

A multicenter randomized trial of personalized acupuncture, fixed acupuncture, letrozole and placebo on live birth for infertility in women with polycystic ovary syndrome

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Study protocol

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Abstract

BACKGROUND Traditional Chinese Medicine (TCM) usually involves syndrome and disease differentiation, and for acupuncture selection of appropriate points and skillful needling techniques. Many clinical trials on acupuncture used fixed acupuncture protocols without accounting for individual differences. We here design a multicenter randomized controlled trial (RCT) to evaluate whether personalized or fixed acupuncture increases the likelihood of live births for infertility in women with polycystic ovary syndrome (PCOS) compared with placebo letrozole. We hypothesize that letrozole is more effective than personalized acupuncture, and personalized acupuncture is more effective than fixed acupuncture, which is more effective than placebo letrozole. Moreover, personalized acupuncture is more likely to reduce miscarriage rate and the risk of pregnancy complications than letrozole.

METHODS / DESIGN The study is a randomized assessor-blind controlled trial. A total of 1,100 infertile women with PCOS will be recruited from 28 hospitals and randomly allocated into four groups: A) letrozole, B) personalized acupuncture, C) fixed acupuncture, or D) placebo letrozole. They will receive treatment for 16 weeks and the primary outcome is live birth. Secondary outcomes include ovulation rate, ongoing pregnancy rate, miscarriage rate, hormonal and metabolic profile, and quality-of-life scores. The side effect profile will be recorded throughout the trial. All statistical analyses will be performed using the SPSS program V.21.0 (SPSS, Chicago, Illinois, USA) and a p value <0.05 will be considered statistically significant.

DISCUSSION This study is the first multicentre RCT to compare personalized or fixed acupuncture, letrozole or placebo letrozole on live birth for infertility in women with PCOS. This findings will inform whether personalized acupuncture therapy could be as an alternative treatment method on live birth for infertile women with PCOS.

Background

Polycystic ovary syndrome (PCOS) is associated with anovulation and infertility^[1]. The managements of anovulatory infertility in women with PCOS involve lifestyle modifications, pharmacological treatment (letrozole, clomid, metformin and gonadotropins), laparoscopic ovarian diathermy and in vitro fertilization^[2]. Letrozole, an aromatase inhibitor, is the first-line therapy for infertility in women with PCOS^[3]. It reduces oestrogen biosynthesis and increases follicle-stimulating hormone (FSH) secretion by modulating the estrogenic negative feedback of the hypothalamus/pituitary axis without adverse endometrial and cervical mucus effects^[4].

Acupuncture has been used in China for more than 3000 years and is a core part of traditional Chinese medicine (TCM). Jedel et al^[5] reported that acupuncture with manual and low-frequency electrical stimulation was superior to exercise and no treatment for improving menstrual frequency and reducing total testosterone levels. Johansson et al^[6] demonstrated that repeated acupuncture resulted in higher ovulation frequency during the treatment period in women with PCOS. Besides, acupuncture can restore dysfunctional glucose homeostasis as well as adipose tissue gene expression and DNA methylation, in part via activation of autonomic nervous system in PCOS patients^[7]. However, a recent large multicenter trial in China demonstrated that acupuncture given in a fixed protocol did not improve the live birth in women with PCOS when compared with placebo acupuncture or clomiphene citrate^[8].

Acupuncture is a complex treatment and tailors to individual patients in TCM. According to TCM should signs and symptoms of each patient be considered in differential symptom diagnosis before selecting the acupoints. While, several studies had concluded that acupuncture was effective for some disease conditions^[9,10], other did not find any differences between personalized, fixed and sham acupuncture on systolic or diastolic blood pressure^[11]. The recent study demonstrating no improvement of live birth rate^[8], used a fixed and followed Western Medical acupuncture theories. Acupuncture points were selected according to innervation of ovaries and uterus and differs from the TCM theory with syndrome differentiation and treatment. Moreover, it is not certain if acupuncture is more effective than no intervention for ovulation induction and live birth rate.

Therefore, we aim to investigate the efficacy of personalized acupuncture based on the TCM theory, fixed acupuncture, letrozole and placebo letrozole on the live birth rate of infertility in women with PCOS.

Objectives

The objectives of the present trial are to test the following four hypotheses for infertility in women with PCOS:

- Letrozole is more likely to induce ovulation and results in live birth than personalized acupuncture.
- Personalized acupuncture is more likely to induce ovulation and results in live birth than fixed acupuncture.
- Fixed acupuncture is more likely to induce ovulation and results in live birth than placebo letrozole.
- Personalized acupuncture is more likely to reduce miscarriage rate and the risk of pregnancy complications compared with letrozole, fixed acupuncture and placebo letrozole.

Methods And Design

Study design

This is a multi-center randomized trial involving four treatment groups. The research protocol is compliant with the Consolidated Standards of Reporting Trials (CONSORT)^[12] guidelines and Standards for Reporting Interventions in Clinical Trials of Acupuncture (STRICTA)^[13], as well as with the Standard Protocol with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT)^[14] statement. The SPIRIT checklist is presented in Additional file 1.

Recruitment

A total of 1,100 infertile women with PCOS will be recruited from 28 hospitals in China. They will be randomly allocated into four groups: personalized acupuncture, fixed acupuncture, letrozole or placebo letrozole. Women will be recruited through advertisements in local newspapers and bulletin boards in each trial center. Eligible women will be approached and sign the consent form after detailed explanation of the study design and comprehensive counseling.

Inclusion criteria are:

- Age of women between 20 and 40 years.
- Chronic oligomenorrhea or amenorrhea: oligomenorrhea is defined as an intermenstrual interval > 35 days or < 8 menstrual bleedings in the past year. Amenorrhea is defined as an intermenstrual interval >90 days.
- Hyperandrogenism (either hirsutism or hyperandrogenemia) or polycystic ovaries on ultrasound. Hirsutism is determined by a modified Ferriman-Gallwey (FG) Score ≥ 5 at screening examination^[15], and biochemical hyperandrogenism is defined as total testosterone (T) >2.6 nmol/L and free testosterone ≥ 6.0 pg/mL (the cutoff level may differ from each study site)^[16]. Polycystic ovaries are present when the number of small antral follicles (2–9 mm in diameter) ≥ 12 or ovarian volume > 10mL on transvaginal scanning ^[17].
- At least one patent tube shown by hysterosalpingogram or diagnostic laparoscopy within 3 years if the patient does not have a history of abortion or pelvic operation. If the patient has a history of pregnancy and no history of pelvic operation within the past 5 years, she is not required to undergo a tubal patency test.
- Sperm concentration $\geq 15 \times 10^6$ /mL and total motility $\geq 40\%$ or total motile sperm count ≥ 9 million in the semen analysis of the husband ^[18].
- The couple agree to have regular intercourse i.e. 2–3 times per week during the study period.

