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Efficacy and safety of autologous adipose tissue-derived stromal vascular fraction in patients with premature ovarian insufficiency: protocol for a single-centre randomised controlled trial

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ABSTRACT

Introduction Premature ovarian insufficiency (POI) is a complicated reproductive endocrine disease seriously affecting physiological function and fertility in women. Its clinical features include amenorrhoea or infrequent menstruation, oestrogen deficiency and elevated levels of gonadotropins. At present, conventional treatments for POI in clinical practice are unable to fundamentally improve ovarian function or solve fertility problems, and often have certain side effects. Adipose tissue-derived stromal vascular fraction (SVF) contains various cell types, including adipose-derived stem/stromal cells, stromal cells, endothelial cells, fibroblasts and macrophages. Recently, SVF has shown tremendous potential in treating many refractory diseases, offering a promising therapeutic option for improving ovarian function. Although SVF has shown therapeutic effects in animal models of POI, there is insufficient evidence demonstrating the efficacy and safety of autologous SVF in women with POI.

Methods and analysis This study is a single-centre randomised controlled trial designed to explore the efficacy and safety of using autologous SVF in improving pregnancy outcomes in patients with infertility diagnosed with POI. A total of 308 women meeting the eligibility criteria will be randomly assigned in a 1:1 ratio to either the SVF group or the control group. The control group will receive conventional assisted reproductive technology treatment, including in vitro fertilisation, embryo transfer and intracytoplasmic sperm injection. In the SVF group, patients will undergo bilateral intraovarian injections of the SVF suspension under ultrasound guidance. Their in vitro fertilisation cycles will commence 4–8 weeks after SVF injection. The primary outcome of this trial is the cumulative clinical pregnancy rate within 6 months. Aside from this, secondary outcomes including menstrual volume and duration, ovarian volume, antral follicle count, and serum levels of anti-mullerian hormone and sex hormone (oestrogen and follicle-stimulating hormone) will be measured. All adverse events will be monitored and recorded within a 6-month follow-up period. Additionally, pregnancy outcomes and the health status of the offspring will be tracked through telephone follow-up for 2 years.

Ethics and dissemination This trial has been reviewed and approved by the Ethics Committee of Peking University Third Hospital (approval number: IRB00006761-M2024330). We will ensure that each patient has signed informed consent before participation in the trial. The findings will be published in a peer-reviewed journal.

Trial registration number NCT06481969.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is a carefully designed randomised controlled trial aiming to generate a high level of evidence.
⇒ The trial will explore the therapeutic effects of adipose tissue-derived stromal vascular fraction in patients with premature ovarian insufficiency.
⇒ The study will also focus on safety and will follow up on the health of the participants' offspring.
⇒ The study is limited to a single research centre, which could limit the generalisability of the findings.

INTRODUCTION

Premature ovarian insufficiency (POI) refers to an irreversible decrease in ovarian function in women of reproductive age under the age of 40, mainly manifested as infrequent menstruation or cessation of menstruation for 4 months or more, elevated serum level

of basal follicle-stimulating hormone (FSH) (>25 IU/L; at least twice and with an interval of more than 4 weeks) and remarkable decreases in serum level of estradiol (E_2), anti-mullerian hormone (AMH) and antral follicle count (AFC). It is also associated with secondary osteoporosis and other perimenopausal symptoms.[1] In recent years, the incidence rate of POI has been rising and increasing with age.[2] The aetiology and pathogenesis of POI are not yet fully understood and may include genetic defects, iatrogenic anticancer treatment, autoimmune disorders, metabolic disorders and environmental factors.[2–4] Premature ovarian failure (POF) is the final stage of POI, where FSH levels rise to menopausal levels (>40 IU/L; measured at least twice and with an interval of more than 4 weeks), accompanied by varying degrees of perimenopausal symptoms.[2] Currently, hormone replacement therapy (HRT) is extensively used in clinical practice to alleviate and prevent perimenopausal symptoms and long-term health risks caused by oestrogen deficiency. However, HRT cannot radically restore the physiological function of the ovaries and female fertility. Studies have shown that patients with POI still have some dormant follicles. In fact, even at menopause, patients still have at least 1000 primordial follicles in their ovaries. However, due to their high level of FSH, the recruitment and consumption of follicles are accelerated. Therefore, it is possible for patients with POI to have a partial recovery of ovarian reserve function through appropriate treatment. Although various methods have been explored to treat POI, including in vitro activation of primordial follicles, mitochondrial activation and platelet-rich plasma (PRP),[5] the clinical therapeutic effects remain unclear, and there are certain ethical concerns and risks. Therefore, more effective treatments are urgently needed.

