has been approved by the Medical Research Ethics Committee of International Peace Maternity and Child Health Hospital (approval no. GKLW2017-84).

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

Funding: This trial received funding from the Shanghai Shenkang Hospital Development Center Clinical Science and Technology Innovation Project (item number SHDC12017X05).

References

1. Pan H, Zeng M, Xu T, et al. The prevalence and risk predictors of cesarean scar defect at 6 weeks postpartum in Shanghai, China: A prospective cohort study. Acta Obstet Gynecol Scand 2018.

2. Antila-Langsjo R, Maenpaa JU, Huhtala H, et al. Comparison of transvaginal ultrasound and saline contrast sonohysterography in evaluation of cesarean scar defect: a prospective cohort study. Acta Obstet Gynecol Scand 2018;**97**(9):1130-36.

3. Betran AP, Ye J, Moller AB, et al. The Increasing Trend in Caesarean Section Rates: Global, Regional and National Estimates: 1990-2014. PLoS One 2016;**11**(2):e0148343.

4. van der Voet LF, Vervoort AJ, Veersema S, et al. Minimally invasive therapy for gynaecological symptoms related to a niche in the caesarean scar: a systematic review. BJOG 2014;**121**(2):145-56.

5. Allornuvor GF, Xue M, Zhu X, et al. The definition, aetiology, presentation, diagnosis and management of previous caesarean scar defects. J Obstet Gynaecol 2013;**33**(8):759-63.

6. Tulandi T, Cohen A. Emerging Manifestations of Cesarean Scar Defect in Reproductive-aged Women. J Minim Invasive Gynecol 2016;**23**(6):893-902.

7. Pomorski M, Fuchs T, Zimmer M. Prediction of uterine dehiscence using ultrasonographic parameters of cesarean section scar in the nonpregnant uterus: a prospective observational study. BMC Pregnancy Childbirth 2014;**14**:365.

8. Timor-Tritsch IE, Monteagudo A, Cali G, et al. Cesarean scar pregnancy is a precursor of morbidly adherent placenta. Ultrasound Obstet Gynecol 2014;**44**(3):346-53.

9. Vervoort AJ, Uittenbogaard LB, Hehenkamp WJ, et al. Why do niches develop in Caesarean uterine scars? Hypotheses on the aetiology of niche development. Hum Reprod 2015;**30**(12):2695-702.

10. Roberge S, Demers S, Berghella V, et al. Impact of single- vs double-layer closure on adverse outcomes and uterine scar defect: a systematic review and metaanalysis. Am J Obstet Gynecol 2014;**211**(5):453-60.

11. Kaelin Agten A, Cali G, Monteagudo A, et al. The clinical outcome of cesarean scar pregnancies implanted "on the scar" versus "in the niche". Am J Obstet Gynecol 2017;**216**(5):510 e1-10 e6.

12. Nieuwenhuis LL, Hermans FJ, Bij de Vaate AJM, et al. Three-dimensional saline infusion sonography compared to two-dimensional saline infusion sonography for the diagnosis of focal intracavitary lesions. Cochrane Database Syst Rev 2017;**5**:CD011126.

13. Osser OV, Jokubkiene L, Valentin L. Cesarean section scar defects: agreement between transvaginal sonographic findings with and without saline contrast enhancement. Ultrasound Obstet Gynecol 2010;**35**(1):75-83.

14. Baranov A, Gunnarsson G, Salvesen KA, et al. Assessment of Cesarean hysterotomy scar in non-pregnant women: reliability of transvaginal sonography with and without contrast enhancement. Ultrasound Obstet Gynecol 2016;**47**(4):499-505.

15. Sholapurkar SL. Etiology of Cesarean Uterine Scar Defect (Niche): Detailed Critical Analysis of Hypotheses and Prevention Strategies and Peritoneal Closure Debate. J Clin Med Res 2018;**10**(3):166-73.

16. Dosedla E, Calda P. Can the final sonographic assessment of the cesarean section scar be predicted 6 weeks after the operation? Taiwan J Obstet Gynecol 2016;**55**(5):718-20.

17. Bamberg C, Hinkson L, Dudenhausen JW, et al. Longitudinal transvaginal ultrasound evaluation of cesarean scar niche incidence and depth in the first two years after single- or double-layer uterotomy closure: a randomized controlled trial. Acta Obstet Gynecol Scand 2017;**96**(12):1484-89.

18. Smaill FM, Grivell RM. Antibiotic prophylaxis versus no prophylaxis for preventing infection after cesarean section. Cochrane Database Syst Rev 2014(10):CD007482.

19. Gyte GM, Dou L, Vazquez JC. Different classes of antibiotics given to women routinely for preventing infection at caesarean section. Cochrane Database Syst Rev 2014(11):CD008726.

20. Allen J, David M, Veerman JL. Systematic review of the cost-effectiveness of preoperative antibiotic prophylaxis in reducing surgical-site infection. BJS Open 2018;**2**(3):81-98.

21. Ward E, Duff P. A comparison of 3 antibiotic regimens for prevention of postcesarean endometritis: an historical cohort study. Am J Obstet Gynecol 2016;**214**(6):751 e1-4.

22. D'Angelo LJ, Sokol RJ. Determinants of postpartum morbidity in laboring monitored patients: a reassessment of the bacteriology of the amniotic fluid during labor. Am J Obstet Gynecol 1980;**136**(5):575-8.

23. Lamey JR, Eschenbach DA, Mitchell SH, et al. Isolation of mycoplasmas and bacteria from the blood of postpartum women. Am J Obstet Gynecol 1982;**143**(1):104-12.

24. Sun J, Ding M, Liu J, et al. Prophylactic administration of cefazolin prior to skin incision versus antibiotics at cord clamping in preventing postcesarean infectious morbidity: a

systematic review and meta-analysis of randomized controlled trials. Gynecol Obstet Invest 2013;**75**(3):175-8.

25. Mackeen AD, Packard RE, Ota E, et al. Timing of intravenous prophylactic antibiotics for preventing postpartum infectious morbidity in women undergoing cesarean delivery. Cochrane Database Syst Rev 2014(12):CD009516.

26. Purba AKR, Setiawan D, Bathoorn E, et al. Prevention of Surgical Site Infections: A Systematic Review of Cost Analyses in the Use of Prophylactic Antibiotics. Front Pharmacol 2018;**9**:776.

27. Tita ATN, Boggess K, Saade G. Adjunctive Azithromycin Prophylaxis for Cesarean Delivery. N Engl J Med 2017;**376**(2):182.

28. Sutton AL, Acosta EP, Larson KB, et al. Perinatal pharmacokinetics of azithromycin for cesarean prophylaxis. Am J Obstet Gynecol 2015;**212**(6):812 e1-6.

29. Skeith AE, Niu B, Valent AM, et al. Adding Azithromycin to Cephalosporin for Cesarean Delivery Infection Prophylaxis: A Cost-Effectiveness Analysis. Obstet Gynecol 2017;**130**(6):1279-84.

30. Harper LM, Kilgore M, Szychowski JM, et al. Economic Evaluation of Adjunctive Azithromycin Prophylaxis for Cesarean Delivery. Obstet Gynecol 2017;**130**(2):328-34.

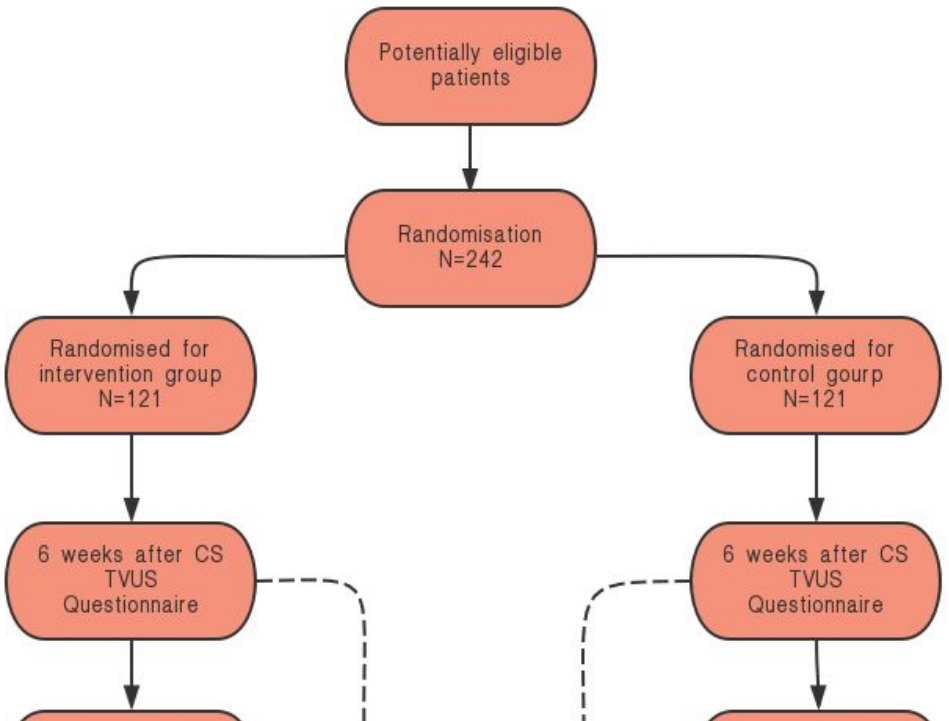

Fig 1 Flowchart of the study. CS, Cesarean-section; TVUS, transvaginal ultrasonography

BMJ Open

Efficacy of adjunctive azithromycin versus single-dose cephalosporin prophylaxis for caesarean scar defect: study protocol for a randomised controlled trial