Exclusion criteria included:

- Exclusion of other endocrine disorders:
 - patients with hyperprolactinemia (defined as two prolactin (PRL) levels at least one week apart ≥ 25 ng/mL)
 - Patients with FSH levels > 15 mIU/mL. A normal level within the last year is adequate for entry
 - Patients with uncorrected thyroid disease (defined as thyroid-stimulating hormone (TSH) < 0.2 mIU/mL or > 5.5 mIU/mL). A normal level within the last year is adequate for entry
 - Patients diagnosed with Type I or Type II diabetes who are poorly controlled (defined as glycated haemoglobin (HbA1c) level > 7.0%), or patients receiving antidiabetic medications such as metformin, insulin, thiazolidinediones, acarbose, or sulfonylureas
 - Patients with suspected Cushing's syndrome.
- Use of other TCM treatments including Chinese herbal prescriptions and acupuncture in the past 3 months.
- Use of other western medications known to affect reproductive function or metabolism in the past 2 months.
- Pregnancy within the past 6 weeks.
- Within 6 weeks postabortion or postpartum.
- Breastfeeding within the last 6 months.
- Not willing to give written consent to the study.
- Additional exclusion criteria are as follows.
 - Patients taking other medications known to affect reproductive function or metabolism. These medications include oral contraceptives, depot progestins, hormonal implants (including Implanon), GnRH agonists and antagonists, antiandrogens, gonadotropins, antiobesity drugs, antidiabetic drugs such as metformin and thiazolidinediones, somatostatin, diazoxide, angiotensin-converting-enzyme inhibitors, and calcium channel blockers. The washout period on these medications will be two months, longer washouts may be necessary for certain depot contraceptive forms or implants, especially where the implants are still in place. A one-month washout will be required for patients on depot progestins.

- Patients with liver disease defined as aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >2 times normal or total bilirubin >2.5 mg/dL. Patients with renal disease defined as blood urea nitrogen (BUN) > 30 mg/dL or serum creatinine >1.4 mg/dL.
- Patients with hemoglobin < 10 g/dL.
- Patients with a history of deep venous thrombosis, pulmonary embolus, or cerebrovascular accident.
- Patients with known heart disease that is likely to be exacerbated by pregnancy.
- Patients with a history of suspected cervical carcinoma, endometrial carcinoma, or breast carcinoma. A normal Pap smear result will be required for women 21 years and over.
- Patients with a current history of alcohol abuse. Alcohol abuse is defined as >14 drinks/week or binge drinking.
- Patients enrolled into other investigative studies that require medications, prescribe the study medications, limit intercourse, or otherwise prevent compliance with the protocol.
- Patients who anticipate taking longer than a one-month break during the protocol should not be enrolled.
- Patients with a suspected adrenal or ovarian tumor secreting androgens.
- Couples with previous sterilization procedures (vasectomy, tubal ligation), which have been reversed. The prior procedure may affect study outcomes, and patients with both a reversed sterilization procedure and PCOS are rare enough that exclusion should not adversely affect recruitment.
- Subjects who have undergone a bariatric surgery procedure in the recent past (< 12 months) and are in a period of acute weight loss or have been advised against pregnancy by their bariatric surgeon.
- Patients with untreated poorly controlled hypertension defined as a systolic blood pressure 160 mmHg or a diastolic 100 mm Hg obtained on two measures obtained at least 60 minutes apart.
- Patients with known congenital adrenal hyperplasia.

Intervention

All participants will be informed oral information about the benefits of regular physical exercise and will be instructed to have regular intercourse every 2 to 3 days.

Women in both personalized and fixed acupuncture groups will receive acupuncture treatment three times a week. Acupuncture treatment will start on day 3 after a spontaneous period or after a withdrawal bleeding following progestin. Each treatment session will last for 30–60 minutes, with a maximum of 48 treatment sessions over 16 weeks. They will be contacted by phone if they miss the scheduled appointment. If they miss appointments during treatment, missed appointments will be clearly documented in the record for analysis later. They will be treated for up to 16 weeks. If they become pregnant, the acupuncture treatment will be stopped. For personalized and fixed acupuncture group, credibility and expectancy questionnaires will be completed on the third acupuncture treatment and the last acupuncture treatment.

Personalized acupuncture protocol

The rationale of personalized acupuncture protocol is based on Zang-fu organ system, Yin-yang theory and clinical rules for PCOS acupoint selection ^[19]. Two sets of acupoints will be selected for the two types (Table 1). The basic acupoint-prescription includes the conception vessel (CV) 4, CV 6, CV 12 and spleen (SP) 6 bilaterally, stomach (ST) 25 bilaterally, [extra point of chest and abdomen \(EX-CA\) 1](#) bilaterally, ST 40 bilaterally and SP 9 bilaterally. Additional point ST 36 bilaterally and moxibustion as adjuvant therapy will be added for the type of yang deficiency of spleen and kidney, while additional points kidney (K) 13, liver (LR) 3 for the type of yin deficiency of liver and kidney. Besides, flexible modifications of 2–3 acupoints will be performed according to patients' special symptoms.

