

# BMJ Open Comparison of PGS2.0 versus conventional embryo morphology evaluation for patients with recurrent pregnancy loss: a study protocol for a multicentre randomised trial

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## ABSTRACT

**Introduction** Pregnancy loss (PL) is an adverse life event, and there is no proven effective treatment for recurrent PL (RPL). Preimplantation genetic screening (PGS) can be performed to reduce the risks of PL; however, there is still no solid scientific evidence that PGS improves outcomes for couples experiencing RPL. Comprehensive chromosome screening (PGS2.0) has become a routine practice in *in vitro* fertilisation (IVF) clinics. Previous studies based on PGS1.0 with a focus on RPL couples where the female is of advanced maternal age have reported contradictory results. Hence, a multicentre randomised trial is needed to provide evidence for the clinical benefits of PGS2.0 treatment for RPL couples.

**Methods and analysis** Overall, 268 RPL couples undergoing IVF cycles will be enrolled. Couples will be randomised according to a unique grouping number generated by a random digital software into (1) PGS2.0 group and (2) non-PGS (conventional embryo morphology evaluation) group. This study aims to investigate whether the live birth rate (LBR) per initiated cycle after PGS2.0 is superior to the LBR per initiated cycle after conventional embryo evaluation (non-PGS group). Live birth will be defined as a live baby born after a gestation period of >28 weeks, with a birth weight of more than 1000 g. A multivariate logistic regression model will be used to adjust for confounding factors.

**Ethics and dissemination** Ethical approval has been granted by the Ethics Committee of Obstetrics and Gynecology Hospital, Fudan University and the participating hospitals. Written informed consent will be obtained from each couple before any study procedure is performed. Data from this study will be stored in the Research Electronic Data Capture. The results of this trial will be presented and published via peer-reviewed publications and presentations at international conferences.

**Trial registration number** NCT03214185; Pre-results.

## INTRODUCTION

A pregnancy loss (PL) or miscarriage is defined as the spontaneous demise of a pregnancy before the fetus reaches viability; that

## Strengths and limitations of this study

- This will be the first multicentre randomised trial to investigate the effectiveness of preimplantation genetic screening (PGS2.0) for the treatment of recurrent pregnancy loss (RPL).
- This is the first trial that seeks to add significantly to the clinical evidence on the positive effects of PGS2.0 on the live birth rate (LBR) in young RPL couples.
- A multivariable prediction model for future pregnancy outcomes of young RPL couples will be provided based on trial data.
- Bias by adjustment for important confounding factors, including maternal and paternal factors, will be made to investigate the independent effect of PGS2.0 on RPL.
- Sample size calculation will be based on a difference of 15% points in the LBR per initiated cycle between the two cohorts, and a smaller difference in the LBR may not be detected.

is, from the time of conception until 28 weeks of gestation in China,<sup>1 2</sup> 24 weeks of gestation in European countries<sup>3</sup> or 22 weeks' gestation according to the international glossary on infertility and fertility care.<sup>4</sup> It also includes non-visualised PLs (biochemical PLs or resolved and treated pregnancies of unknown location), and excludes ectopic and molar pregnancies.<sup>3</sup> Recurrent PL (RPL) is defined as two or more PLs.<sup>3 5</sup> Approximately 1%–5% of couples trying to conceive experience RPL.<sup>6</sup> Little is known about the cause of RPL; however, this condition is believed to have a multifactorial pathogenesis. Miscarriage specimen examinations have revealed that 50%–70% of early PLs are due to chromosomal abnormalities,<sup>7</sup> which can either be of parental origin or arise *de novo* in the embryo from parents with normal karyotypes,<sup>8</sup> often

as a random event. Among these, aneuploidy is considered as the main chromosomal abnormality; it is also the main abnormality found in normally developing monospermic embryos during *in vitro* fertilisation (IVF).<sup>9</sup> Recently, a large genetic survey of embryos supported the finding that aneuploidy is the leading chromosomal abnormality in IVF, and it primarily occurs due to errors in maternal meiosis and mitosis.<sup>10</sup> The association between aneuploidy and increasing maternal age has been recognised for a long time<sup>11</sup>; however, the underlying molecular basis has remained elusive. Some studies have provided evidence that the age-related increase in maternal errors is not attributable to one single factor.<sup>12</sup> However, when the female patient in couples with a history of RPL is of relatively young age, the reasons for frequent aneuploidy cannot be attributed to advanced age alone, and the mechanisms remain unclear.