Recently, adipose-derived stromal vascular fraction (SVF) has become a hot topic in the treatment of various diseases. SVF has shown unique potential in treating a variety of refractory diseases, such as diabetic foot and Crohn's disease.[6] In rodent models of Asherman syndrome, the transplantation of SVF into the uterine cavity resulted in significant increases in both endometrial thickness and angiogenesis.[7] Moreover, the preparation process of SVF is relatively simple and does not involve in vitro culturing, thus eliminating the risk of immune rejection.[6 8] These studies of SVF have preliminarily demonstrated its efficacy and safety, offering a potential therapeutic option for improving ovarian reserve and function. Recently, the potential of SVF in treating POI has also been explored. Adipose-derived stem/stromal cells (ADSCs) within SVF suspensions derived from adipose tissue possess regeneration and migration characteristics, as well as the ability to proliferate, differentiate and perform paracrine activities.[9] In mouse or rat models of POF, ADSCs injected through the tail vein or into the ovaries can improve the microenvironment for follicle development, increase follicle count, promote follicle development and ovulation and improve the quality of oocytes, thus improving ovarian reserve.[10 11] In addition, ADSCs can also inhibit granulosa cell apoptosis, increase serum oestrogen level and decrease FSH level, thereby improving ovarian function.[12 13] SVF suspensions also contain endothelial cells, stromal cells, fibroblasts, macrophages and other cell groups, which play a role in regulating the microenvironment for follicle development,[14] thereby improving the fertility of patients with POI. Additionally, in a study by Mashayekhi *et al*, nine patients with POF were treated with autologous adipose tissue-derived SVF, with four experiencing menstrual recovery and a significant decrease in serum level of FSH.[15] However, there is still insufficient robust evidence to prove the efficacy of SVF in the treatment of women with POI. In light of this, we plan to conduct a well-powered, single-centre randomised controlled trial to explore the efficacy and safety of SVF in patients with POI. This trial aims to confirm the effectiveness of SVF in improving pregnancy outcomes among patients with infertility with POI. To our knowledge, no similar study has been proposed yet. The results of this study can help clinicians standardise the treatment strategies for SVF in patients with POI, facilitating clinical promotion and application.

METHODS AND ANALYSIS

Trial design and setting

Based on clinical phenomena and problems, this study will explore the efficacy and safety of autologous adipose tissue-derived SVF in the treatment of POI. While some studies have been conducted on this topic, most previous studies have consisted of case reports and observational studies. Therefore, we have designed a single-centre randomised controlled clinical trial with 1:1 treatment ratio. This study will be conducted at Peking University Third Hospital, in close collaboration with the reproductive medical centre, plastic surgery department and stem cell department.

This study aims to evaluate the hypothesis that autologous adipose tissue-derived SVF can improve ovarian reserve function and pregnancy outcomes in patients with infertility with POI. All qualified participants will be assessed and followed up (see figure 1). The procedures for this study, including enrolment, interventions, treatments and follow-up assessment, are shown in online supplemental table S1. Adverse events (AEs), including local and systemic reactions, will be monitored before, during and after the procedures. Outcomes such as changes in menstruation and serum level of sex hormone will be measured. Additionally, we will follow up pregnancy outcomes and the health status of offspring through telephone. The results obtained from this study will provide high-level evidence and will be suitable for clinical transformation and standardised application.