The additional points ST 36, K 13, LR 3 and all the basic points will be inserted with disposable needles (Huanqiu, Suzhou Acupuncture Goods Co., Suzhou, China. Size: 0.30×25mm and 0.30×40mm). The depth and degree of puncture for each acupoint is described in Table 1, which could be adjusted according to body shape of participants as shallower for thin women and deeper for fat women. The needles for abdominal acupoints should be inserted until resistance without manual stimulation. After inserting the needles, the acupuncturist will rotate the needles to evoke needle sensation (de qi). De-qi (a dull aching and irradiating sensation indicative of effective needle placement) refers to a sensation of [numbness](#), distension, or electrical tingling at the needling site that might radiate along the corresponding [meridian](#). Once de-qi is achieved, further techniques might be utilized which aim to “influence” the de-qi. Simultaneously, the other techniques of acupuncture with thumb and forefinger to tonify or sedate qi. Tonifying is used to reinforce deficiency while sedating is to clear excess, which are the two manners to stimulate qi in the acupuncture in China. The former is the needles rotate slower and gentle in clockwise direction. Conversely, the latter is turning faster and more forcefully in counterclockwise rotation. Furthermore, the points SP 6 to SP 9 bilaterally for both types will be connected to electrical stimulator (SDZ-IIb, Huatuo, China) and stimulated with low-frequency at 2 Hz, continuous wave and the intensity is adjusted to produce local muscle contractions without pain or discomfort.

Abdominal and back moxibustion will be used for the type of yang deficiency of spleen and kidney (Figure 1). Ignited 1.5 moxa sticks (Hanyi, Nanyang, China; Size: 18mm×200mm) in a special moxa box (19.5×29.5×16.5cm) will be used for a treatment session. The moxa box will be placed onto patients' abdomen with acupuncture treatment simultaneously for 30 minutes. The box of abdominal moxibustion will cover from the midpoint of sternal body xiphoid junction and umbilicus to superior margin of pubic symphysis. After 30 minutes, the moxa box on the back will be continue for another 30 minutes subsequently without acupuncture. And the box will cover from 11th thoracic vertebra to the 4th sacral posterior foramen. Moxibustion will be stopped at any time if the participants feel mouth parched, canker sore and sore throats. Needles not connected to the electrical stimulator or not given moxibustion will be manually stimulated to evoke de-qi every 10 minutes, in total four times.

Fixed acupuncture protocol

The fixed acupuncture protocol is based on the previous study^[8]. Two sets of acupuncture points will be alternated every second treatment (Table 2). The first set consists of CV 3, CV 6, ST 29 bilaterally, SP 6 bilaterally, SP 9 bilaterally, governor vessel (GV) 20 and large intestine (LI) 4 bilaterally. In total, 11 needles will be placed. The needles for abdominal acupoints should be inserted until resistance without manual stimulation. Other needles will be manipulated manually by rotation to evoke needle sensation, de qi. CV 3, CV 6 (mid-line), ST 29 bilaterally, SP 6 bilaterally and SP 9 bilaterally will be connected to an electrical stimulator (SDZ-IIB, Huatuo, China). The second set consists of 13 needles: ST 25 bilaterally, ST 29 bilaterally, CV 3, CV 6, SP 6 bilaterally, LR 3 bilaterally, pericardium meridian (PC) 6 bilaterally and GV 20. The following points will be connected to an electrical stimulator: ST 25 bilaterally, ST 29 bilaterally, SP 6 bilaterally, LR 3 bilaterally. Electrical stimulation is given with low-frequency electric acupuncture (EA) of 2Hz, continuous wave, with an intensity adjusted to produce local muscle contractions without pain or discomfort. Needles not connected to the electrical stimulator and not for abdominal acupoints will be manually stimulated to evoke de-qi every 10 minutes, in total four times.

Letrozole

Women in the letrozole group will be given letrozole (Femara, Novartis Pharmaceuticals, Basel, Switzerland) 2.5 mg (1 pill) daily from day 3 to day 7 of the spontaneous menstrual cycle or after a withdrawal bleeding following progestin. If there is response with ovulation (ie, the serum progesterone level on day 21 or day 28 of the cycle is higher than 3 ng/mL) this dose will be maintained. In those with no ovulatory response, letrozole tablets of the next ovulation cycle will be take on the day 28 of the menstrual cycle and the letrozole dose will be increased to 5 mg (2 pills) a day for 5 days. If there is still no response, the dose will be increased to 7.5 mg (3 pills) per day for 5 days in the next cycle. The maximum daily dose of letrozole will be 7.5 mg daily for five days.

Placebo letrozole

Women will receive placebo letrozole with no acupuncture. Placebo letrozole will have the same appearance as letrozole (Dongyangguang pharmaceutical Co. LTD, Guangdong, China). Placebo letrozole will be given in the same way as letrozole, women will receive 1 tablet a day of placebo letrozole from the day 3 to day 7 of the menstrual cycle and placebo letrozole dose will be increased 2 tablets a day in the next cycle if there is no response. The maximum daily dose of placebo letrozole will be 3 tablets daily for five days.

Randomization and patient allocation

An online computerized program of web-based Research Management (ResMan) database system will be used to allocate patients in 1: 1: 1: 1 ratio. The data coordinating center (DCC) statisticians generated and validated the randomization scheme for the study before it was implemented in ResMan database system. When a participant is enrolled, an investigator of participating site will take out sealed envelope and enter the ID number into the ResMan database system, and login a password protected secured website designed by the DCC to find the group allocation.

Blinding

The acupuncture groups will be known only to the acupuncturists besides the ResMan data manager. The letrozole and the placebo letrozole having the same appearance will be organized in a kit consisting of one cycle tablets for each subject and one subject will at most take 45 tablets in total for 4 cycles. The sealed envelope with 4 cycles tablets will be labeled with an ID number mapped to the letrozole or placebo letrozole known only to the ResMan personnel. Participants and investigators will be blinded to the letrozole or placebo letrozole allocation. All outcome assessors will be blinded to group allocation.

Study-specific visits and procedures (Figure 2)

Women will attend up to five visits, including the screening visit, baseline visit, treatment visit, pregnancy visit and end of treatment visit (Table 3). Adverse events and concomitant medications will be recorded during every visit. Face-to-face adherence reminder sessions will take place at each study visit thereafter.

Screening visit

Women are screened in the morning after an overnight 12-h fast. Detailed information about the study design is given.

Physical examination

Complete physical examinations will be performed including height, weight, hip, waist measurements, and vital signs. Height and weight will be recorded to the nearest 0.1 cm and 0.1 kg respectively. Waist and hip circumference will be recorded to the nearest 1 cm. Hirsutism assessment by FG, acne by standard acne lesion counts.