Owing to the high frequency of aneuploidy in patients with RPL, preimplantation genetic screening (PGS)—now called preimplantation genetic testing-aneuploidy—which aims to detect aneuploidy before transfer, is applied to these patients. In the past two decades, fluorescence in situ hybridisation (FISH) technology using limited probes has been applied to detect the 5–10 most common aneuploidies in one or two blastomeres biopsied at day 3 in cleaving embryos. Although this has been applied to reduce the miscarriage rate and increase the live birth rate (LBR) in IVF (PGS1.0), a few randomised clinical trials have shown a significant decrease in pregnancy outcomes after PGS1.0.<sup>13–14</sup> This disappointing result might be due to three reasons: first, the cleavage stage biopsy harms the embryo development potential<sup>15</sup>; second, FISH can detect only a limited number of aneuploidies; third, mosaicism of the cleaving embryo leads to incorrect assessment of the embryo. Therefore, a new generation of PGS (PGS2.0) has been introduced to IVF centres; this favours trophoctoderm (TE) biopsy and comprehensive chromosome aneuploidy screening.<sup>16–17</sup> Hence, many reports of PGS2.0 have shown increased ongoing pregnancy rates and LBRs.<sup>18–20</sup> However, the beneficial effect of PGS2.0 has not been proven yet in randomised controlled trials (RCTs).<sup>21</sup>

Conventional morphological blastocyst grading systems recommended by Gardner and Schoolcraft, which include the degree of blastocoele expansion, inner cell mass (ICM) and TE cells, are used to predict the ploidy status of blastocysts.<sup>9</sup> More importantly, this grading is completely non-invasive and has no adverse effects on implantation. Observational studies report a correlation between good morphology and euploidy embryos,<sup>22–23</sup> and many researchers propose embryo morphology as an alternative marker of chromosomal status,<sup>24</sup> given the positive correlation between morphological grading and the euploid state of the embryo. However, it has been reported that morphology analysis cannot accurately predict the genetic status of embryos, because about 50%–60% of excellent and good quality embryos are aneuploid.<sup>25</sup>

In Europe in 2012, the reported mean delivery rates per aspiration for IVF, intracytoplasmic sperm injection (ICSI) and frozen-thawed embryo transfer (FET) were 21.9%, 20.1% and 16.0%, respectively.<sup>26</sup> In 2013, the rates were 22.2%, 20.1% and 18.0%, respectively.<sup>27</sup> In Europe in 2017, delivery rates after PGS per oocyte retrieval and per embryo transfer were 13% and 22%, respectively.<sup>28</sup> These data might be analysed by FISH (PGS1.0). Simon *et al* reported LBR per transfer of 64.5% and per retrieval of 45.1% in 1621 non-donor frozen cycles with PGS in 2018.<sup>29</sup> Lee *et al* also reported LBR per initiated cycle of 46.3% in 82 cycles of RPL couples with PGS in 2019.<sup>30</sup> These data might be analysed by comprehensive chromosome testing (PGS2.0). We have conducted a retrospective analysis and found LBR per initiated cycle of 26.6% in RPL couples with PGS, and 15.4% in RPL couples without PGS.<sup>31</sup>

For RPL couples who require IVF to help them conceive, we know that PGS might increase the LBR per transfer, but whether PGS2.0 could increase the LBR per start cycle or the cumulative LBR remains unknown. PGS2.0 is thought to be a good treatment for patients with RPL, but whether it should be routinely applied for all couples with RPL remains controversial. The present protocol describes a multicentre randomised trial assessing PGS2.0 in the treatment of patients with RPL. The results are very important for clinicians involved in RPL treatment, and for patients who experience RPL.

## METHODS AND ANALYSIS

### Study design

This is a multicentre randomised controlled clinical trial which is designed to compare LBR per initiated oocyte retrieval cycle, per patient (cumulative LBR) and per embryo transfer in 268 RPL couples undergoing ICSI. Participants will be enrolled at three hospitals in Shanghai, China. This study has been approved by the ethics committees at the three hospitals. Informed consent will be obtained from the enrolled couples before any study procedures are performed. Reporting of the study results will follow the 2010 revised Consolidated Standards of Reporting Trials statement<sup>32</sup> and updated guidelines, 2012.<sup>33</sup>

### Study population/participants and recruitment

The following inclusion criteria will be applied:

1. Couples who have experienced two or more PLs.
2. Normal karyotypes of both husband and wife (polymorphic chromosomes are considered normal as well).
3. Females aged between 20 and 38 years ( $\geq 20$  and  $< 38$  years).