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-032379.R1
Article Type:	Protocol
Date Submitted by the Author:	01-Nov-2019
Complete List of Authors:	CAI, Yanqing; International Peace Maternity and Child Health Hospital, Obstetrics and Gynecology pan, hongjie; International Peace Maternity and Child Health Hospital, Obstetrics and Gynecology; Zhejiang University School of Medicine Sir Run Run Shaw Hospital, Obstetrics and Gynecology Zhang, Jian; International Peace Maternity and Child Health Hospital, Obstetrics and Gynecology CHENG, Weiwei; International Peace Maternity and Child Health Hospital, Obstetrics and Gynecology shi, yiru; International Peace Maternity and Child Health Hospital, Obstetrics and Gynecology Zeng, Min; International Peace Maternity and Child Health Hospital, Ultrasound shi, liye; International Peace Maternity and Child Health Hospital, Ultrasound Yu, Jin; International Peace Maternity and Child Health Hospital, Obstetrics and Gynecology shen, ying; International Peace Maternity and Child Health Hospital, Obstetrics and Gynecology Chen, Shan; International Peace Maternity and Child Health Hospital, Obstetrics and Gynecology Zhu, Qian; International Peace Maternity and Child Health Hospital, Obstetrics and Gynecology Mol, Ben; Monash University, Obstetrics and Gynecology; The University of Adelaide, Robinson Research Institute,School of Medicine Huang, Ding; International Peace Maternity and Child Health Hospital, Obstetrics and Gynecology
Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Surgery, Infectious diseases
Keywords:	caesarean section, caesarean scar defect, azithromycin, RCT, antibiotic

Efficacy of adjunctive azithromycin versus single-dose cephalosporin prophylaxis for caesarean scar defect: study protocol for a randomised controlled trial

Yanqing Cai[1,*], Hongjie Pan[1,5*], Jian Zhang[1], Weiwei Cheng[1], Yiru Shi[1], Min Zeng[2], Liye Shi[2], Jin Yu[1], Ying Shen[1], Sha Chen[1], Qian Zhu[1], Ben WJ Mol[3,4], Ding Huang[1]

[1]Department of Obstetrics and Gynecology, International Peace Maternity & Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

[2]Department of Ultrasound, International Peace Maternity & Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

[3]Department of Obstetrics and Gynaecology, Monash University, Melbourne, Victoria, Australia

[4]Robinson Research Institute, School of Medicine, The University of Adelaide, South Australia, Australia

[5]Department of Obstetrics and Gynecology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Jianggan District, Hangzhou, China;

Correspondence author

Department of Obstetrics and Gynecology, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiaotong University, No. 910 Hengshan Road, Xuhui District, Shanghai, China, 200020

Email: dingding123hos@163.com; Tel: +86 13817771640; fax: +86 021-64078220

Word count: 4474

*These authors contributed equally to this work.

ABSTRACT

Introduction Perioperative infections may be considered predictors of caesarean scar defect (CSD), and multi-dose antibiotics have a protective effect against CSD. However, the ability of adjunctive azithromycin combined with cephalosporin to reduce the prevalence of CSD remains unclear. The planned study aims to clarify the protective effect of antibiotics against CSD and to assess the effectiveness of adjunctive azithromycin prophylaxis for CSD.

Methods and analysis This study is a double-blind, parallel-control randomised clinical trial that will be carried out at the International Peace Maternity & Child Health Hospital. A total of 220 eligible patients will be randomised (1:1) to receive either

adjunctive azithromycin or single-dose cephalosporin 30 min before the incision. The evaluation criteria are the prevalence and characteristics of CSD as assessed by transvaginal ultrasound (TVU) and saline infusion sonohysterography (SIS) at 42 days, 6 months and 12 months after delivery. The primary outcome will be the prevalence of CSD, and the characteristics of CSD will be assessed by TVU and SIS 42 days after delivery; all other outcomes are secondary.

Ethics and dissemination This protocol received authorisation from the Medical Research Ethics Committee of International Peace Maternity and Child Health Hospital on 25 April 2018 (approval no. GKLW2017-84). The findings will be reported in peer-reviewed publications and presentations at international scientific meetings.

Trial registration number ChiCTR-INR-17013272

Keywords: caesarean section, caesarean scar defect, azithromycin, randomised control trial, antibiotic

ARTICLE SUMMARY

Strengths and limitations of this study:

1. This is the first interventional randomised controlled trial with the primary aims of assessing the protective effects of antibiotics against CSD and investigating the relationship between CSD occurrence and preoperative infection.
2. This study is based on our previous cohort study, and we want to study the effects of infection and antibiotics on CSD directly.
3. The study is a double-blinded randomised controlled trial with a relatively large sample size and a long follow-up period. In this set, we can find the optimal timepoint for the early detection of CSD.
4. The trial is based in a single centre, which might limit the generalisability of the findings.
5. This study is not a randomised controlled trial of different surgical techniques for uterine incision closure, which may be as or more important than antibiotics.

INTRODUCTION

The rate of caesarean section (CS) delivery is increasing worldwide, especially in China due to the prevalence of maternally requested caesarean deliveries and the 30-year national one-child policy[1]. According to the data from official figures of the China National Bureau of Statistics, there were approximately 17 million new-borns in China

in 2017, with a caesarean delivery rate of approximately 40%, which means that there are approximately 7 million CS deliveries each year[2].

There is no universal definition of CSD. Most studies refer to the thinning of the myometrium or a triangular defect in the myometrium that is contiguous with the endometrial cavity. The remaining myometrial thickness (RMT) is defined as the distance from the delineation of the endometrium to the serosal surface at the level of the caesarean scar, and total myometrial thickness is measured at the myometrium adjacent to the scar (AMT)[3 4]. Some authors have suggested the existence of a niche, described as an indentation of the myometrium that is at least 2 mm. Large niches are uncommon, with a reported incidence that varies from 11-45% depending on the definition used. Some authors have suggested that a large CSD penetrates to one involving a depth of at least 50-80% of the anterior myometrium or a RMT that is less than 2.2 mm when evaluated by TVU and less than 2.5mm when evaluated by SIS[5] . Small niches may indeed be quite common but would be clinically unimportant. Large niches are most likely to give rise to long-term sequelae; hence, the focus of research and discussion should be on the causes of large niches and on prevention strategies[6 7]. In our study, we will also focus on the differences in the prevalence rates and outcomes of CSD and large CSD. Measured parameters such as the height, length, width, RMT, AMT and niche volume of CSD will be collected to present the characteristics of CSD.

In a random population of women with a history of CS, the prevalence of CSD ranges from 24% to 70% and 56% to 84% when assessed by TVU with and without contrast enhancement, respectively[7]. Saline infusion sonohysterography (SIS) is better than traditional TVU for characterising CSD and has a higher sensitivity[8 9]. Moreover, sonohysterography can more precisely evaluate the size and shape of the defect and thus the severity[2]. In this study, we will apply and compare the differences between TVU and SIS.

Caesarean scar defect (CSD) is associated with abnormal uterine bleeding (75-82%), postmenstrual spotting (29-34%) caesarean scar ectopic pregnancies (1:1,800-1:2,216) and infertility (32/92)[10 11]. Uterine dehiscence, uterine rupture, caesarean scar pregnancy and morbidly adherent placenta are also associated with CSD[12-14]. Annually, there are thousands of fatalities and near-death cases caused by uterine rupture or haemorrhage from the CSD[15].

Our previously published data first revealed a CSD prevalence of 43.4% (95% confidence interval (CI)=39.1-47.7%, N=514) in Shanghai, which is a cause for concern[16]. Our cohort study also showed that infection might be an important risk factor for CSD and that multi-dose antibiotic administration has a significant protective effect compared with single-dose antibiotic administration (CSD prevalence 31.1%, 95% CI=23.8-38.3% versus 49.0%, 95% CI=43.8-54.3%; aOR=0.4, 95% CI=0.3-0.7).

Furthermore, the single-dose cefuroxime subgroup (n=190) was found to have a significantly lower CSD prevalence than the single-dose cefradine subgroup (n=162) (38.9%, 95% CI=34.7-48.6% vs 56.8%, 95% CI=51.3-66.7%; aOR=0.5, 95% CI=0.3-0.8, P=0.006). These results strongly indicate that antibiotics may be a protective factor for CSD[17-19].

According to the results of a study involving pathogen monitoring for nosocomial infections, Ureaplasma urealyticum, *Escherichia coli* , Enterococcus and Streptococcus are the most common infectious agents[20 21]. Second-generation cephalosporins have a stronger antibacterial effect against *E. coli* and *Enterobacteriaceae* than first-generation cephalosporin, while azithromycin has stronger antibacterial and bacteriostatic effects against atypical pathogens, such as *Mycoplasma chlamydia* and anaerobic bacteria[22 23]. Current recommendations for antibiotic prophylaxis in caesarean delivery include the standard administration of a broad-spectrum antibiotic, most commonly a first-generation cephalosporin, before the skin incision[24]. Recent reports on adjunctive azithromycin prophylaxis for caesarean delivery showed a significant protective effect against maternal endometritis (3.8% vs 6.1%, P=0.02), wound infection (2.4% vs 6.6%, P<0.001), and serious maternal adverse events (1.5% vs 2.9%, P=0.03) but no significant between-group differences in secondary neonatal composite outcomes[25]. In other words, the addition of azithromycin to caesarean delivery prophylaxis is less costly and leads to better maternal outcomes in index and subsequent deliveries[26-29].