Transvaginal ultrasound of ovaries

Ovaries, including the ovarian size in three dimensions, the size of the largest ovarian follicle/cyst and size of every follicle with a mean diameter greater than 10 mm, and total antral follicle count (small follicles with mean diameter of 2–9 mm) of each ovary will be obtained through transvaginal ultrasound.

Pregnancy test

A urine and serum pregnancy test will be performed to exclude pregnancy.

Laboratory tests

Blood samples will be collected for the following laboratory: T, sex hormone binding Globulin (SHBG), FSH, luteinising hormone (LH), progesterone (P), estradiol (E2), PRL, fasting glucose, fasting insulin, HbA1C, C-peptide, TSH, T3, free T4, and lipid profile (cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C)). PCOS safety screen: liver profile (ALT, AST, total bilirubin), renal profile (BUN and creatinine), complete blood cell count (CBC), HIV, cervical Pap smear or TCT. Every woman should have T and SHBG assay in local labs. Free androgen index will be calculated.

Progestin withdrawal

Subjects without evidence of ovulation on baseline screening will be provided progestin to induce withdrawal bleeding, with instructions to begin medication once eligibility has been determined.

They will sign the consent form after comprehensive counseling.

Baseline visit

The baseline visit will be around day 2–5 of a spontaneous period or after a withdrawal bleeding if a woman fulfills the selection criteria and has signed the consent form. Fasting blood samples will be obtained from the patients and will be shipped to the core laboratory for analysis. Urine or serum pregnancy test will be done at this visit.

Questionnaires

Women will fill in the following questionnaires: the short form 36 (SF 36) of health-related quality of life (QOL), the Polycystic Ovary Syndrome Questionnaire (PCOS-QOL), the Chinese Quality of life (ChQOL), Zung Self-Rating Anxiety Scale (SAS) and Zung Self-reported Depression Scale (SDS).

TCM differentiation of syndromes of PCOS is mainly for the deficiency of liver- kidney-yin type, and the deficiency of spleen-kidney-yang type. The TCM syndrome diagnosis will be made by principal investigator and acupuncturist in each participating site according to a standard questionnaire during baseline visit and end of treatment visit. If the diagnosis is differ from each other, they will discuss until reaching an agreement. Syndrome

differentiation in TCM is the comprehensive analysis of clinical information gained by the four-main diagnostic TCM procedures: observation, listening, questioning, and pulse analysis.

All baseline measures will be repeated in all subjects at the end of treatment visit.

Treatment visit

The women will be instructed to have intercourse once every 2 or 3 days. A serum progesterone level test will be performed in local laboratory on day 21 of the cycle. An elevated level of progesterone >3 ng/mL is considered to indicate evidence of ovulation. If there is no ovulation, serum progesterone level will be checked every week until ovulation. If ovulation, serum pregnancy test will be performed after a week. Women should make a urine pregnancy test at any time if they are suspected to be pregnant.

We will determine whether the women have anovulation response based on the serum progesterone level. There will be three possible scenarios: ovulation, delayed ovulation and no ovulation (1). Ovulation: serum progesterone level on day 21 of the cycle >3 ng/mL. A serum pregnancy test will be performed after a week to exclude pregnancy, letrozole or placebo letrozole will be started on day 3 of the period, and the letrozole or placebo letrozole dose will be maintained. (2) Delayed ovulation: serum progesterone level in the third week <3 ng/mL. In this case, we will wait for one more week, and check serum progesterone level again. A serum pregnancy test will be performed after a week if ovulation to exclude pregnancy. Letrozole or placebo letrozole will be started on day 3 of the period, and the letrozole or placebo letrozole dose will be maintained. (3) No ovulation: It will be defined as negative serum progesterone levels on day 21 and day 28. In the letrozole and placebo letrozole groups, women will take letrozole or placebo letrozole on day 28 of the period and one more tablet per day will be given for 5 days until the maximum daily dose of 3 tablets a day. Day 28 will be defined as day 1 of next cycle, and serum progesterone test will be done every week until ovulation.

Every period will be recorded, including the date, amount and duration of menstruation.

End of treatment visit

End of treatment visit will be performed when women conceive or finish four cycle treatments. Baseline measures will be repeated in all subjects including vital signs, height, weight, hip and waist measurements, hirsutism and acne assessments, sex hormone steroids (FSH, LH, T for women not pregnant, and HCG, P, E2, T for pregnant women), SHBG, metabolic profile (glucose and insulin concentrations, HbA1C, C-peptide, cholesterol, triglycerides, HDL-C, LDL-C), and safety profile (AST, ALT, total bilirubin, BUN, creatinine). Subjects will return menstrual and intercourse journal logs, and their adverse events and concomitant medications will be recorded. Fasting blood samples will be obtained, and TCM syndrome diagnosis and questionnaires SF36, PCOS-QOL, ChQOL, SAS and SDS will be repeated.

Pregnancy visit (only with conception)

Serum progesterone and hCG levels will be determined at local sites to document ovulation and pregnancy. Serial serum hCG level will be checked on a weekly basis if pregnant. Serum beta hCG > 10 IU/L indicates pregnancy. Pelvic ultrasound will be arranged to determine the location, number and viability of the pregnancy when serum hCG is 2000–4000 IU/L.

Women who conceive will be followed till around 9 weeks gestational age and will be referred to their prior or referring doctors for antenatal care. Subsequent scans will be performed at week 18–24, 32, and 36 or at the prescription of the obstetrician. In the present trial, pregnant women who present with threatened miscarriage or are considered to have a significant risk for miscarriage can be given oral Duphaston (10 mg three times a day, Solvay Pharmaceuticals B. V.) till 12 weeks of gestation or 1 week after the vaginal bleeding stops. This information will be documented in the record form. No other medications including hCG, herbal medications, and acupuncture will be given.

Follow-up of pregnancies

For those women who have an ongoing pregnancy, arrangements will be made to follow the outcome of the pregnancy at the end of the first trimester and also after delivery or termination of gestation. All pregnancies (including multiples) will be followed to monitor weight, glucose tolerance, blood pressure and fetal growth and to determine the pregnancy outcomes. The glucose tolerance will be measured by oral glucose tolerance test in all pregnancies at 24–28 weeks of pregnancy. Women will be instructed to inform the study personnel of the outcomes of the pregnancy, and we will obtain delivery record from the obstetricians to determine the birth weight, length of gestation, and any prenatal complications of the mother or neonatal complications of the infant. Phone contacts will be initiated if the pregnant woman has not contacted the study personnel within 6 weeks of the original estimated date of childbirth.