Eligibility criteria

Within 4 weeks before the interventions, the participants will undergo transvaginal ultrasound examination and tests for serum level of sex hormone to determine

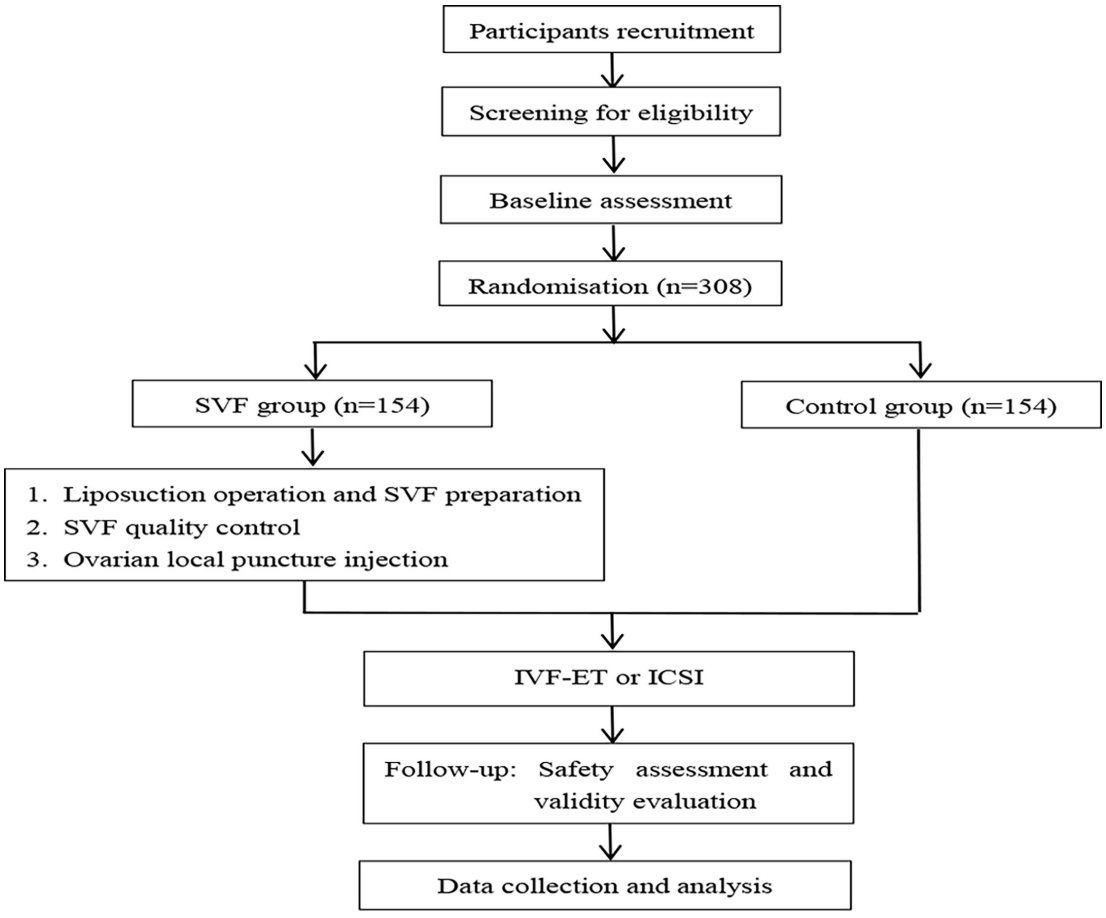

Figure 1 Trial flow chart. ICSI, intracytoplasmic sperm injection; IVF-ET, in vitro fertilisation and embryo transfer; SVF, stromal vascular fraction.

eligibility for enrolment. The following information will be recorded: (1) medical history and physical examination; (2) menstrual cycle, length of menstrual period and menstrual volume before the intervention; (3) ovarian volume and AFC; and (4) serum level of AMH and sex hormone (E_2 and FSH). In order to control variability caused by the differences in operating skills and technical proficiency among sonographers, each patient's transvaginal ultrasound examination will be performed by two sonographers of different seniority, following standardised procedures and specifications. Additionally, all ultrasound measurement data will undergo consistency checks to ensure accuracy and control errors.