Therefore, we designed a prospective, randomised, double-blind, parallel-group study to determine whether the prophylactic use of cephalosporin plus azithromycin combination is superior to that of a single dose of cephalosporin in reducing the prevalence of CSD. Our study has a strong theoretical basis and foundation, and this trial may provide evidence of the effective use of antibiotics in clinical practice and data indicating a reduction in the rate of CSD.

Objective and hypothesis

The objective of this trial is to clarify the protective effects of antibiotics against CSD and to assess the effectiveness of adjunctive azithromycin prophylaxis for CSD. Our hypothesis is that the prophylactic use of cephalosporins combined with azithromycin is superior to a single dose of cephalosporin and can reduce the prevalence of CSD.

METHODS AND ANALYSIS

Study design and setting

The study design is that of a double-blind, parallel-control randomised clinical trial. Pregnant women with singleton pregnancies in cephalic presentation who have ruptured

membranes or signs of labour will be informed about the study and the need to undergo CS by the medical team. The subjects will be randomly divided into two groups. The trial group (group A) will be administered the regular single-dose cefuroxime sodium (1.5 g/50 ml 0.9% NaCl) plus azithromycin (0.5 g/250 ml 0.9% NaCl). The control group (group B) will be administered the regular single-dose cefuroxime sodium (1.5 g/50 ml 0.9% NaCl) plus an additional placebo (250 ml 0.9% NaCl) as an intravenous drip 30 min before surgery. The prevalence and clinical symptoms of CSD at 6 weeks, 6 months, and 12 months after delivery will be observed with TVU and SIS. We will include 220 women in total. The primary outcome measure will be the prevalence of CSD, and the characteristic of CSD will be assessed by TVU and SIS 42 days after delivery. The secondary outcome measures will be infection indexes and labour results, as shown in Figure 1.

Participants

Patients will be included in this study if they meet all the following criteria:

1. Han Chinese ethnicity with a maternal age of 18 years or older,
2. Singleton pregnancy with a gestational age ≥37 weeks,
3. Fibrinogen≥2 g/L, platelet count ≥$100*10^9$, and haemoglobin ≥90 g/L before surgery,
4. Non-elective caesarean delivery during labour or after membrane rupture,
5. Labour defined as regular contractions with cervical dilation of 3 cm or with documented cervical changes of at least 1 cm of dilation or at least 50% effacement (women with membrane rupture for at least 4 hours will be eligible, regardless of whether labour has started).

Patients will be excluded from participating in the study if one of the following criteria is met:

1. The patient declines to participate in the trial,
2. The patient is known to be allergic to cefuroxime sodium or azithromycin (including an allergy to any kind of macrolide or cephalosporin),
3. The patient was administered azithromycin within 7 days before randomisation,
4. The patient is positive for Group B Streptococcus haemolyticus (GBS) on screening at approximately 36 weeks,
5. The patient has a diagnosis of a non-reproductive tract infection (appendicitis, upper respiratory infection, urinary tract infection) and needs additional antibiotic treatment,
6. The patient has a severe maternal disease (severe liver or renal dysfunction; pulmonary oedema; cardiac structural abnormality or a condition requiring antiarrhythmic drug use; systematic lupus erythaematosus; or inadequately

controlled diabetes),
7. The patient has a stillbirth,
8. The patient has a preoperative diagnosis of uterine abnormalities (such as uterine malformation, adenomyosis, or myoma of the uterus), or
9. The patient has undergone a previous CS.

Randomisation

Demographic, medical and obstetrical information will be collected at baseline. The patient will then be randomised between the trial group (group A) and the control group (group B) according to her serial number (number 1 to 250, depending on the time of trial entry). The randomisation codes will be generated by the block of the statistical software at a 1:1 ratio (SPSS, version 22.0, IBM, Armonk, NY, USA). We will generate a list of 250 randomisation codes (A or B). Each code will correspond to one serial number, and each code will be placed in a black envelope with the serial number printed on the outside. Anyone who will have contact with the patient or other researchers will be blinded to the list. Antibiotic prophylaxis will be administered within 30 min before incision. The medicine will be dissolved in the hospital dispensary, where the patient's envelope will be opened by an independent pharmacist, and the containers will be identical so that no one else can identify the set. The opened envelope will be locked in a non-transparent box until the trial is finished. These settings will ensure that both the participants and investigators or surgeons are blinded to the treatment.

Intervention

All participants will undergo a standard CS with a lower segment uterine incision and double-layer suturing of the uterine wound. The first layer will be the decidua and total myometrium, and the second layer will be the serosa and the upper 1/2 layer of myometrium. All sutures will be continuously unlocked sutures with absorbable suture material. We have three experienced surgeons with the same technical standards who will be responsible for the trial. Each of them will perform approximately 70 CSs randomly in the trial. All the participants will be cared for in the wards, and additional antibiotics will be given if there is a confirmed infection before the patient checks out.

Withdrawal of participants

The participants will be able to leave the study at any time for any reason if they wish to do so, without any consequences. The investigator will be able to decide to withdraw a subject from the study for urgent medical reasons. After randomisation, if a woman wishes to change her assigned protocol, she will be considered a crossover subject. All

subjects will remain in the study for analysis based on the intention-to-treat principle.

Participant timeline

The schedules for enrolment, interventions and assessments are summarised in Table 1.

Table 1 Patient's characteristics and data collection

	Days								
Data collection	BL	0	1	2	3	4	42	6months	12months
Age	×								
BMI	×								
Gestational age	×								
Screen	×								
Informed consent	×								
Randomization	×								
TVU							×	×	×
SIS							×	×	×
Antibiotics	×	×							
Blood infection index	×		×						
Blood coagulation function	×		×						
Secretion culture	×	×					×		
Temperature	×	×	×	×	×	×			
The skin infection of incision		×	×	×	×	×			
Endometritis		×	×	×	×	×			
Puerperal fever		×	×	×	×	×			
Other postoperative complicatioins		×							
Questionnaire							×	×	×

BL, baseline; BMI, body mass index; TVU, transvaginal ultrasound; SIS, saline infusion sonohysterography.

The CS will be performed on day 0, and antibiotic treatment, which will be randomly assigned, will be started on day 1.

All included patients will undergo randomisation and treatment. We will conduct preoperative and postoperative routine blood tests and detect C-reactive protein (CRP) levels, procalcitonin (PCT) levels, and blood coagulation function. We will also culture vaginal secretions before surgery and at 42 days postpartum.

Clinical evaluations of the efficacy and safety of anti-infection prophylaxis will be performed during hospitalisation and will include evaluations of the incidence of

puerperal fever, skin infections at the incision site, endometritis and other postoperative complications.

Clinical symptoms as well as the prevalence and characteristics of CSD at 6 weeks, 6 months, and 12 months after caesarean delivery will be documented.

Primary outcome measure

The prevalence of CSD,and the characteristic of CSD will be assessed by TVU and SIS 42 days after delivery.

Secondary outcome measure

1. The changes in infection indexes, such as routine blood examinations including CRP and PCT before and after surgery;
2. Pre-surgical vaginal secretion culture results and intraoperative uterine cavity culture results;
3. Body temperature 6 hours before and 72 hours after CS;
4. Postoperative morbidity, endometritis and incisional infection after surgery and severe infection 42 days after delivery;
5. Type and dosage of all antibiotics administered postoperatively according to standard processes;
6. Responses to postpartum questionnaires at approximately 6 weeks, 6 months and 12 months;
7. Follow-up data from TVU and SIS evaluations performed at 6 months and 12 months after delivery; and
8. The size and position of the uterus and the height, length, width, RMT, AMT and niche volume of the defects.

STATISTICAL CONSIDERATIONS

Sample size calculation

Based on the findings of previous studies, we concluded that the occurrence of CSD decreases from 47.9% to 29% after the administration of multi-dose antibiotics during emergency caesarean delivery. We will predict the occurrence of CSD after using two prophylactic antibiotics with PASS sample prediction software version 11.0. We will need to include 220 women in total (two groups of 110 women), with an alpha error of 0.05. Assuming a 10% drop-out rate, we will need to randomise a total of 242 participants (121 participants per group).

Statistical analysis

For analysis, the adopted EDC system will be used to collect data uniformly and record all relevant personal information for data collection points. The method of automatic computer verification, which will include data that clearly do not conform to the conventional standards and among which values are missing, will be adopted. Researchers will be required to check the original data, and all changes in the data will be managed with traces. Query tables will be checked if necessary.

For the analysis of the data, t-tests, analysis of variance, chi-square tests and other differential tests will be carried out with SPSS software.

Descriptive statistics will be used to analyse demographic data and laboratory indicators, such as basic information from the preoperative period, from the postoperative hospitalisation period, and at 6 weeks post-surgery. Preoperative and postoperative indicators will be used for baseline data, and the normality of the distribution of continuous variables from the baseline data will be tested. Normally distributed data will be reported as the mean and standard deviation, and the two groups will be assessed by the independent samples t-test; if the data are nonnormally distributed, a non-parametric analysis will be performed, and the data will be reported as the median and interquartile range. We will use the chi-square test or Fisher's exact test to analyse categorical variables.

We will apply the chi-square test to assess the primary outcome to compare the occurrence rate of the CSD in two groups.

We will apply the t-test, analysis of variance and chi-square test to evaluate the secondary outcome according to the characteristics of the endpoint.

Patient and public involvement

Neither the patients nor the public will be involved in the study design. They will also not be involved in the recruitment process or conduction of the study. The results will be disseminated to patients via an open access publication and our local trials teams.