We will collect pregnancy outcome data and track the outcomes of all women who have a positive serum pregnancy screen during the course of this study. We will record biochemical pregnancies (defined as positive serum pregnancy screens without ultrasonically detected pregnancies), ectopic pregnancies and all intrauterine pregnancy losses both before and after 20 weeks, including miscarriages, abortions, fetal deaths and stillbirths.

Outcomes

Primary outcome

The primary outcome is the live birth rate.

Secondary outcomes include

- Ovulation rate
- Ongoing pregnancy rate (around 8–10 weeks gestation)
- Miscarriage rate: loss of an intrauterine pregnancy before 20 completed weeks of gestation
- Hormonal profile: FSH, LH, total T, SHBG
- Metabolic profile: glucose and insulin concentrations, cholesterol, triglycerides, HDL-C and LDL-C
- Quality-of-life scores
- Side effect profile

Sample size calculations

The overall live birth proportion for women who received letrozole for six month was 36.3%^[20], while for women who received fixed acupuncture twice a week with a maximum of 32 acupuncture treatments was 13.9%^[8]. Thus, we hypothesise that the live birth rate is 35% for letrozole treatments with four cycles and 15% for fixed acupuncture with three times a week. We chose 10% as the minimal clinically detectable difference that is likely to change clinical practice. Assuming a 35% live birth rate with letrozole, a 25% live birth rate with personalized acupuncture, a 15% live birth rate with fixed acupuncture and a 5% live birth rate with placebo letrozole. 80% power at a significance level of 0.05, we calculated that 250 subjects per treatment group were required. The sample size were increased from 250 to 275 per group considering a 10% dropout rate, 1100 women for four groups would be enrolled. If there is no statistical significance on the live birth rate between personalized acupuncture and fixed acupuncture, we will combine two groups to analyze.

Analysis of data

The primary analysis will use an intent-to-treat approach to examine differences in the live birth rate among the four treatment groups. Primary efficacy analysis will be done by comparing the treatment groups with respect to the primary outcome of live birth using the Pearson chi-square test. For the secondary supportive analysis, we will fit a logistic regression model to compare the treatment arms with respect to the primary outcome of live birth, adjusting for other factors such as randomization stratification of study site and prior exposure to study medications. Comparisons of variables such as live birth rate, ovulation rate, conception rate, pregnancy rate, multiple pregnancy rate and miscarriage rate will include relative risk and 95% CIs in addition to the χ^2 test. Changes in laboratory values were assessed by ANCOVA.

The analysis of other secondary (supplemental) outcomes measured over time will entail the application of statistical methods that have been developed for correlated data since repeated observations will be made over time on each individual. For secondary outcomes such as hormone levels, a linear mixed-effect model will be fit where the main independent variables will be treatment group, time, and their interaction as well as the designed randomization stratification factors as covariates. Logistic regression models will be used in secondary analyses to evaluate the predictive value of treatment arm, clinical site, body mass index and other explanatory variables on binary outcomes (e.g., live birth, abortion). Cox proportional hazards models and a Kaplan-Meier method will be applied to compare time to pregnancy in the treatment groups. Effects on QoL were tested as the change from baseline of the Medical Outcomes Study Short Form physical and mental component scores by univariate and multivariate ANCOVA. Adequacy of masking was tested by Fisher's exact test comparing true assignments to participant guesses, including "don't know." Confidence scores were tested by ordinal logistic regression. Differences in adverse event rates were analyzed in a negative-binomial generalized linear model. A $P < 0.05$ with 2-tailed test is considered significant. All statistical analyses will be performed using the SPSS program V.21.0 (SPSS, Chicago, Illinois, USA).

Imputation procedure for missing data

We will report reasons for withdrawal for different randomisation groups and compare the reasons qualitatively. The effect that any missing data might have on the results will be assessed via sensitivity analysis of augmented data sets. Dropouts (essentially participants who withdraw consent for continued follow-up) will be included in the analysis by modern imputation methods for missing data.

Quality assurance

To ensure that treatments are of a high standard and delivered in accordance with the trial protocol, all acupuncturists with TCM certificate and 1-year experience receive a study specific theoretical course and a study specific practical training, totally 3 times before the beginning of the study, 1–3 days per time, and 1 time, four hours after one month of the study beginning. They are graduate students in Chinese medicine and have received general acupuncture training during their studies. All acupuncturists have to pass the theory and practical tests.

Safety analysis

Adverse events will be categorized and the percentage of patients experiencing adverse events and serious adverse events during the treatment period and follow-up period will be documented and reported to the Data and Safety Monitoring Board (DSMB). These will be reviewed on a quarterly basis by the DSMB, and serious adverse events will be immediately reviewed.

Data management and quality control of data

Both the Case record form (CRF) and web-based electronic database will be used to manage individual participant data. Quality control of the data will be handled at three different levels: the investigators will be required to ensure the accuracy of the data as the first level of control when they input the records in CRF. The second level will include data monitoring and validation that will be carried out by an independent group. The third level will be the site visits, during which data in ResMan will be compared with the source documents. Identified errors will be resolved by ResMan and clinical sites.

A web-based electronic database, ResMan (www.medresman.org), is used as a double input database. Finally, the individual participant data except the private information will be allowed to be share with the public within 6 months after the trial completion.

Data monitoring and access

We will establish an independent DSMB. The responsibility of the DSMB will be to review and interpret the data generated from the study, and its primary objectives will be to ensure the safety of the study participants and the integrity of the research data. The DSMB will hold regular conference calls to review the protocol with respect to ethical and safety standards, to monitor the safety of the trials, to monitor the integrity of the data with respect to the original study design, and to provide advice on study conduct. It will also review the progress of the trial, adjudicate adverse events and decide on any premature closure of the study.

The ResMan will oversee the intra-study data sharing process. All principal investigators will be given access to the cleaned data sets. Project data sets will be housed on the ResMan database system for the study, and all data sets will be password protected. The principal investigators will have direct access to their own site's data sets as well as access to other sites' data by request. To ensure confidentiality, data dispersed to project team members will be blinded to any identifying participant information.