Inclusion criteria

1. Women of childbearing age (20-39 years old) with fertility needs.
2. Cessation of menstruation or oligomenorrhoea lasting for at least 4 consecutive months.
3. Serum level of basal FSH >25 IU/L for at least two times (during the second to the fourth day of the menstrual cycle or during amenorrhoea; the interval between the two tests is 4 weeks).
4. Serum level of AMH ≤1.1 ng/mL.
5. Body mass index of 22–29 kg/m^2 and sufficient abdominal subcutaneous fat reserve.
6. Women with intact uterus and bilateral adnexa.
7. Voluntary participation and informed consent obtained.

Exclusion criteria

1. Women with autoimmune diseases.
2. Women with abnormal and uncontrolled thyroid function.
3. Women with tumours in bilateral adnexa that are not clearly benign or malignant.
4. Women with a history of malignant tumours, radiation therapy or chemotherapy.
5. Women with a history of venous thrombosis or pulmonary embolism during the screening period.
6. Women with severe illnesses that are not suitable for pregnancy, such as diseases of the circulatory, urinary, digestive, endocrine, respiratory, immune, mental or neurological system.
7. Women who have used systemic glucocorticoids or other immunosuppressants continuously for ≥3 weeks within 6 months before administration, or who have used systemic glucocorticoids (prednisone ≥20 mg/day or equivalent dose) or other immunosuppressants within 3 days before administration.
8. Women who are allergic to the active ingredients or excipients of test drugs.

9. Women with a family history of severe genetic diseases or gynaecological malignancies.

Withdrawal criteria

1. Participants who refuse to continue the trial procedures or are lost to follow-up.
2. Researchers deem it necessary to stop their study. Specific cases include but are not limited to the following:
   1. Participants exhibit poor compliance.
   2. Researchers deem it necessary to stop the trial from a medical ethics perspective.
   3. Participants experienced serious adverse events (SAEs) and it is not appropriate for them to continue the trial.
   4. Researchers judge that withdrawal from the trial is most beneficial to the participant.
   5. Participants engage in other behaviours that affect the outcomes of this study.

Termination criteria

1. In view of the number and severity of SAEs, researchers consider that the clinical trial must be stopped in advance.
2. There is evidence that SVF is ineffective, or it is meaningless to continue the trial.
3. The ethics committee or the drug regulatory department orders the termination of the trial for some reason.

Patient recruitment

We will attract more patients through advertising on social media channels and distributing flyers in outpatient clinics. The clinical team with rich clinical experience will preliminarily screen patients through medical history collection, physical examination and simple auxiliary examinations. Eligible patients will then be informed about some details of the trial, including the purpose, procedures, benefits and potential risks, etc. After being introduced to the trial, patients will be allowed to consider and decide whether to participate in the trial. If they agree to enrol in the trial, a researcher will schedule an appointment for them to sign the consent form and proceed with the standard trial procedures. The data and the reasons for exclusion of all excluded patients will also be saved and recorded.

Sample size estimation

The cumulative incidence of clinical pregnancy in patients with POI/POF prescribed with hormone replacement therapy (HRT) has been reported at 3.5%–4.4%.[16 17] Additionally, the cumulative clinical pregnancy rate for women with POI undergoing in vitro fertilisation and embryo transfer (IVF-ET) has been reported at 7.2%.[18] Moreover, historical data from our study sites indicate a clinical pregnancy rate in women with decreased ovarian reserve (DOR) of 4%. Based on this literature and the data from our centre, we now estimate that the cumulative clinical pregnancy rate within 6 months for women with POI receiving conventional assisted reproductive technology (ART) is approximately 5%. Currently, there are limited clinical studies on the application of SVF for treatment of POI. In a clinical trial investigating the use of unilateral intraovarian injection in umbilical cord mesenchymal stem cells for treatment of POF, the clinical pregnancy rate was reported to reach 14.3%.[19] Additionally, to enhance clinical practice, we used the historical data from our study sites, which indicate that the cumulative clinical pregnancy rate within 6 months for women with POI after platelet-rich plasma (PRP) or SVF intraovarian injection can reach up to 15%. Based on these findings, we hypothesised that the cumulative clinical pregnancy rate within 6 months for women with POI after SVF treatment would rise to 15%. Using two-sided test, 5% alpha-error and 80% statistical power, the sample size was calculated as 138 participants in each group. Taking a dropout rate of 10% into consideration, we plan to enrol a total of 308 participants, with 154 participants in each group (1:1 randomisation ratio).