DISCUSSION

Since the mechanism of wound healing in uterine caesarean incisions is not fully known and since the natural development of CSD over time is still unclear, uterine scars and CSD may change over time. One prospective observational study showed that CSD occurred from 6 weeks to 6 months or longer after surgery[30]. Another prospective study reported that the statuses of some women with or without CSD showed a reversal between 6 weeks and 6 months after surgery (90.7% at 6 weeks and 93.1% at 6 months

(OR 0.96 (0.61-1.49), P=0.91))[31]. This trial is based on our previous cohort study in which CSD was observed at 6 weeks postpartum. We will set the initial CSD assessment timepoint as 6 weeks postpartum and follow patients for 1 year, aiming to find the optimal timepoint for the early detection of CSD. Our sample size calculation is also based on previously published data, which were based on the measurement of CSD with TVU and may have resulted in an underestimation[16]. Since we believe that the difference between the two groups will be more significant when CSD is measured with SIS, the sample size could be large enough to observe a difference. We believe that surgical techniques of uterine incision and closure may be important in the occurrence of CSD, and there are many trials that are on-going or that already have results[32 33]. We will focus on the effects of infection and antibiotics against CSD based on the strong indication of our previous study.

ETHICS AND DISSEMINATION

The study was approved by the Medical Research Ethics Committee of International Peace Maternity and Child Health Hospital (GKLW2017-84). The findings will be reported in peer-reviewed publications and presentations at international scientific meetings.

All women who are eligible for participation in the study will be invited for additional counselling by a research doctor to ensure that they are fully informed regarding the nature of the study by means of both oral and written information. Women who agree to participate will be asked to sign written informed consent forms, of which they will receive a copy.

The protocol of the trial is registered in the Chinese Clinical Trials Register (ChiCTR) as ChiCTR-INR-17013272.

Author's contributions:

Yanqing Cai, Hongjie Pan and Ben WJ Mol are responsible for the overall logistical aspects of the trial and the drafting of this paper. Ding Huang, Ben WJ Mol, Weiwei Chen and Jian Zhang designed the trial and were responsible for the development of the protocol. The senior doctors Ding Huang and Yiru Shi are the main operators of the RCT. Min Zeng and Liye Shi, senior ultrasound doctors, are responsible for the ultrasonographic measurements of the uterus diverticulum. Jinyu is responsible for the design of the randomisation codes and production of the black envelopes. Ying Shen, Sha Chen, Qian Zhu is contributing to the protocol by carrying out literature retrieval, specimen collection, data analysis, and follow-up visits. Ding Huang is responsible for the project and is contributing to the organisation and operation of the trial.

Competing interests: None declared

Patient consent: Obtained

Ethics approval: The study was approved by the Medical Research Ethics Committee of International Peace Maternity and Child Health Hospital (approval no. GKLW2017-84).

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

Funding: This trial received funding from the Shanghai Shenkang Hospital Development Center Clinical Science and Technology Innovation Project (item number SHDC12017X05). And it was supported by the medical engineering cross youth funds from Shanghai Jiao Tong University (item number YG2017QN38).

Acknowledgements The authors acknowledge Yanfei Zhang for dissolving the medicine for free and for his confidentiality regarding all the participants and personnel.

References

1. Pan H, Zeng M, Xu T, et al. The prevalence and risk predictors of cesarean scar defect at 6 weeks postpartum in Shanghai, China: A prospective cohort study. Acta Obstet Gynecol Scand 2018.

2. Antila-Langsjo R, Maenpaa JU, Huhtala H, et al. Comparison of transvaginal ultrasound and saline contrast sonohysterography in evaluation of cesarean scar defect: a prospective cohort study. Acta Obstet Gynecol Scand 2018;**97**(9):1130-36.

3. Betran AP, Ye J, Moller AB, et al. The Increasing Trend in Caesarean Section Rates: Global, Regional and National Estimates: 1990-2014. PLoS One 2016;**11**(2):e0148343.

4. Tower AM, Frishman GN. Cesarean scar defects: an underrecognized cause of abnormal uterine bleeding and other gynecologic complications. J Minim Invasive Gynecol 2013;20(5):562-72. doi: 10.1016/j.jmig.2013.03.008

5. Tower AM, Frishman GN. Cesarean scar defects: an underrecognized cause of abnormal uterine bleeding and other gynecologic complications. J Minim Invasive Gynecol 2013;20(5):562-72. doi: 10.1016/j.jmig.2013.03.008

6. Osser OV, Jokubkiene L, Valentin L. Cesarean section scar defects: agreement between transvaginal sonographic findings with and without saline contrast enhancement. Ultrasound Obstet Gynecol 2010;**35**(1):75-83.

7. Bij de Vaate AJ, van der Voet LF, Naji O, et al. Prevalence, potential risk factors for development and symptoms related to the presence of uterine niches following Cesarean section: systematic review. Ultrasound Obstet Gynecol 2014;43(4):372-82. doi: 10.1002/uog.13199

8. Yao M, Wang W, Zhou J, et al. Cesarean section scar diverticulum evaluation by saline contrast-enhanced magnetic resonance imaging: The relationship between variable parameters and longer menstrual bleeding. J Obstet Gynaecol Res 2017;43(4):696-704. doi: 10.1111/jog.13255

9. Nieuwenhuis LL, Hermans FJ, Bij de Vaate AJM, et al. Three-dimensional saline infusion sonography compared to two-dimensional saline infusion sonography for the diagnosis of focal intracavitary lesions. Cochrane Database Syst Rev 2017;**5**:CD011126.

10. Baranov A, Gunnarsson G, Salvesen KA, et al. Assessment of Cesarean hysterotomy scar in non-pregnant women: reliability of transvaginal sonography with and without contrast enhancement. Ultrasound Obstet Gynecol 2016;**47**(4):499-505.

11. van der Voet LF, Vervoort AJ, Veersema S, et al. Minimally invasive therapy for gynaecological symptoms related to a niche in the caesarean scar: a systematic review. BJOG 2014;**121**(2):145-56.

12. Tulandi T, Cohen A. Emerging Manifestations of Cesarean Scar Defect in Reproductive-aged Women. J Minim Invasive Gynecol 2016;**23**(6):893-902.

13. Allornuvor GF, Xue M, Zhu X, et al. The definition, aetiology, presentation, diagnosis and management of previous caesarean scar defects. J Obstet Gynaecol 2013;**33**(8):759-63.

14. Timor-Tritsch IE, Monteagudo A, Cali G, et al. Cesarean scar pregnancy is a precursor of morbidly adherent placenta. Ultrasound Obstet Gynecol 2014;**44**(3):346-53.

15. Vervoort AJ, Uittenbogaard LB, Hehenkamp WJ, et al. Why do niches develop in Caesarean uterine scars? Hypotheses on the aetiology of niche development. Hum Reprod 2015;**30**(12):2695-702.

16. Kaelin Agten A, Cali G, Monteagudo A, et al. The clinical outcome of cesarean scar pregnancies implanted "on the scar" versus "in the niche". Am J Obstet Gynecol 2017;**216**(5):510 e1-10 e6.

17. Smaill FM, Grivell RM. Antibiotic prophylaxis versus no prophylaxis for preventing infection after cesarean section. Cochrane Database Syst Rev 2014(10):CD007482.

18. Gyte GM, Dou L, Vazquez JC. Different classes of antibiotics given to women routinely for preventing infection at caesarean section. Cochrane Database Syst Rev 2014(11):CD008726.

19. Allen J, David M, Veerman JL. Systematic review of the cost-effectiveness of preoperative antibiotic prophylaxis in reducing surgical-site infection. BJS Open 2018;**2**(3):81-98.

20. D'Angelo LJ, Sokol RJ. Determinants of postpartum morbidity in laboring monitored patients: a reassessment of the bacteriology of the amniotic fluid during labor. Am J Obstet Gynecol 1980;**136**(5):575-8.

21. Lamey JR, Eschenbach DA, Mitchell SH, et al. Isolation of mycoplasmas and bacteria from the blood of postpartum women. Am J Obstet Gynecol 1982;**143**(1):104-12.

22. Mackeen AD, Packard RE, Ota E, et al. Timing of intravenous prophylactic antibiotics for preventing postpartum infectious morbidity in women undergoing cesarean delivery. Cochrane Database Syst Rev 2014(12):CD009516.

23. Purba AKR, Setiawan D, Bathoorn E, et al. Prevention of Surgical Site Infections: A Systematic Review of Cost Analyses in the Use of Prophylactic Antibiotics. Front Pharmacol 2018;**9**:776.

24. Sun J, Ding M, Liu J, et al. Prophylactic administration of cefazolin prior to skin incision versus antibiotics at cord clamping in preventing postcesarean infectious morbidity: a systematic review and meta-analysis of randomized controlled trials. Gynecol Obstet Invest 2013;**75**(3):175-8.

25. Tita ATN, Boggess K, Saade G. Adjunctive Azithromycin Prophylaxis for Cesarean Delivery. N Engl J Med 2017;**376**(2):182.

26. Ward E, Duff P. A comparison of 3 antibiotic regimens for prevention of postcesarean endometritis: an historical cohort study. Am J Obstet Gynecol 2016;**214**(6):751 e1-4.

27. Sutton AL, Acosta EP, Larson KB, et al. Perinatal pharmacokinetics of azithromycin for cesarean prophylaxis. Am J Obstet Gynecol 2015;**212**(6):812 e1-6.

28. Skeith AE, Niu B, Valent AM, et al. Adding Azithromycin to Cephalosporin for Cesarean Delivery Infection Prophylaxis: A Cost-Effectiveness Analysis. Obstet Gynecol 2017;**130**(6):1279-84.

29. Harper LM, Kilgore M, Szychowski JM, et al. Economic Evaluation of Adjunctive Azithromycin Prophylaxis for Cesarean Delivery. Obstet Gynecol 2017;**130**(2):328-34.