The exclusion criteria will include:

1. Females with uterine abnormalities such as uterine malformations (uterus unicorns and duplex uterus), untreated septate uterus, adenomyoma, submucous uterine fibroids, endometrial polyps or untreated intrauterine adhesions.

2. Females with medical conditions that contraindicate assisted reproductive technology or pregnancy such as deep vein thrombosis, pulmonary embolism, cardiac disease, carcinoma and severe anaemia.

In order to achieve adequate participant enrolment to reach the target sample size, we will use the following strategies:

1. At the waiting rooms of the three IVF centres, posters will be put to let more people know this study.
2. The doctors at the three IVF centres will be encouraged to introduce the study to their patients to let more people know this study.
3. A study contact will be designated for any person who wants to know the details of this study.

### Interventions

Randomisation will take place during the couple's first visit to the clinic or on the first day of stimulation. All included couples will be informed of the study procedures and written informed consent will be signed before controlled ovarian hyperstimulation (COH) is implemented and any procedures are performed. The included couples will be randomised 1:1 into either of two groups: group A (PGS2.0 group) and group B (non-PGS group, conventional embryo morphology evaluation group). Group A will undergo conventional embryo morphology evaluation and TE biopsy before blastocyst cryopreservation, and group B will undergo conventional embryo morphology evaluation before blastocyst cryopreservation. All patients will undergo an FET once a good quality embryo or an euploid embryo after PGS2.0 is chosen. Evaluation of blastocyst stage embryos is based on three aspects: the expansion of the blastocoele cavity (the expansion and hatching (EH) stage), the number and cohesiveness of the ICM (ICM grade) and TE cells (TE grade) according to the Gardner and Schoolcraft grading system.<sup>34–36</sup> The EH stage is assessed as one of the following: (1) an early blastocyst with the volume of the blastocoele is less than half of that of an embryo; (2) a blastocyst with the volume of the blastocoele is at least half that of the embryo; (3) a full blastocyst with a completely filling blastocoele of the embryo; (4) an expanded, thinning zona blastocyst with the volume of the blastocoele larger than that of the full blastocyst; (5) a hatching blastocyst with the TE starting to herniate through the zona; and (6) a hatched blastocyst completely escaped from the zona. ICM and TE grade are evaluated after EH stage is assessed. The ICM is assessed as one of the following: (A) tightly packed, many cells; (B) loosely grouped, several cells; and (C) very few cells. The TE is assessed as one of the following: (A) many cells forming a cohesive epithelium; (B) few cells forming a loose epithelium; and (C) very few, large cells.

### Randomisation

At the start of the study, the grouping results will be generated by random digital software corresponding to a unique grouping number. The couples will be given a unique

grouping number when they have signed the informed consent form; subsequently, they will be randomly divided into group A or group B. Both the investigators and the patients will be aware of the grouping information and interventions. There will be no blinding of the treatment allocation to the doctors and participants in the study. The embryologist performing the embryo quality evaluation will be blinded to the allocated treatment.

### Questionnaire

A questionnaire will be developed for collating the basic characteristics of the couple; this will include the date of birth of the couple, ethnicity, education, annual income level, occupation and lifestyle. The participants will address these questions on the Research Electronic Data Capture (REDCap) platform. REDCap is a widely used secure web interface for ensuring data quality; it checks data accuracy during data entry.

### Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our trial.