Randomisation

Formulation of random allocation scheme

Eligible patients will be allocated to the SVF treatment group and the control group through stratified block randomisation (1:1 treatment ratio). An independent statistician will use the R software to generate the random assignment tables, with block lengths dynamically set from 4 to 6.

Concealment of random allocation scheme

Platform managers will deploy the random allocation scheme to the REDCap V.11.0 system (provided by the Clinical Epidemiology Research Center of Peking University Third Hospital) to ensure the concealment of centralised random allocation scheme.

Blinding

Because of the particularity of the therapeutic schedule, the evaluator, specifically the follow-up personnel who collect postoperative medical histories and the ultrasound evaluator, will be blinded in this trial to conceal the grouping results. However, blinding of participants and researchers is not possible.

Interventions

SVF group

After lower abdominal liposuction (with a volume of about 100 mL), which will be performed under general anaesthesia by a plastic surgeon, the preparation and quality inspection of SVF will be immediately completed by a qualified stem cell laboratory. The adipose tissue will be cut into pieces of about 1 mm^3, and an equal volume of 0.1% collagenase I will be added for digestion. The digestion process will last for 40 min. Subsequently, the SVF suspension will be obtained through procedures such as isolation, filtration, centrifugation, resuspension and dilution. Then, it will be tested for bacteria, fungi, viruses, endotoxin and so on. According to the quality control criteria for the preparation of autologous adipose

tissue-derived SVF, bacteria, fungi, viruses, *Mycoplasma* and other micro-organisms should not be detected in SVF. Additionally, we will also detect the expression of surface markers of SVF to ensure quality. Qualified SVF must meet the following criteria: endotoxin <0.5 EU/mL; cell viability ≥90%; cell count ≥1×10^7; positive surface markers such as CD13, CD29, CD44, CD73 and CD90 >40% and CD34 >20%; and negative surface markers such as CD31, CD45 and CD11b <50% and CD105 <20%. After about 4 hours of quality control and detection, the SVF suspension will be transported to the operating room on ice in a sealed incubator. Under ultrasound guidance, the SVF suspension will be transplanted into the patient's bilateral ovaries by a reproductive endocrinologist. Briefly, both ovaries will be intramedullary injected on multiple sites using a 17-gauge single-lumen needle. A total of $1–2\times10^7$ cells from autologous adipose tissue will be injected into each ovary. Approximately 330±10 µL of SVF suspension will be administered at each point, 1 mL per ovary in total. Following the infusion procedure, the pelvis will be thoroughly examined via ultrasonography in order to check for total vascular integrity. Anaesthetic drugs will not be used during this procedure, but analgesics may be administered if necessary. After the injection, patients will be required to lie flat for 30 min to monitor for any AEs. Their in vitro fertilisation (IVF) cycles will begin 4–8 weeks after SVF intraovarian injection.

Control group

The patients in the control group will receive conventional ART treatment including IVF-ET and intracytoplasmic sperm injection.

Follow-up strategy

To evaluate the therapeutic effect, patients will be asked to go back to the outpatient clinic for four visits within 6 months, followed by telephone follow-up for 2 years.

Safety evaluation and AEs monitoring

To explore the safety of this trial, we will continuously monitor and record the onset date, duration, severity and frequency of all AEs in detail, including local or systemic adverse reactions.

Preintervention stage

1. Transvaginal ultrasonography: Some patients may experience mild discomfort or pain when the ultrasound probe is inserted into the vagina. However, this discomfort is typically brief and usually resolves shortly after the examination. In rare cases, minor damage to the vaginal mucosa may occur. Although this type of injury usually heals naturally, it may slightly increase the risk of infection.
2. Pain, bruising or other discomfort may occur at the blood collection site. A very small number of patients may experience fainting or infection at the puncture site.