30. Bamberg C, Hinkson L, Dudenhausen JW, et al. Longitudinal transvaginal ultrasound evaluation of cesarean scar niche incidence and depth in the first two years after single- or double-layer uterotomy closure: a randomized controlled trial. Acta Obstet Gynecol Scand 2017;**96**(12):1484-89.

31. Dosedla E, Calda P. Can the final sonographic assessment of the cesarean section scar be predicted 6 weeks after the operation? Taiwan J Obstet Gynecol 2016;**55**(5):718-20.

32. Roberge S, Demers S, Berghella V, et al. Impact of single- vs double-layer closure on adverse outcomes and uterine scar defect: a systematic review and metaanalysis. Am J Obstet Gynecol 2014;**211**(5):453-60.

33. Sholapurkar SL. Etiology of Cesarean Uterine Scar Defect (Niche): Detailed Critical Analysis of Hypotheses and Prevention Strategies and Peritoneal Closure Debate. J Clin Med Res 2018;**10**(3):166-73.

Figure Legend:

Fig 1 Flowchart of the study.

CS, Cesarean-section; TVUS, transvaginal ultrasonography

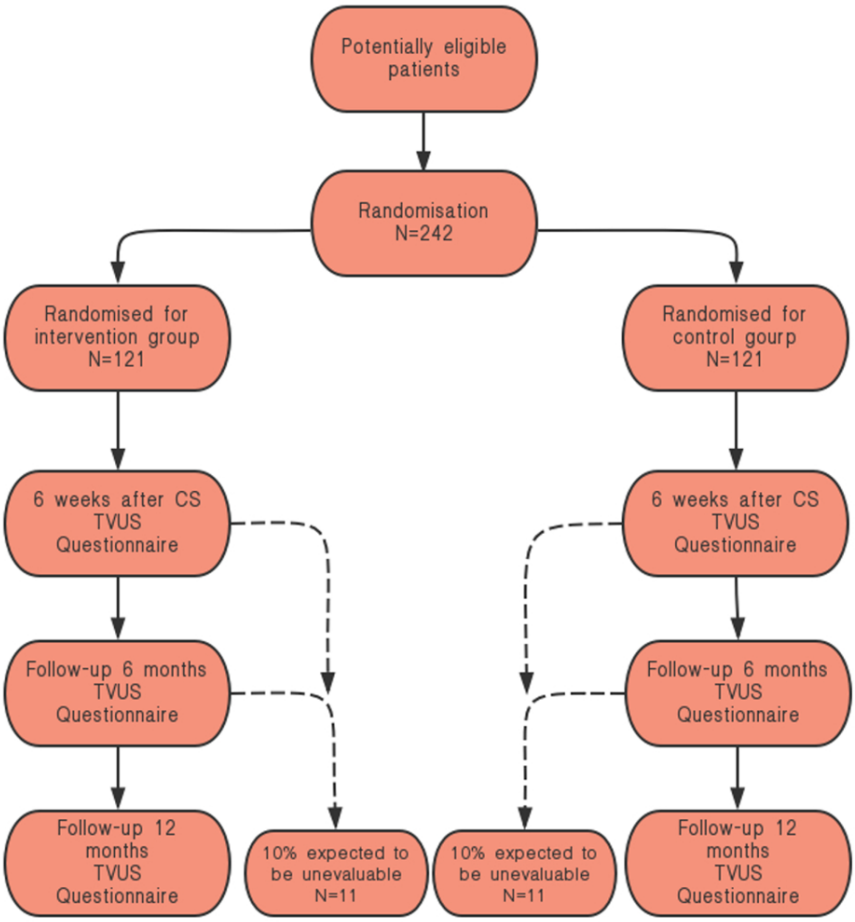

Fig 1 Flowchart of the study.
CS, Cesarean-section; TVUS, transvaginal ultrasonography

90x90mm (300 x 300 DPI)

BMJ Open

Efficacy of adjunctive azithromycin versus single-dose cephalosporin prophylaxis for caesarean scar defect: study protocol for a randomised controlled trial

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-032379.R2
Article Type:	Protocol
Date Submitted by the Author:	21-Nov-2019
Complete List of Authors:	CAI, Yanqing; International Peace Maternity and Child Health Hospital, Obstetrics and Gynecology pan, hongjie; International Peace Maternity and Child Health Hospital, Obstetrics and Gynecology; Zhejiang University School of Medicine Sir Run Run Shaw Hospital, Obstetrics and Gynecology Zhang, Jian; International Peace Maternity and Child Health Hospital, Obstetrics and Gynecology CHENG, Weiwei; International Peace Maternity and Child Health Hospital, Obstetrics and Gynecology shi, yiru; International Peace Maternity and Child Health Hospital, Obstetrics and Gynecology Zeng, Min; International Peace Maternity and Child Health Hospital, Ultrasound shi, liye; International Peace Maternity and Child Health Hospital, Ultrasound Yu, Jin; International Peace Maternity and Child Health Hospital, Obstetrics and Gynecology shen, ying; International Peace Maternity and Child Health Hospital, Obstetrics and Gynecology Chen, Shan; International Peace Maternity and Child Health Hospital, Obstetrics and Gynecology Zhu, Qian; International Peace Maternity and Child Health Hospital, Obstetrics and Gynecology Mol, Ben; Monash University, Obstetrics and Gynecology; The University of Adelaide, Robinson Research Institute,School of Medicine Huang, Ding; International Peace Maternity and Child Health Hospital, Obstetrics and Gynecology
Primary Subject Heading:	Obstetrics and gynaecology
Secondary Subject Heading:	Surgery, Infectious diseases
Keywords:	caesarean section, caesarean scar defect, azithromycin, RCT, antibiotic

Efficacy of adjunctive azithromycin versus single-dose cephalosporin prophylaxis for caesarean scar defect: study protocol for a randomised controlled trial

Yanqing Cai[1,*], Hongjie Pan[1,5*], Jian Zhang[1], Weiwei Cheng[1], Yiru Shi[1], Min Zeng[2], Liye Shi[2], Jin Yu[1], Ying Shen[1], Sha Chen[1], Qian Zhu[1], Ben WJ Mol[3,4], Ding Huang[1]

[1]Department of Obstetrics and Gynecology, International Peace Maternity & Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

[2]Department of Ultrasound, International Peace Maternity & Child Health Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China

[3]Department of Obstetrics and Gynaecology, Monash University, Melbourne, Victoria, Australia

[4]Robinson Research Institute, School of Medicine, The University of Adelaide, South Australia, Australia

[5]Department of Obstetrics and Gynecology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Jianggan District, Hangzhou, China;

Correspondence author

Department of Obstetrics and Gynecology, International Peace Maternity and Child Health Hospital, School of Medicine, Shanghai Jiaotong University, No. 910 Hengshan Road, Xuhui District, Shanghai, China, 200020

Email: dingding123hos@163.com; Tel: +86 13817771640; fax: +86 021-64078220

Word count: 4474

*These authors contributed equally to this work.

ABSTRACT

Introduction Perioperative infections may be considered predictors of caesarean scar defect (CSD), and multi-dose antibiotics have a protective effect against CSD. However, the ability of adjunctive azithromycin combined with cephalosporin to reduce the prevalence of CSD remains unclear. The planned study aims to clarify the protective effect of antibiotics against CSD and to assess the effectiveness of adjunctive azithromycin prophylaxis for CSD.

Methods and analysis This study is a double-blind, parallel-control randomised clinical trial that will be carried out at the International Peace Maternity & Child Health Hospital. A total of 220 eligible patients will be randomised (1:1) to receive either

adjunctive azithromycin or single-dose cephalosporin 30 min before the incision. The evaluation criteria are the prevalence and characteristics of CSD as assessed by transvaginal ultrasound (TVU) and saline infusion sonohysterography (SIS) at 42 days, 6 months and 12 months after delivery. The primary outcome will be the prevalence of CSD, and the characteristics of CSD will be assessed by TVU and SIS 42 days after delivery; all other outcomes are secondary.

Ethics and dissemination This protocol received authorisation from the Medical Research Ethics Committee of International Peace Maternity and Child Health Hospital on 25 April 2018 (approval no. GKLW2017-84). The findings will be reported in peer-reviewed publications and presentations at international scientific meetings.

Trial registration number ChiCTR-INR-17013272

Keywords: caesarean section, caesarean scar defect, azithromycin, randomised control trial, antibiotic

ARTICLE SUMMARY

Strengths and limitations of this study:

1. This is the first interventional randomised controlled trial with the primary aims of assessing the protective effects of antibiotics against CSD and investigating the relationship between CSD occurrence and preoperative infection.
2. This study is based on our previous cohort study, and we want to study the effects of infection and antibiotics on CSD directly.
3. The study is a double-blinded randomised controlled trial with a relatively large sample size and a long follow-up period. In this set, we can find the optimal timepoint for the early detection of CSD.
4. The trial is based in a single centre, which might limit the generalisability of the findings.
5. This study is not a randomised controlled trial of different surgical techniques for uterine incision closure, which may be as or more important than antibiotics.

INTRODUCTION

The rate of caesarean section (CS) delivery is increasing worldwide, especially in China due to the prevalence of maternally requested caesarean deliveries and the 30-year national one-child policy[1]. According to the data from official figures of the China National Bureau of Statistics, there were approximately 17 million new-borns in China

in 2017, with a caesarean delivery rate of approximately 40%, which means that there are approximately 7 million CS deliveries each year.