### COH protocol

1. All patients will undergo up to three COH cycles unless they indicate that they wish to stop treatment. If the patient is not pregnant after three COH cycles and has no surplus embryos for transfer, she will be automatically withdrawn from the study.
2. A 2D pelvic ultrasound will be performed before the start of COH, and basal hormone levels, including serum follicle-stimulating hormone (FSH), luteinising hormone (LH), prolactin, oestradiol (E2), progesterone (P4), testosterone and anti-Mullerian hormone, will be examined.
3. Conventional GnRH antagonist COH protocols will be used in all patients either by using daily recombinant FSH (rFSH) or human menopausal gonadotropin (hMG).<sup>37</sup> The gonadotropin stimulation will be performed according to the routine methods used in the clinics of the three hospitals involved in the study. Generally, rFSH or hMG will begin on day 2 or day 3 of the menstrual period; the latter occurring either naturally or induced by exogenous administration of progesterone or contraceptive pill/oral contraceptives. The initial doses will be 150–300 IU/day according to female age, body mass index (BMI), number of antral follicles and basal hormone levels.<sup>38</sup> On the sixth day of receiving the rFSH or hMG, transvaginal ultrasound will be performed to examine the diameter of the follicles, and a blood test for serum E2, P and LH levels will be performed. rFSH or hMG doses will be adjusted according to ovarian response. Subsequently, such monitoring will be performed either every other day or every day. The antagonist regimen is as follows: Antagonist regimen 1=rFSH (150–300 IU intramuscular) from day 2 or day 3 followed by rFSH (150–300 IU

intramuscular)+Cetrotide (0.25 mg/day subcutaneous) from day 8 or day 9.

Antagonist regimen 2=hMG (150–300IU intramuscular) from day 2 or day 3 followed by hMG (150–300IU intramuscular)+Cetrotide (0.25 mg/day subcutaneous) from day 8 or day 9.

4. When at least one follicle reaches a mean diameter of 14 mm, or the serum E2 reaches 1000 pg/mL, the patient will receive 0.25 mg/day of GnRH antagonist (Cetrotide, Merck Serono, Shanghai, China) and this will be continued daily until the trigger day.
5. Human chorionic gonadotropin (hCG) trigger or a GnRH agonist for final oocyte maturation: when the mean diameter of at least one follicle is  $\geq 18$ mm or two follicles are  $\geq 16$ mm, an intramuscular injection of hCG (hCG, HCG, Zhuhai Livzon Pharmaceutical Group, Zhuhai, China) 5000–10 000IU or triptorelin (Triptorelin Pamoate, Ferring, Switzerland) 0.1 mg will be administered to the patient. Subsequently, 36 hours after hCG or triptorelin injection, the oocytes will be retrieved under transvaginal ultrasound guidance. On the trigger day, the endometrial thickness and morphology, as well as the number and size of follicles ( $\geq 15$ , 10–15 and  $< 10$ mm) will be documented.

#### ICSI and embryo culture

A single sperm will be injected within 4 hours after the follicular aspiration. Embryos will be cultured in sequential medium with 5% carbon dioxide in the atmosphere. The fertilisation state of the embryo will be observed 16–18 hours after ICSI. The observation of blastomere formation (cleavage rate) and scoring of the effective cleavage stage embryos will be performed 72 hours after ICSI; however, the day 3 cleaving embryos will continue to be cultured to blastocysts.

#### Good quality embryo evaluation

Group A: Blastocysts in group A will first be evaluated according to a widely used grading system (Gardner and Schoolcraft) as previously described.<sup>35 39</sup> Subsequently, 3–10 TE cells will be biopsied and immediately transported to the genetics laboratory for chromosome screening analysis. The day of TE biopsy will be dependent on blastocyst development and recorded as day 5 or day 6. The amplified products will be preserved according to the requirements of the genetic laboratory. Blastocysts will be cryopreserved immediately after the biopsy procedure is finished. Embryos will be classified as euploid, aneuploid, mosaic or not classifiable. Consequently, only one euploid and good morphology embryo will be transferred. If no euploid embryo is detected, the transfer cycle will be cancelled.

Group B: Blastocysts in group B will be evaluated according to the Gardner grading system as described above and then cryopreserved. One good quality embryo will be transferred in the next frozen-thawed cycle.

The freeze-all strategy used here is to reduce the potential risk of ovarian hyperstimulation syndrome which

could happen on some of these patients. If that was happened, we will record these adverse events and give appropriate and timely treatment.