Ethics and dissemination

All women and their husbands will be asked to sign a consent form prior to joining the study, and they will be made fully aware that they are free to withdraw from the study at any time. The results of this trial will be disseminated in peer-reviewed journals and presented at international meetings.

Discussion

To the best of our knowledge, no study has investigated whether personalized acupuncture has the potential to increase the live birth rate in women with PCOS. Our study is the first multicentre RCT to compare personalized or fixed acupuncture, letrozole or placebo letrozole on live birth for infertility in women with PCOS. The hypothesis is letrozole is more effective than personalized acupuncture, and personalized acupuncture is more effective than fixed acupuncture, which is more effective than placebo letrozole. Moreover, personalized acupuncture is more likely to reduce miscarriage rate and the risk of pregnancy complications than letrozole.

In our trial, considering the treatment principle based on Zang-fu organ system, Yin-yang theory and syndrome/pattern differentiation in TCM, the therapeutic proposal should be reinforcing liver-kidney-yin deficiency, promoting spleen-kidney-yang circulation and resolving phlegm. We will divide the individual group into two types as the theory of TCM, "liver-yin and kidney-yin deficiency" and "spleen-yang and kidney-yang deficiency". This method is similar the actual clinical practice and can be used in future studies. The fixed acupuncture regimen is controversial based on the theory of TCM's acupuncture.

Trial Status

This trial was approved on March 9, 2018. The recruitment began on August 13, 2018 and is expected to be completed by the August 13, 2021. The trial procedures are expected to be completed by the end of May, 2022 when considering ten month pregnancies follow up until collecting the information of live birth. This is version 3.0 of the protocol, dated May 12, 2019.

Abbreviations

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BUN: Blood urea nitrogen; CBC: Complete blood cell count; ChQOL: Chinese Quality of life; CONSORT: Consolidated Standards of Reporting Trials; CRF: Case record form; CV: Conception vessel; DCC: Data coordinating center; DSMB: Data and Safety Monitoring Board; E2: Estradiol; EA: Electric acupuncture; EX-CA: Extra point of chest and abdomen; FG: Ferriman-Gallway; FSH: Follicle-stimulating hormone; GV: Governor vessel; HbA1c: Glycated haemoglobin; HDL-C: High-density lipoprotein cholesterol; K: Kidney; LDL-C: Low-density lipoprotein cholesterol; LH: Luteinising hormone; LI: Large intestine; LR: Liver; P: Progesterone; PC: Pericardium meridian; PCOS: Polycystic ovary syndrom; PCOS-QOL: Polycystic Ovary Syndrome Questionnaire; PRL: Prolactin; QOL: Quality of life; RCT: Randomized controlled trial; ResMan: Research Management; SAS: Self-Rating Anxiety Scale; SDS: Self-reported Depression Scale; SF 36: Short form 36; SHBG: Sex hormone binding Globulin; SP: Spleen; SPIRIT: Standard Protocol with the Standard Protocol Items: Recommendations for Interventional Trials; ST: Stomach; STRICTA: Standards for Reporting Interventions in Clinical Trials of Acupuncture; T: Total testosterone; TCM: Traditional Chinese Medicine; TSH: Thyroid-stimulating hormone.

Declarations

Ethics approval and consent to participate

Central ethical approval has been confirmed from the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University (Reference approval no. 2018019) and we will not begin recruiting at other centres in the trial until local ethical approval has been obtained. Any protocol modifications will be approved by this ethics committee before its implementation. All participants and their husbands will sign the informed consent prior to participation, and they will be made fully aware that they are free to withdraw from the study at any time. Adverse events and serious adverse events during the treatment period will be documented.

This study is referred as the Clinical trial of Personalized Acupunctue on PCOS (PPCOSAct). It has been registered at [Clinicaltrials.gov](https://clinicaltrials.gov) (NCT03625531) and Chinese Clinical Trials Registry (ChiCTR1800017304).

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

EHYN, ESV and HM conceived and designed the study. SH and MH drafted the manuscript for important intellectual content. HM sought funding and ethical approval. MH, JQ, EHYN and ESV critically revised the manuscript and designed the acupuncture protocol. SH, YZ, QW, JL, CY, JL, KQ, CW, ML, XW, SL, HS, HM and JQ recruited subjects and carried out acupuncture treatment. All authors read and approved the final manuscript.