There is no universally accepted definition or criterion for caesarean scar defect (CSD). In most studies, CSD is defined as "the thinning of the myometrium or a triangular defect in the myometrium that is contiguous with the endometrial cavity"[2]. It was stated in the research by Di Spiezio Sardo et al[3] that remaining myometrial thickness (RMT) is defined as "the distance from the delineation of the endometrium to the serosal surface at the level of the Caesarean scar, and total myometrial thickness was measured at the myometrium adjacent to the scar", which refers to adjacent myometrium thickness (AMT). Some researchers have also indicated that a niche should be described as the indentation of myometrium with a depth of at least 2 mm[4 5]. Additionally, according to Osser et al[6], a large CSD was defined as "thickness of the remaining myometrium over the defect≤2.2 mm by TVU and ≤2.5 mm by SIS , ratio between the thickness of the remaining myometrium over the defect and the myometrial thickness adjacent to the defect≤23% by TVU and ≤29% by SIS for women who had undergone only one Cesarean section". Small CSDs may be definitely common and not considered significant. Nevertheless, large CSDs may lead to some long-term potential sequelae [7]. In our study, we will focus on the differences in the prevalence rates and outcomes of CSDs and large CSDs. Data on measured parameters such as the height, length, width, RMT, AMT and niche volume of the CSD will be collected to present the characteristics of CSD.

In a random population of women with a history of CS, the prevalence of CSD ranges from 56% to 84% and 24% to 70% when assessed by TVU with and without contrast enhancement, respectively[8]. Saline infusion sonohysterography (SIS) is better than traditional TVU for characterising CSD and has a higher sensitivity[9]. Moreover, sonohysterography can more precisely evaluate the size and shape of the defect and thus the severity[10]. In this study, we will apply and compare the differences between TVU and SIS.

Caesarean scar defect (CSD) is associated with abnormal uterine bleeding (75-82%), postmenstrual spotting (29-34%) caesarean scar ectopic pregnancies (1:1,800-1:2,216) and infertility (32/92)[4 11 12]. Uterine dehiscence, uterine rupture, caesarean scar pregnancy and morbidly adherent placenta are also associated with CSD[13-15]. Annually, there are thousands of fatalities and near-death cases caused by uterine rupture or haemorrhage from the CSD[16].

Our previously published data first revealed a CSD prevalence of 43.4% (95% confidence interval (CI)=39.1-47.7%, N=514) in Shanghai, which is a cause for concern[17]. Our cohort study also showed that infection might be an important risk factor

for CSD and that multi-dose antibiotic administration has a significant protective effect compared with single-dose antibiotic administration (CSD prevalence 31.1%, 95% CI=23.8-38.3% versus 49.0%, 95% CI=43.8-54.3%; aOR=0.4, 95% CI=0.3-0.7)[18-20]. Furthermore, the single-dose cefuroxime subgroup (n=190) was found to have a significantly lower CSD prevalence than the single-dose cefradine subgroup (n=162) (38.9%, 95% CI=34.7-48.6% vs 56.8%, 95% CI=51.3-66.7%; aOR=0.5, 95% CI=0.3-0.8, P=0.006). These results strongly indicate that antibiotics may be a protective factor for CSD.

According to the results of a study involving pathogen monitoring for nosocomial infections, Ureaplasma urealyticum, *Escherichia coli* , Enterococcus and Streptococcus are the most common infectious agents[21 22]. Second-generation cephalosporins have a stronger antibacterial effect against *E. coli* and *Enterobacteriaceae* than first-generation cephalosporin, while azithromycin has stronger antibacterial and bacteriostatic effects against atypical pathogens, such as *Mycoplasma chlamydia* and anaerobic bacteria[23 24]. Current recommendations for antibiotic prophylaxis in caesarean delivery include the standard administration of a broad-spectrum antibiotic, most commonly a first-generation cephalosporin, before the skin incision[25]. Recent reports on adjunctive azithromycin prophylaxis for caesarean delivery showed a significant protective effect against maternal endometritis (3.8% vs 6.1%, P=0.02), wound infection (2.4% vs 6.6%, P<0.001), and serious maternal adverse events (1.5% vs 2.9%, P=0.03) but no significant between-group differences in secondary neonatal composite outcomes[26]. In other words, the addition of azithromycin to caesarean delivery prophylaxis is less costly and leads to better maternal outcomes in index and subsequent deliveries[27-30].

Therefore, we designed a prospective, randomised, double-blind, parallel-group study to determine whether the prophylactic use of cephalosporin plus azithromycin combination is superior to that of a single dose of cephalosporin in reducing the prevalence of CSD. Our study has a strong theoretical basis and foundation, and this trial may provide evidence of the effective use of antibiotics in clinical practice and data indicating a reduction in the rate of CSD.

Objective and hypothesis

The objective of this trial is to clarify the protective effects of antibiotics against CSD and to assess the effectiveness of adjunctive azithromycin prophylaxis for CSD. Our hypothesis is that the prophylactic use of cephalosporins combined with azithromycin is superior to a single dose of cephalosporin and can reduce the prevalence of CSD.

METHODS AND ANALYSIS

Study design and setting

The study design is that of a double-blind, parallel-control randomised clinical trial. Pregnant women with singleton pregnancies in cephalic presentation who have ruptured membranes or signs of labour will be informed about the study and the need to undergo CS by the medical team. The subjects will be randomly divided into two groups. The trial group (group A) will be administered the regular single-dose cefuroxime sodium (1.5 g/50 ml 0.9% NaCl) plus azithromycin (0.5 g/250 ml 0.9% NaCl). The control group (group B) will be administered the regular single-dose cefuroxime sodium (1.5 g/50 ml 0.9% NaCl) plus an additional placebo (250 ml 0.9% NaCl) as an intravenous drip 30 min before surgery. The prevalence and clinical symptoms of CSD at 6 weeks, 6 months, and 12 months after delivery will be observed with TVU and SIS. We will include 220 women in total. The primary outcome measure will be the prevalence of CSD, and the characteristic of CSD will be assessed by TVU and SIS 42 days after delivery. The secondary outcome measures will be infection indexes and labour results, as shown in Figure 1.

Participants

Patients will be included in this study if they meet all the following criteria:

1. Han Chinese ethnicity with a maternal age of 18 years or older,
2. Singleton pregnancy with a gestational age ≥37 weeks,
3. Fibrinogen≥2 g/L, platelet count ≥$100*10^9$, and haemoglobin ≥90 g/L before surgery,
4. Non-elective caesarean delivery during labour or after membrane rupture,
5. Labour defined as regular contractions with cervical dilation of 3 cm or with documented cervical changes of at least 1 cm of dilation or at least 50% effacement (women with membrane rupture for at least 4 hours will be eligible, regardless of whether labour has started).

Patients will be excluded from participating in the study if one of the following criteria is met:

1. The patient declines to participate in the trial,
2. The patient is known to be allergic to cefuroxime sodium or azithromycin (including an allergy to any kind of macrolide or cephalosporin),
3. The patient was administered azithromycin within 7 days before randomisation,
4. The patient is positive for Group B Streptococcus haemolyticus (GBS) on screening at approximately 36 weeks,
5. The patient has a diagnosis of a non-reproductive tract infection (appendicitis, upper respiratory infection, urinary tract infection) and needs additional antibiotic treatment,

6. The patient has a severe maternal disease (severe liver or renal dysfunction; pulmonary oedema; cardiac structural abnormality or a condition requiring antiarrhythmic drug use; systematic lupus erythaematosus; or inadequately controlled diabetes),
7. The patient has a stillbirth,
8. The patient has a preoperative diagnosis of uterine abnormalities (such as uterine malformation, adenomyosis, or myoma of the uterus), or
9. The patient has undergone a previous CS.

Randomisation

Demographic, medical and obstetrical information will be collected at baseline. The patient will then be randomised between the trial group (group A) and the control group (group B) according to her serial number (number 1 to 250, depending on the time of trial entry). The randomisation codes will be generated by the block of the statistical software at a 1:1 ratio (SPSS, version 22.0, IBM, Armonk, NY, USA). We will generate a list of 250 randomisation codes (A or B). Each code will correspond to one serial number, and each code will be placed in a black envelope with the serial number printed on the outside. Anyone who will have contact with the patient or other researchers will be blinded to the list. Antibiotic prophylaxis will be administered within 30 min before incision. The medicine will be dissolved in the hospital dispensary, where the patient's envelope will be opened by an independent pharmacist, and the containers will be identical so that no one else can identify the set. The opened envelope will be locked in a non-transparent box until the trial is finished. These settings will ensure that both the participants and investigators or surgeons are blinded to the treatment.

Intervention

All participants will undergo a standard CS with a lower segment uterine incision and double-layer suturing of the uterine wound. The first layer will be the decidua and total myometrium, and the second layer will be the serosa and the upper 1/2 layer of myometrium. All sutures will be continuously unlocked sutures with absorbable suture material. We have three experienced surgeons with the same technical standards who will be responsible for the trial. Each of them will perform approximately 70 CSs randomly in the trial. All the participants will be cared for in the wards, and additional antibiotics will be given if there is a confirmed infection before the patient checks out.

Withdrawal of participants

The participants will be able to leave the study at any time for any reason if they wish

to do so, without any consequences. The investigator will be able to decide to withdraw a subject from the study for urgent medical reasons. After randomisation, if a woman wishes to change her assigned protocol, she will be considered a crossover subject. All subjects will remain in the study for analysis based on the intention-to-treat principle.

Participant timeline

The schedules for enrolment, interventions and assessments are summarised in Table 1.