#### Embryo transfer and luteal phase support

Endometrial preparation will be hormonally induced. Oral E2 valerate (E2V, Progynova, Bayer Schering Pharma, Shanghai, China) will be given to patients at a dose of 4 mg daily from menstrual day 3. The E2V dose will remain unchanged for 10 days and will then be increased to approximately 6–8 mg/day if the endometrial thickness is still less than 8 mm. When the endometrial thickness is  $\geq 8$ mm, 60 mg of progesterone (progesterone injection, Xianju Pharma, Zhejiang, China) will be injected intramuscularly per day. Six days after the progesterone injections, the blastocyst will be frozen thawed and transferred. One good quality embryo will be transferred through a catheter guided by transabdominal ultrasound. The patients will lie in bed for half an hour or be free to walk around after transfer. The dose of E2V and progesterone will be unchanged until the day on which serum  $\beta$ -hCG levels are measured. If the patient is pregnant, luteal phase support will continue until 11 weeks of gestation and 8% progesterone sustained-release vaginal gel (Crinone, Merck Serono, Shanghai, China; 90 mg per day) will be added.

#### Pregnancy evaluation

Serum  $\beta$ -hCG will be measured to determine pregnancy 14 days after embryo transfer. If a biochemical pregnancy has been detected, a transvaginal ultrasound scan will be performed 28 days after embryo transfer. If a gestational sac is detected and a heartbeat is seen, a clinical pregnancy is confirmed. The ultrasound scan will be repeated every 2 weeks until 11 weeks. Ongoing pregnancy will be confirmed if the fetal heartbeat is confirmed at 12 weeks of gestation.

#### Follow-up evaluation

At 12 weeks of gestation, first-trimester pregnancy complications (miscarriage, ectopic pregnancy and gestational trophoblastic neoplasia) will be documented in the case report form (CRF) for the first pregnancy follow-up time point. Antenatal care will be referred for these women when the ongoing pregnancy is beyond 12 weeks.

At 28 weeks of gestation, the situation of mothers and fetuses will be documented in the CRF at the second pregnancy follow-up time point. If the patient fails to have a live birth, another FET will be arranged and followed up. Perinatal care will be introduced to these mothers when the pregnancy is beyond 28 weeks.

At 42 weeks of gestation, delivery information (gestational age, delivery mode, placenta abnormality and delivery complications) and the newborn information (baby sex, birth weight, Apgar score and birth defects) will be documented in the CRF for the third pregnancy follow-up time point. Postpartum care will be introduced to these mothers to help with postpartum recovery.

Six weeks after delivery, the postpartum information and neonatal disease information will be documented in the CRF for the fourth and final pregnancy follow-up time points.

### Primary objective

The primary objective of the study is to investigate if the LBR per initiated cycle after PGS is superior compared with the conventional embryo morphology evaluation strategy in the treatment of patients with RPL. Live birth will be defined as a live-born baby with a gestational period beyond gestational week 28, and birth weight more than 1000 g. Investigation of the cumulative LBR, which is the LBR per patient, and LBR per blastocyst transfer, is also considered a primary aim of the study.

### Secondary objectives

The secondary objectives are as follows:

1. To analyse clinical pregnancy rate per transfer, per initiative and cumulative pregnancy rate in the two groups. Clinical pregnancy will be defined as the presence of an intrauterine gestation sac 4 weeks after embryo transfer.
2. To measure time to pregnancy from the date of starting COH to the date of the first ongoing pregnancy in the two groups (the longest follow-up time will be 2 years; hence, failure will be defined as no pregnancy over the 2-year period from the start of COH).
3. To measure the miscarriage rate in the two groups. Miscarriage will be defined as the termination of the pregnancy at <28 weeks of gestation with a miscarried fetal weight less than 1000 g.

### Sample size calculation

The three study centres had an average 15% LBR per initiated retrieval cycle and an average 30% LBR per initiated cycle following PGS and FET strategy for the last 3 years. For the sample size calculations, we aim to detect an increase of 15% of LBR following PGS strategy with an alpha error level of 0.05 and a beta error level of 0.2. The number will be set to 1:1 in each group, and the minimum sample size will be 242 participants. Considering a dropout rate of 10%, we expect to have a total of 268 participants, with 134 participants in each group.

### Outcome measurements (primary and secondary)

Four investigators from the three centres have composed a data monitoring group (DMG), which is responsible for data integrity and accuracy. All the data will be stored in the REDCap, and this interface will automatically ensure accuracy during data entry. We included data obtained from participants completing the self-administered basic characteristics survey questionnaire. We included outcome data from the whole COH cycle and follow-up evaluations. We will use the full analysis set, an intent-to-treat approach, to examine differences in the LBR per initiated cycle in the two treatment arms in the primary analysis using a Pearson  $\chi^2$  test. Clinical pregnancy rate and other rates will be analysed using the Pearson  $\chi^2$  test

and logistic regression. Cox proportional hazards models and the Kaplan-Meier method will be used to compare differences of time to pregnancy and cumulative LBR. Multiple imputation will be conducted for analysis of missing data. The DMG will audit the data quarterly.