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Tables

Table 1 Personalized acupuncture protocol

Point	Stimulation	Location	Anatomy	Muscle innervation	Method
Spleen-yang and kidney-yang deficiency					
Sanyinjiao (bilateral) (SP6)	EA, (+)	3 cun proximal to the medial malleolus	Mm. flexor digitorum longus, tibialis posterior	L4-5, S1	Puncture perpendicularly 0.5-1.0 cun
Tianshu (bilateral) (ST25)	A	2 cun lateral to the midline at the level of the umbilicus	M rectus abdominis	Th6-12	Puncture perpendicularly 0.7-1.2 cun
Zigong (bilateral) (EX-CA1)	A	1 cun cranial to the pubic bone and 3 cun lateral to the midline	Mm. obliquus externus abdominis, obliquus internus abdominis	Th12, L1	Puncture perpendicularly 0.8-1.2 cun
Guanyuan (CV4)	A	3 cun caudal to the umbilicus	Fibrous tissue , linea alba	Th12	Puncture perpendicularly 0.8-1.2 cun
Zhongwan (CV12)	A	4 cun superior to the umbilicus	Fibrous tissue , linea alba	Th8	Puncture perpendicularly 0.8-1.2 cun
Qihai (CV6)	A	1.5 cun caudal to the umbilicus	Fibrous tissue, linea alba	Th11	Puncture perpendicularly 0.8-1.2 cun
Fenglong (bilateral) (ST40)	Deqi 4 times, (-)	8 cun superior to lateral malleolus	Mm. extensor digitorum longus, Peroneus M. M.gastrocnemius	L4-5, S1-2	Puncture perpendicularly 0.5-1.0 cun
Yinlingquan (bilateral) (SP9)	EA, (-)	Below medial tibia chondyle	M. gastrocnemius	S1-2	Puncture perpendicularly 0.5-1.0 cun
Zusanli (bilateral) (ST36)	Deqi 4 times, (+)	On the anterior lateral side of the leg, 3 cun below Dubi (ST35), one finger width (middle finger) from the anterior crest of the tibia	Mm. tibialis anterior, extensor digitorum longus, musculi tibialis posterior	L2-4	Puncture perpendicularly 0.5-1.0 cun
Liver-yin and kidney-yin deficiency					
Sanyinjiao (bilateral) (SP6)	EA, (+)	3 cun proximal to the medial malleolus	Mm. flexor digitorum longus, tibialis posterior	L4-5, S1	Puncture perpendicularly 0.5-1.0 cun
Tianshu(bilateral) (ST25)	A	3 cun caudal to the umbilicus	Fibrous tissue, linea alba	Th12	Puncture perpendicularly 0.7-1.2 cun
Zigong(bilateral) (EX-CA1)	A	1 cun cranial to the pubic bone and 3 cun lateral to the midline	Mm. obliquus externus abdominis, obliquus internus abdominis	Th12, L1	Puncture perpendicularly 0.8-1.2 cun
Guanyuan (CV4)	A	3 cun caudal to the umbilicus	Fibrous tissue , linea alba	Th12	Puncture perpendicularly 0.8-1.2 cun
Zhongwan (CV12)	A	4 cun superior to the umbilicus	Fibrous tissue , linea alba	Th8	Puncture perpendicularly 0.5-1.2 cun
Qihai (CV6)	A	1.5 cun caudal to the umbilicus	Fibrous tissue, linea alba	Th11	Puncture perpendicularly 0.8-1.2 cun
Fenglong (bilateral) (ST40)	Deqi 4 times, (-)	8 cun superior to lateral malleolus	Mm. extensor digitorum longus, Peroneus M. M.gastrocnemius	L4-5, S1-2	Puncture perpendicularly 0.5-1.0 cun
Yinlingquan (bilateral) (SP9)	EA, (-)	Below medial tibia chondyle	M. gastrocnemius	S1-2	Puncture perpendicularly 0.5-1.0 cun
Taixi (bilateral) (K13)	Deqi 4 times, (+)	Behind the medial malleolus	Fibrous tissue	L3-4	Puncture perpendicularly 0.5-1.0 cun
Taichong (bilateral) (LR3)	Deqi 4 times, (-)	Between metatarsal I and II, just distal to the caput	M. Interosseous dorsalis I	S2-3	Puncture perpendicularly 0.5-1.0 cun

+/-, signifies that these points have been rotated bi-directionally to tonifying (+) or sedating (-).

A, acupuncture; CV, conception vessel; EA, electroacupuncture; EX-CA, extra point of chest and abdomen; GV, governor vessel; K, kidney; L, lumbar vertebra; LI, large intestine; LR, liver; M., muscoli; Mm., musculus; S, sacral vertebra; ST, stomach; SP,

spleen; Th, thoracic vertebra.

Table 2 Fixed acupuncture protocol

Point	Stimulation	Location	Muscle	Muscle innervation	Method
Set 1					
Zhongji (CV3)	EA	4 cun caudal to the umbilicus	Fibrous tissue , linea alba	L1	Puncture perpendicularly 0.5-1.0 cun
Qihai (CV6)	EA	1.5 cun caudal to the umbilicus	Fibrous tissue, linea alba	Th11	Puncture perpendicularly 0.8-1.2 cun
Guilai (bilateral) (ST29)	EA	1 cun cranial to the pubic bone and 2 cun lateral to the midline	M. rectus abdominis	Th6-12	Puncture perpendicularly 0.7-1.2 cun
Sanyinjiao (bilateral) (SP6)	EA	3 cun proximal to the medial malleolus	Mm. flexor digitorum longus, tibialis posterior	L4-5, S1-2	Puncture perpendicularly 0.5-1.0 cun
Yinlingquan (bilateral) (SP9)	EA	Below medial tibia chondyle	M. gastrocnemius	S1-2	Puncture perpendicularly 0.5-1.0 cun
Hegu (bilateral) (LI4)	DeQi 4 times	On the highest point at the muscoli interosseus dorsalis	Mm. interosseus dorsalis I, lumbricalis II, adductor pollicis	C8, Th1	Puncture perpendicularly 0.5-1.0 cun
Baihui (GV20)	DeQi 4 times	On the top of head	Aponeurosis epicranii	C2-3,N trigeminus	Puncture subcutaneously 0.3-0.5 cun
Set 2					
Tianshu (bilateral) (ST25)	EA	2 cun lateral to the midline at the level of the umbilicus	M rectus abdominis	Th6-12	Puncture perpendicularly 0.7-1.2 cun
Guilai (bilateral) (ST29)	EA	1 cun cranial to the pubic bone and 2 cun lateral to the midline	M. rectus abdominis	Th6-12	Puncture perpendicularly 0.7-1.2 cun
Zhongji (CV3)	A	4 cun caudal to the umbilicus	Fibrous tissue , linea alba	L1	Puncture perpendicularly 0.5-1.0 cun
Qihai (CV6)	A	1.5 cun caudal to the umbilicus	Fibrous tissue, linea alba	Th11	Puncture perpendicularly 0.8-1.2 cun
Sanyinjiao (bilateral) (SP6)	EA	3 cun proximal to the medial malleolus	Mm. flexor digitorum longus, tibialis posterior	L4-5, S1-2	Puncture perpendicularly 0.8-1.2 cun
Taichong (bilateral) (LR3)	EA	Between metatarsal I and II, just distal to the caput	M. interosseus dorsalis I	S2-3	Puncture perpendicularly 0.3-0.5 cun
Neiguan (bilateral) (PC6)	DeQi 4 times	2 cun proximal to the processus styloideus radii, between the tendons of the palmaris longus and the flexor carpi radialis	M. flexor digitorum superficialis	C8, Th1	Puncture perpendicularly 0.5-0.8 cun
Baihui (GV20)	DeQi 4 times	On the top of head	Aponeurosis epicranii	C2-3,N trigeminus	Puncture subcutaneously 0.3-0.5 cun

The two sets will be alternated for every other treatment.