Table 1 Patient's characteristics and data collection

	Days								
Data collection	BL	0	1	2	3	4	42	6months	12months
Age	×								
BMI	×								
Gestational age	×								
Screen	×								
Informed consent	×								
Randomization	×								
TVU							×	×	×
SIS							×	×	×
Antibiotics	×	×							
Blood infection index	×		×						
Blood coagulation function	×		×						
Secretion culture	×	×					×		
Temperature	×	×	×	×	×	×			
The skin infection of incision		×	×	×	×	×			
Endometritis		×	×	×	×	×			
Puerperal fever		×	×	×	×	×			
Other postoperative complicatioins		×							
Questionnaire							×	×	×

BL, baseline; BMI, body mass index; TVU, transvaginal ultrasound; SIS, saline infusion sonohysterography.

The CS will be performed on day 0, and antibiotic treatment, which will be randomly assigned, will be started on day 1.

All included patients will undergo randomisation and treatment. We will conduct preoperative and postoperative routine blood tests and detect C-reactive protein (CRP) levels, procalcitonin (PCT) levels, and blood coagulation function. We will also culture vaginal secretions before surgery and at 42 days postpartum.

Clinical evaluations of the efficacy and safety of anti-infection prophylaxis will be performed during hospitalisation and will include evaluations of the incidence of puerperal fever, skin infections at the incision site, endometritis and other postoperative complications.

Clinical symptoms as well as the prevalence and characteristics of CSD at 6 weeks, 6 months, and 12 months after caesarean delivery will be documented.

Primary outcome measure

The prevalence of CSD,and the characteristic of CSD will be assessed by TVU and SIS 42 days after delivery.

Secondary outcome measure

1. The changes in infection indexes, such as routine blood examinations including CRP and PCT before and after surgery;
2. Pre-surgical vaginal secretion culture results and intraoperative uterine cavity culture results;
3. Body temperature 6 hours before and 72 hours after CS;
4. Postoperative morbidity, endometritis and incisional infection after surgery and severe infection 42 days after delivery;
5. Type and dosage of all antibiotics administered postoperatively according to standard processes;
6. Responses to postpartum questionnaires at approximately 6 weeks, 6 months and 12 months;
7. Follow-up data from TVU and SIS evaluations performed at 6 months and 12 months after delivery; and
8. The size and position of the uterus and the height, length, width, RMT, AMT and niche volume of the defects.

STATISTICAL CONSIDERATIONS

Sample size calculation

Based on the findings of previous studies, we concluded that the occurrence of CSD decreases from 47.9% to 29% after the administration of multi-dose antibiotics during emergency caesarean delivery. We will predict the occurrence of CSD after using two prophylactic antibiotics with PASS sample prediction software version 11.0. We will need to include 220 women in total (two groups of 110 women), with an alpha error of 0.05. Assuming a 10% drop-out rate, we will need to randomise a total of 242

participants (121 participants per group).

Statistical analysis

For analysis, the adopted EDC system will be used to collect data uniformly and record all relevant personal information for data collection points. The method of automatic computer verification, which will include data that clearly do not conform to the conventional standards and among which values are missing, will be adopted. Researchers will be required to check the original data, and all changes in the data will be managed with traces. Query tables will be checked if necessary.

For the analysis of the data, t-tests, analysis of variance, chi-square tests and other differential tests will be carried out with SPSS software.

Descriptive statistics will be used to analyse demographic data and laboratory indicators, such as basic information from the preoperative period, from the postoperative hospitalisation period, and at 6 weeks post-surgery. Preoperative and postoperative indicators will be used for baseline data, and the normality of the distribution of continuous variables from the baseline data will be tested. Normally distributed data will be reported as the mean and standard deviation, and the two groups will be assessed by the independent samples t-test; if the data are nonnormally distributed, a non-parametric analysis will be performed, and the data will be reported as the median and interquartile range. We will use the chi-square test or Fisher's exact test to analyse categorical variables.

We will apply the chi-square test to assess the primary outcome to compare the occurrence rate of the CSD in two groups.

We will apply the t-test, analysis of variance and chi-square test to evaluate the secondary outcome according to the characteristics of the endpoint.

Patient and public involvement

Neither the patients nor the public will be involved in the study design. They will also not be involved in the recruitment process or conduct of the study. The results will be disseminated to patients via an open access publication and our local trials teams.

DISCUSSION

Since the mechanism of wound healing in uterine caesarean incisions is not fully known and since the natural development of CSD over time is still unclear, uterine scars and CSD may change over time. One prospective observational study showed that CSD

occurred from 6 weeks to 6 months or longer after surgery[30]. Another prospective study reported that the statuses of some women with or without CSD showed a reversal between 6 weeks and 6 months after surgery (90.7% at 6 weeks and 93.1% at 6 months (OR 0.96 (0.61-1.49), P=0.91))[31]. This trial is based on our previous cohort study in which CSD was observed at 6 weeks postpartum. We will set the initial CSD assessment timepoint as 6 weeks postpartum and follow patients for 1 year, aiming to find the optimal timepoint for the early detection of CSD. Our sample size calculation is also based on previously published data, which were based on the measurement of CSD with TVU and may have resulted in an underestimation[16]. Since we believe that the difference between the two groups will be more significant when CSD is measured with SIS, the sample size could be large enough to observe a difference. We believe that surgical techniques of uterine incision and closure may be important in the occurrence of CSD, and there are many trials that are on-going or that already have results[32 33]. We will focus on the effects of infection and antibiotics against CSD based on the strong indication of our previous study.

ETHICS AND DISSEMINATION

The study was approved by the Medical Research Ethics Committee of International Peace Maternity and Child Health Hospital (GKLW2017-84). The findings will be reported in peer-reviewed publications and presentations at international scientific meetings.

All women who are eligible for participation in the study will be invited for additional counselling by a research doctor to ensure that they are fully informed regarding the nature of the study by means of both oral and written information. Women who agree to participate will be asked to sign written informed consent forms, of which they will receive a copy.

The protocol of the trial is registered in the Chinese Clinical Trials Register (ChiCTR) as ChiCTR-INR-17013272.

Author's contributions:

Yanqing Cai, Hongjie Pan and Ben WJ Mol are responsible for the overall logistical aspects of the trial and the drafting of this paper. Ding Huang, Ben WJ Mol, Weiwei Chen and Jian Zhang designed the trial and were responsible for the development of the protocol. The senior doctors Ding Huang and Yiru Shi are the main operators of the RCT. Min Zeng and Liye Shi, senior ultrasound doctors, are responsible for the ultrasonographic measurements of the uterus diverticulum. Jinyu is responsible for the design of the randomisation codes and production of the black envelopes. Ying Shen, Sha Chen, Qian Zhu is contributing to the protocol by carrying out literature retrieval, specimen collection, data analysis, and follow-up visits. Ding Huang is responsible for

the project and is contributing to the organisation and operation of the trial.

Competing interests: None

Patient consent: Obtained

Ethics approval: The study was approved by the Medical Research Ethics Committee of International Peace Maternity and Child Health Hospital (approval no. GKLW2017-84).

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

Funding: This trial received funding from the Shanghai Shenkang Hospital Development Center Clinical Science and Technology Innovation Project (item number SHDC12017X05). And it was supported by the medical engineering cross youth funds from Shanghai Jiao Tong University (item number YG2017QN38).

Acknowledgements The authors acknowledge Yanfei Zhang for dissolving the medicine for free and for his confidentiality regarding all the participants and personnel.

References

1. Betran AP, Ye J, Moller AB, et al. The Increasing Trend in Caesarean Section Rates: Global, Regional and National Estimates: 1990-2014. PLoS One 2016;**11**(2):e0148343.

2. Tower AM, Frishman GN. Cesarean scar defects: an underrecognized cause of abnormal uterine bleeding and other gynecologic complications. J Minim Invasive Gynecol 2013;20(5):562-72. doi: 10.1016/j.jmig.2013.03.008

3. Di Spiezio Sardo A, Saccone G, McCurdy R, et al. Risk of Cesarean scar defect following single- vs double-layer uterine closure: systematic review and meta-analysis of randomized controlled trials. Ultrasound Obstet Gynecol 2017;50(5):578-83. doi: 10.1002/uog.17401

4. Allornuvor GF, Xue M, Zhu X, et al. The definition, aetiology, presentation, diagnosis and management of previous caesarean scar defects. J Obstet Gynaecol 2013;33(8):759-63.

5. Sholapurkar SL. Etiology of Cesarean Uterine Scar Defect (Niche): Detailed Critical Analysis of Hypotheses and Prevention Strategies and Peritoneal Closure Debate. J Clin Med Res 2018;10(3):166-73. doi: 10.14740/jocmr3271w

6. Osser OV, Jokubkiene L, Valentin L. Cesarean section scar defects: agreement between transvaginal sonographic findings with and without saline contrast enhancement. Ultrasound Obstet Gynecol 2010;**35**(1):75-83.

7. Yao M, Wang W, Zhou J, et al. Cesarean section scar diverticulum evaluation by saline contrast-enhanced magnetic resonance imaging: The relationship between variable parameters and longer menstrual bleeding. J Obstet Gynaecol Res 2017;43(4):696-704. doi: 10.1111/jog.13255

8. Bij de Vaate AJ, van der Voet LF, Naji O, et al. Prevalence, potential risk factors for development and symptoms related to the presence of uterine niches following Cesarean section: systematic review. Ultrasound Obstet Gynecol 2014;43(4):372-82. doi: 10.1002/uog.13199

9. Nieuwenhuis LL, Hermans FJ, Bij de Vaate AJM, et al. Three-dimensional saline infusion sonography compared to two-dimensional saline infusion sonography for the diagnosis of focal intracavitary lesions. Cochrane Database Syst Rev 2017;**5**:CD011126.