Abdominal liposuction

1. Risks and AEs associated with general anaesthesia include respiratory complications, cardiovascular issues (eg, postinduction hypotension), allergic reactions, perioperative nausea and emesis, etc.
2. Intraoperative adverse reactions include pain, bleeding, fat embolism, pulmonary embolism, etc.
3. Postoperative adverse reactions include subcutaneous congestion, bruising, ecchymosis, induration, local skin depression or relaxation, incision scar formation, skin infection or necrosis, etc.

Ovarian local puncture injection

1. Abdominal pain: The primary manifestation is dull pain in the lower abdomen. Most patients' abdominal pain will ease naturally after rest. If the pain does not subside but worsen, analgesic drugs can be given in the case of a clear diagnosis or cause.
2. Anaphylaxis: Although extremely rare, anaphylaxis can occur, ranging from mild-like rash and pruritus to severe reactions such as dyspnoea and even death.
3. Infection: Any invasive operation carries the risk of postoperative infection. The main measures to prevent infection include strictly adhering to aseptic techniques and prophylactic use of antibiotics.
4. Bleeding: The primary causes of bleeding include surgical injury of ovarian vessels and coagulation disorders. Haemostatics and haematinics can be given according to the actual situation.

Outcome measurements

Primary outcome

The primary outcome of this trial is the cumulative clinical pregnancy rate within 6 months in women with POI undergoing IVF. This will be calculated as: cumulative clinical pregnancy rate (%)= number of participants obtain clinical pregnancy within 6 months/total number of participants×100%). Clinical pregnancy refers to the condition where ultrasound can detect one or more viable gestational sacs. In most instances, intrauterine gestational sac and yolk sac are usually visible from 5 to 6 weeks of gestation, and embryonic bud and embryonic heart beat are visible from 6 to 7 weeks of gestation.

Secondary outcomes

The secondary outcomes include the measurement of ovarian reserve function. Ovarian reserve refers to the quantity and developmental potential of follicles in the ovaries. It will be primarily assessed by serum AFC, AMH, FSH levels and ovarian volume. Ovarian function reflects the ovulatory and endocrine capabilities of the ovaries. It will be evaluated through serum FSH, estradiol levels, menstrual status and ovulation patterns observed during the study. Additionally, we will also monitor and record all AEs and follow up on the pregnancy outcomes and the health status of the offspring.

1. Serum level of AMH: The efficacy rate will be calculated as: efficacy rate (%)=number of participants with

two consecutive increases in serum level of AMH/total number of participants×100%.
2. AFC: The efficacy rate will be calculated as: efficacy rate (%)=number of participants whose bilateral ovarian AFC increases by at least two follicles/total number of participants×100%.
3. Serum level of sex hormone (E_2 and FSH): The efficacy rate will be calculated as: efficacy rate (%)=number of participants with two consecutive increases or decreases in serum level of E_2 or FSH/total number of participants×100%.
4. Ovarian volume: Ovarian volume will be recorded before and after the intervention. Changes in ovarian volume between the control group and the SVF group will be analysed and compared using paired sample t-test or independent sample t-test. A change in ovarian volume is considered significant at $p<0.05$.
5. Menstrual status: The frequency, duration and volume of menses within 6 months after the intervention will be recorded to evaluate whether the patient's menstrual status improved. The number of sanitary napkins needed will be used to approximate the menstrual volume. We define the recovery of menstrual status as the resumption of menstrual cycles (at least two consecutive uterine bleeds at an interval of 3–6 weeks) without any hormonal treatment, and normal menstruation duration and volume. This will be calculated as: menstrual recovery rate (%)=number of participants with menstrual recovery/total number of participants×100%.
6. Pregnancy outcomes: Live births or miscarriage of patients with successful pregnancy within 6 months will be followed up. Live birth refers to the delivery of one or more viable fetus after 24 weeks of gestation. Miscarriage refers to a loss of pregnancy that occurs before 24 weeks of gestation. The rate of live birth will be calculated as: live birth rate (%)=number of participants with live births/total number of participants×100%. The rate of miscarriage will be calculated as: miscarriage rate (%)=number of participants with miscarriage/total number of participants×100%.
7. Health status of offspring: The health status (survival, physiological and intellectual development) of the offspring will also be recorded at the following follow-up times: 1 year, 1 year and 6 months, and 2 years after the intervention.
8. AEs: The incidence of AEs will be calculated as: incidence of AEs (%)=number of participants with AEs/total number of participants×100%. The gold standard for reporting AEs is the Common Terminology Criteria for Adverse Events version 5.0,[20] which provides a standardised framework for evaluating the severity and impact of AE. Clinicians will interpret, diagnose and grade AEs using a scale from 1 to 5 over the duration of the trial: grade 1 (mild): asymptomatic or mild symptoms, clinical or diagnostic observations only, intervention not indicated; grade 2 (moderate): minimal, local or non-invasive intervention indicated, limiting age-appropriate instrumental activities of daily living (ADL); grade 3 (severe): severe or medically significant but not immediately life-threatening, hospitalisation or prolongation of hospitalisation indicated, disabling, limiting self-care ADL; grade 4: life-threatening consequences, urgent intervention indicated; grade 5: death related to AE.