A, acupuncture; C, cervical vertebra; CV, conception vessel; EA, electroacupuncture; GV, governor vessel; L, lumbar vertebra; LI, large intestine; LR, liver; M., muscoli; Mm., musculus; PC, pericardium; S, sacral vertebra; SP, spleen; ST, stomach; Th, thoracic vertebra

Table 3. Overview of the study visits

	Screening	Baseline	Treatments Visit (16 weeks)				End of Treatment Visit	Pregnancy visit
	Visit	Visit	Pesonalized Acupuncture	Fixed Acupuncture	Letrozole	Placebo letrozole		
Sign Consent	x							
History	x						x	
Urine pregnancy test	x	x	x	x	x	x		
Physical Exam	x						x	
Transvaginal ultrasound	x							x
Semen Analysis	x							
Hysterosalpin-gogram	x							
Cervical Pap smear or TCT	x							
Preconception counselling	x							
Fasting blood for study eligibility Lads	x						x	
Blood samples collection		x					x	
Serum progesterone assay	x		x	x	x	x	x	x
Serum HCG assay	x	x	x	x	x	x	x	x
Questionnaires		x					x	
Acupuncture Visits (three times weekly)			x	x				
Assess adverse events and concomitant medications			x	x	x	x	x	x
Pregnancy and neonatal records								x

Physical examination: weight, height, waist circumference, hip circumference, FG/acne.

Fasting blood for study eligibility Lads: FSH, LH, SHBG, T, free testosterone, E2, PRL, FGLU, FINS, C-peptide, HbA1c, cholesterol, triglycerides, HDL-C, LDL-C, CBC, thyroid profile, renal profile and liver profile.

Transvaginal ultrasound: endometrial thickness, ovarian volume, antral follicle count, and size of ovarian cysts or developing follicles.

Preconception counselling: TORCH and HIV screening.

Blood samples collection: serum for the central core laboratory.

Abbreviation: CBC, complete blood count; E2, estradiol; FG, Ferriman-Gallwey; FGLU, fasting blood glucose; FINS, fasting insulin; FSH, follicle-stimulating hormone; HbA1c, glycated haemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LH, luteinising hormone; PRL, prolactin; SHBG, sex hormone-binding globulin, T, total testosterone; TCT, thinprep cytologic test; TORCH, Toxoplasmosis, Rubella, Cytomegalovirus, and Herpes virus.

Figures

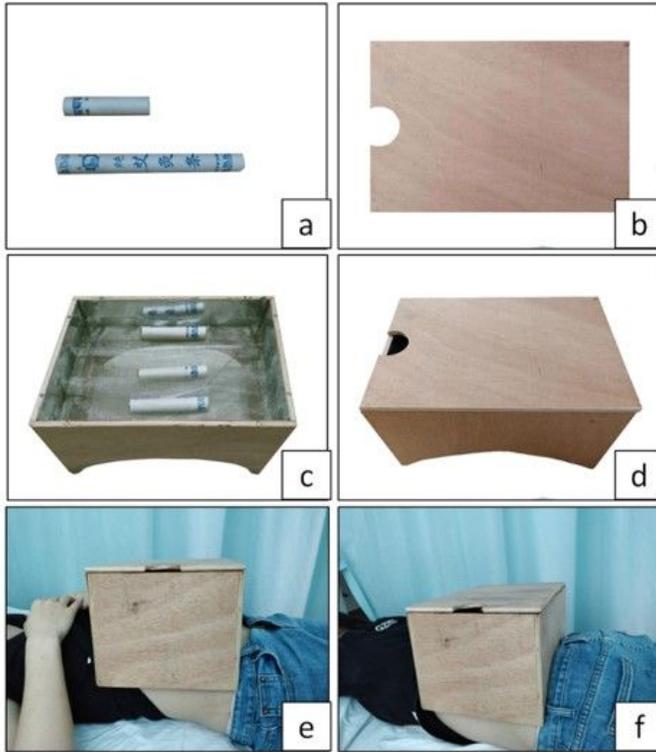


Figure 1

Moxibustion in personalized acupuncture group. (a) 1.5 moxa sticks for a treatment session. (b) Safety cap of moxa box. (c) Ignited moxa sticks placed into moxa box. (d) The appearance of the whole moxa box. (e) Moxa box is on the abdomen of patient from the midpoint of sternal body xiphoid junction and umbilicus to superior margin of pubic symphysis. (f) Moxa box is on the back of patient from 11th thoracic vertebra to the 4th sacral posterior foramen.

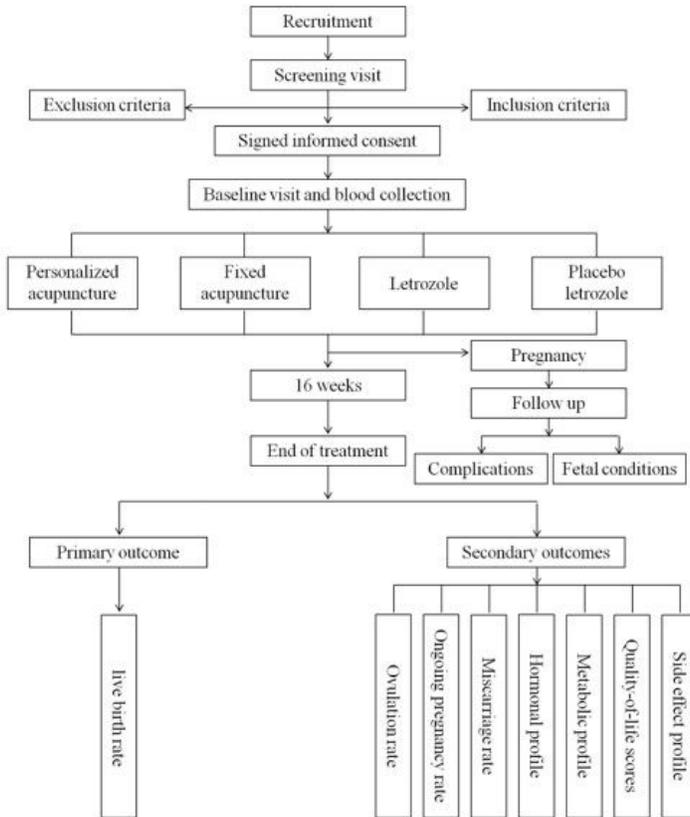


Figure 2

The study flow chart main document. PCOS, polycystic ovary syndrome.

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