### Ethics and dissemination

RPL is unexplained in about 50% of young couples, and the effectiveness of treatments, such as anticoagulation,<sup>40</sup> corticosteroids<sup>41</sup> and other such treatments, is controversial. In current practice, RPL is considered an issue derived mostly from embryo causes. However, it is questionable whether this embryo-centred approach is correct.

In this trial, we hypothesise that euploid embryos will increase the LBR for young RPL couples. Many observational studies have shown that PGS can increase the LBR per transfer, but may decrease the LBR per initiated cycle in women of advanced age.<sup>13 25</sup> To the best of our knowledge, this trial is the first RCT to analyse LBR in young RPL couples.

The limitations of this RCT are that the sample size calculation is based on a difference in the LBR per initiated cycle of 15% between the two cohorts; hence, it may not be able to detect smaller differences in LBR. Larger effect sizes may be achieved in more controlled settings; however, this is a trade-off for studying the complex, heterogeneous RPL population who might receive other individualised and complex treatment. Additionally, the centres included in this RCT are all in Shanghai, although included couples may come from all over the country. Therefore, the generalisability of the results may be limited and the inclusion of sites and patient populations from around the country may have provided a more diverse and larger sample size. We will try to minimise this by using randomisation and by choosing young couples who have travelled from other parts of China for treatment.

No blinding of the treatment allocation to the doctors in the study might cause the doctors to choose a higher stimulation dose in the PGS2.0 group in order to get more oocytes for selection. However, the dose of the gonadotropins and euploidy rate is controversial.<sup>38 42</sup> The initiative doses will be 150–300 IU/day according to female age, BMI, number of antral follicles and basal hormone levels. To choose PGS or not is not considered when choosing the initiative stimulation dose, and the adjustment of dose will be based on the women's ovarian response. We use the randomised trial to reduce confounders.

Counselling of young couples confronted with unexplained RPL regarding its aetiology and prognosis is an essential part of the treatment process, and the advice will allow them to choose their treatment modalities and decide for or against future attempts. This study may prove that PGS is a quick and safe future treatment option.

Amendments to the protocol will be agreed on by the ethics committee, data and safety monitoring committee

and will be approved by the ethics committee prior to implementation.

Ethical approval has been granted by the Ethics Committees of Obstetrics and Gynecology Hospital, Fudan University (2017-85), the Shanghai JiAi Genetics & IVF Institute (JIAIE2017-15), the coordinated centres of Renji Hospital, Shanghai Jiao Tong University (2017072101) and the International Peace Maternity and Child Health Hospital of China Welfare Institute, Shanghai Jiao Tong University (GKLW2017-13). Written informed consent will be obtained from each couple before any study procedure is performed. Data from this study are/will be stored in the REDCap. To improve adherence to intervention protocols, the investigators will keep the proper scientific research attitude, and be able to answer the participants' various questions to increase participants' compliance. The personal information of the enrolled participants will be removed during collecting, sharing and maintaining in order to protect the confidentiality of the participants, and all COH cycles assigned to the participants will be identified by a consistent patient identification. There will be no interim analysis during the study period. The results of this trial will be presented and published via peer-reviewed publications and presentations at international conferences.

### Trial status

The study was designed in July 2017, and the first participant was randomised on 22 March 2018. At the time of the manuscript preparation, we have recruited 100 couples and the recruitment is ongoing. We aim to complete the recruitment by 31 March 2021.

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**Contributors** CLei, YSui and XSun from the sponsor hospital designed the whole study. XSun was responsible for the whole project. CLei, XSun, YSun and LJin were responsible for patient recruitment and randomisation. CLei, YLu, JXi and JYe formed the data management team responsible for collecting and analysing all data. CLei, JYe, XSun, YSun and LJin supervised the data. The manuscript was drafted by CLei. All authors participated in reviewing, curating and the approval of the final manuscript.

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**Competing interests** None declared.

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