10. Baranov A, Gunnarsson G, Salvesen KA, et al. Assessment of Cesarean hysterotomy scar in non-pregnant women: reliability of transvaginal sonography with and without contrast enhancement. Ultrasound Obstet Gynecol 2016;**47**(4):499-505.

11. van der Voet LF, Vervoort AJ, Veersema S, et al. Minimally invasive therapy for gynaecological symptoms related to a niche in the caesarean scar: a systematic review. BJOG 2014;**121**(2):145-56.

12. Tulandi T, Cohen A. Emerging Manifestations of Cesarean Scar Defect in Reproductive-aged Women. J Minim Invasive Gynecol 2016;**23**(6):893-902.

13. Pomorski M,Fuchs T,Zimmer M. Prediction of uterine dehiscence using ultrasonographic parameters of cesarean scar in the nonpregnant uterusa prospective observational study. BMC Pregnancy Childbirth. 2014;14:365-371.

14. Timor-Tritsch IE, Monteagudo A, Cali G, et al. Cesarean scar pregnancy is a precursor of morbidly adherent placenta. Ultrasound Obstet Gynecol 2014;**44**(3):346-53.

15. Vervoort AJ, Uittenbogaard LB, Hehenkamp WJ, et al. Why do niches develop in Caesarean uterine scars? Hypotheses on the aetiology of niche development. Hum Reprod 2015;**30**(12):2695-702.

16. Kaelin Agten A, Cali G, Monteagudo A, et al. The clinical outcome of cesarean scar pregnancies implanted "on the scar" versus "in the niche". Am J Obstet Gynecol 2017;**216**(5):510 e1-10 e6.

17. Pan H, Zeng M, Xu T, et al. The prevalence and risk predictors of cesarean scar defect at 6 weeks postpartum in Shanghai, China: A prospective cohort study. Acta Obstet Gynecol Scand 2018.

18. Smaill FM, Grivell RM. Antibiotic prophylaxis versus no prophylaxis for preventing infection after cesarean section. Cochrane Database Syst Rev 2014(10):CD007482.

19. Gyte GM, Dou L, Vazquez JC. Different classes of antibiotics given to women routinely for preventing infection at caesarean section. Cochrane Database Syst Rev 2014(11):CD008726.

20. Allen J, David M, Veerman JL. Systematic review of the cost-effectiveness of preoperative antibiotic prophylaxis in reducing surgical-site infection. BJS Open 2018;**2**(3):81-98.

21. D'Angelo LJ, Sokol RJ. Determinants of postpartum morbidity in laboring monitored patients: a reassessment of the bacteriology of the amniotic fluid during labor. Am J Obstet Gynecol 1980;**136**(5):575-8.

22. Lamey JR, Eschenbach DA, Mitchell SH, et al. Isolation of mycoplasmas and bacteria from the blood of postpartum women. Am J Obstet Gynecol 1982;**143**(1):104-12.

23. Mackeen AD, Packard RE, Ota E, et al. Timing of intravenous prophylactic antibiotics for preventing postpartum infectious morbidity in women undergoing cesarean delivery. Cochrane Database Syst Rev 2014(12):CD009516.

24. Purba AKR, Setiawan D, Bathoorn E, et al. Prevention of Surgical Site Infections: A Systematic Review of Cost Analyses in the Use of Prophylactic Antibiotics. Front Pharmacol 2018;**9**:776.

25. Sun J, Ding M, Liu J, et al. Prophylactic administration of cefazolin prior to skin incision versus antibiotics at cord clamping in preventing postcesarean infectious morbidity: a systematic review and meta-analysis of randomized controlled trials. Gynecol Obstet Invest 2013;**75**(3):175-8.

26. Tita ATN, Boggess K, Saade G. Adjunctive Azithromycin Prophylaxis for Cesarean Delivery. N Engl J Med 2017;**376**(2):182.

27. Ward E, Duff P. A comparison of 3 antibiotic regimens for prevention of postcesarean endometritis: an historical cohort study. Am J Obstet Gynecol 2016;**214**(6):751 e1-4.

28. Sutton AL, Acosta EP, Larson KB, et al. Perinatal pharmacokinetics of azithromycin for cesarean prophylaxis. Am J Obstet Gynecol 2015;**212**(6):812 e1-6.

29. Skeith AE, Niu B, Valent AM, et al. Adding Azithromycin to Cephalosporin for Cesarean Delivery Infection Prophylaxis: A Cost-Effectiveness Analysis. Obstet Gynecol 2017;**130**(6):1279-84.

30. Harper LM, Kilgore M, Szychowski JM, et al. Economic Evaluation of Adjunctive Azithromycin Prophylaxis for Cesarean Delivery. Obstet Gynecol 2017;**130**(2):328-34.

31. Bamberg C, Hinkson L, Dudenhausen JW, et al. Longitudinal transvaginal ultrasound evaluation of cesarean scar niche incidence and depth in the first two years after single-

or double-layer uterotomy closure: a randomized controlled trial. Acta Obstet Gynecol Scand 2017;**96**(12):1484-89.

32. Dosedla E, Calda P. Can the final sonographic assessment of the cesarean section scar be predicted 6 weeks after the operation? Taiwan J Obstet Gynecol 2016;**55**(5):718-20.

33. Roberge S, Demers S, Berghella V, et al. Impact of single- vs double-layer closure on adverse outcomes and uterine scar defect: a systematic review and metaanalysis. Am J Obstet Gynecol 2014;**211**(5):453-60.

Figure Legend:

Fig 1 Flowchart of the study.

CS, Cesarean-section; TVUS, transvaginal ultrasonography

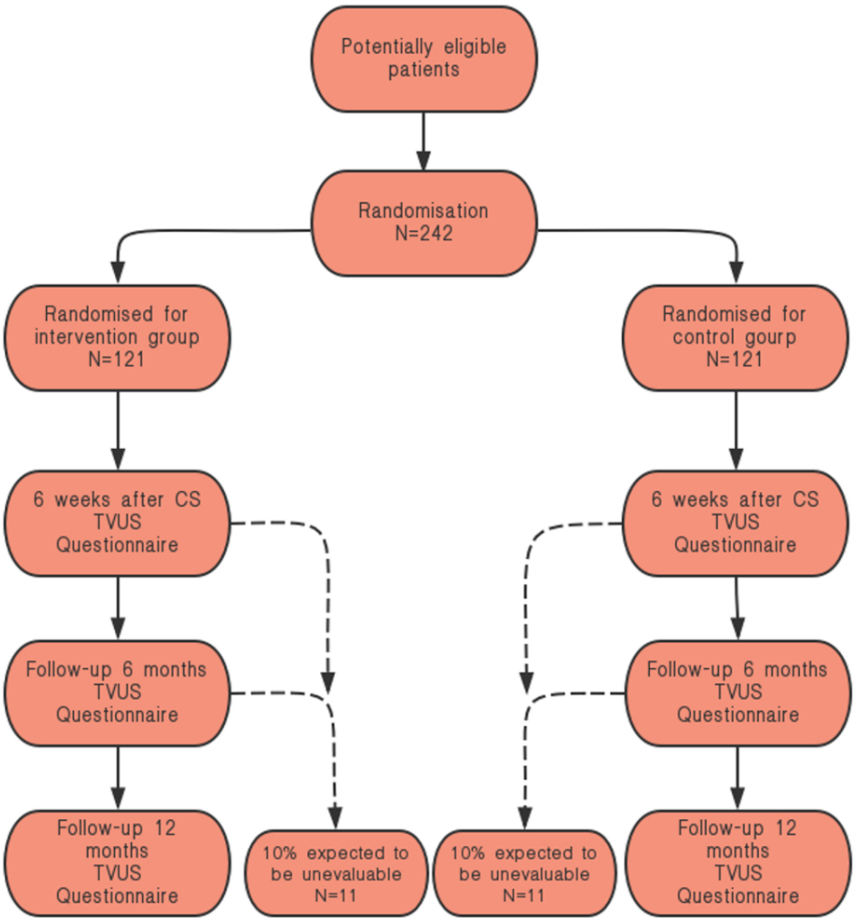

Fig 1 Flowchart of the study.
CS, Cesarean-section; TVUS, transvaginal ultrasonography

90x90mm (300 x 300 DPI)

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
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym (Page1,line1)
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry (Page2,line13)
	2b	All items from the World Health Organization Trial Registration Data Set (Page2,line13)
Protocol version	3	Date and version identifier (N/A)
Funding	4	Sources and types of financial, material, and other support (Page11,line9)
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors (Page1,line4)
	5b	Name and contact information for the trial sponsor (Page11,line9)
	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 (Page2,line31)
	6b	Explanation for choice of comparators (Page3,line5)
Objectives	7	Specific objectives or hypotheses (Page4,line30)
Trial design	7	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) (Page4,line36)

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 (Page5,line5)
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) (Page5,line14)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered (Page6,line25)
	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) (N/A)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) (N/A)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial (Page7,line1)

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 (Page8,line8)
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) (Page7,line8)
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 (Page8,line28)
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size (N/A)
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 (Page6,line14)
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 (Page6,line15)
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions (Page6,line13)
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how (Page6,line23)

	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial (N/A)
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 (Page9,line8)
	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 (N/A)
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 (Page9,line13)
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 (Page9,line22)
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) (N/A)
	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) (Page9,line5)
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 (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 (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 (N/A)
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor (N/A)
Ethics and dissemination		
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval (Page10,line17)
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) (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) (Page10,line21)
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable (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 (N/A)
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site (Page11,line3)
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 (N/A)
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 (N/A)
	31b	Authorship eligibility guidelines and any intended use of professional writers (N/A)
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code (N/A)
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates (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 (N/A)

*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.