Data collection and management

Each selected case must have a case report form (CRF) filled out by professionally trained researchers. In addition, all research data related to the participants, including original medical records, CRFs, signed informed consent, etc, are properly stored by specialised researchers. After the completed CRF is reviewed and approved by the inspector, the data will be entered into a dedicated electronic database, which is only accessible to our researchers. Each participant will receive a personal trial ID as an identifier, and all information related to the participant's actual identity will be kept confidential to protect privacy.

Statistical analysis

We will collect and analyse the relevant data of all enrolled patients. Descriptive analysis will be used to depict the baseline characteristics. The Kolmogorov-Smirnov test will be used to test the normality of continuous variables. Quantitative data that follow a normal distribution will be expressed as mean and SD, and the independent sample t-test will be used to compare the differences between the groups. Conversely, quantitative data that follow a skewed distribution will be presented as median and IQR, and the Mann-Whitney U test will be used. Additionally, qualitative variables will be presented as proportion (%), and the χ^2 test or Fisher's exact test will be used to compare the differences between the groups when appropriate. Generalised linear model and binomial regression will be used to determine the effects of SVF on the clinical outcomes of patients, and potential confounding variables will be controlled. The difference will be considered statistically significant at $p<0.05$. The R software (V.4.1.0) will be used for statistical analysis.

Patient and public involvement

None.

ETHICS AND DISSEMINATION

This study is designed following good clinical practice as well as the Declaration of Helsinki. This trial has been approved by the Ethics Committee of Peking University Third Hospital (approval number: IRB00006761-M2024330) and registered on the ClinicalTrials.gov registry (NCT06481969). Each participant will be required to sign the informed consent before entering the trial (see online supplemental file 2 for a copy of the consent form). Researchers will allow for supervision, auditing and regulatory inspections related to the study,

as well as unrestricted access to source data and files. The findings of this trial will be presented in a peer-reviewed journal.

TRIAL STATUS

The recruitment started in July 2024. The estimated end date of the last recruitment for this study is December 2026.

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Contributors JQ, PL, RL, CM, YY and RY conceived and initiated the study design. HZ, HX, HL, QG, XL, CZ and HB participated in the design of the study. YZ and YL drafted the first edition of the manuscript. HL, RY, YY and CM helped with the critical revision of this protocol. CM, XZ, SY, HZ, JiaL and WY will participate in the recruitment of the participants and the assessment of clinical outcomes. JQ, PL and RL will supervise patient diagnosis and recruitment. Patients will be followed up by YZ, YL, JialL, CL and WY. YZ, YL, JialL and CL will be responsible for recording, entering and analysing the data. LZ and TT will design the statistical analysis plan and oversee data collection and analysis. All authors critically reviewed the article and approved the final manuscript. RY is the guarantor.

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Competing interests None declared.

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