




BMJ Open Association between anti-Müllerian hormone levels during pregnancy and pregnancy outcomes in infertile patients undergoing in vitro fertilisation/ intracytoplasmic sperm injection: protocol for a multicentre prospective cohort study

Qiong Liu ^{1,2}, Kai-Lun Hu ^{1,2,3}, Jing Shi,⁴ Shulin Yang,⁵ Yifan Chu,⁵ Honghong Wang,⁶ Yiwen Zhang,⁷ Jing Yue,⁵ Guimin Hao,⁷ Xueqing Wu,⁶ Jie Zhao,^{1,2,3,8,9} Rong Li ^{1,2,3,8,9}

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QL and K-LH contributed equally.

QL and K-LH are joint first authors.

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For numbered affiliations see end of article.

Correspondence to

Dr Rong Li;
roseli001@sina.com and
Dr Jie Zhao;
2358044941@qq.com

ABSTRACT

Introduction Polycystic ovary syndrome (PCOS), recognised as the predominant aetiological factor in ovulatory dysfunction-related infertility, accounts for approximately 70% of anovulatory infertility cases. Patients with PCOS have significantly higher anti-Müllerian hormone (AMH) levels than their counterparts undergoing in vitro fertilisation (IVF) for non-PCOS indications (eg, male/tubal factors). Several studies have suggested that a high AMH level is associated with adverse pregnancy outcomes in IVF, particularly preterm delivery. However, most of these studies are retrospective studies, and their results are inconsistent. The majority of AMH measurements are conducted before pregnancy; however, AMH levels fluctuate dynamically during pregnancy. There is a pressing need for a well-structured prospective study to definitively establish whether high AMH levels during pregnancy are associated with IVF/intracytoplasmic sperm injection (ICSI) pregnancy outcomes in PCOS patients.

Methods and analysis This prospective cohort study will be conducted at four reproductive medicine centres. The plan is to enrol 1,320 PCOS patients and 1320 non-PCOS women who undergo IVF/ICSI and achieve singleton clinical pregnancies. Serum samples will be collected at about 6 weeks of gestation to measure the serum AMH level. Follow-up visits will be conducted at 12, 28 and 37 weeks of gestation, delivery and 6 weeks after delivery to obtain information about pregnancy outcomes and complications. The primary outcome is preterm delivery.

Ethics and dissemination The study was approved by the Medical Research Ethics Committee of Peking University Third Hospital (M2022618). Informed consent will be obtained from all patients. The results of this clinical study will be presented at scientific conferences and submitted to a peer-reviewed journal.

Trial registration number ChiCTR2300068554.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ This large-scale (n=2640) multicentre cohort study prospectively examines the association between anti-Müllerian hormone (AMH) levels and preterm delivery risk.
- ⇒ While current AMH assessments focus on pre-pregnancy stages, this study emphasises gestational AMH measurement at 6 weeks of gestation to elucidate its effect on maternal–foetal health.
- ⇒ Preterm delivery will be used as the main observation indicator, while current studies rarely focus on this aspect.
- ⇒ While high AMH levels may impair follicle development and in vitro fertilisation/intracytoplasmic sperm injection outcomes, follicle status is not included in our observational parameters.
- ⇒ Baseline discrepancies (eg, differences in the proportion of frozen-thawed embryo transfer or hormonal levels) between polycystic ovary syndrome (PCOS) and non-PCOS groups may introduce confounding effects despite multivariate and stratified analyses.

INTRODUCTION

Polycystic ovary syndrome (PCOS), one of the most prevalent endocrine and metabolic disorders in reproductive-aged women, is clinically defined by the presence of any two of the following criteria: oligo-ovulation or anovulation, hyperandrogenism and polycystic ovary morphology. The prevalence of PCOS in Chinese women of reproductive age is 5.67%,¹ with up to 70% of affected women remaining undiagnosed worldwide.² Notably, PCOS represents a leading cause of infertility,

contributing to 70% of cases marked by anovulatory infertility. Beyond reproductive implications, PCOS is closely linked to glucose and lipid metabolism disorders, significantly affecting patients' quality of life, long-term health and even offspring health.³ Thus, PCOS is regarded as a significant public health concern for women of child-bearing age.

In vitro fertilisation/intracytoplasmic sperm injection and embryo transfer (IVF/ICSI-ET) is a widely used assisted reproductive technology (ART) for PCOS patients, who are 8–10 times more likely to require such interventions to achieve pregnancy compared with non-PCOS individuals.⁴ However, retrospective studies have indicated that although more oocytes are retrieved after ovarian stimulation in PCOS patients, their high-quality embryo rate is lower. Furthermore, the biochemical pregnancy rate, early abortion rate and incidence of adverse pregnancy outcomes, such as gestational diabetes mellitus and premature delivery, also increase significantly.^{5–7} Currently, these outcomes are partially attributed to hyperandrogenism, obesity and insulin resistance in PCOS patients.^{8–10} Nevertheless, addressing the above factors cannot improve early IVF outcomes and reduce the rate of adverse pregnancy outcomes.¹¹

The anti-Müllerian hormone (AMH) is a member of the transforming growth factor- β family. In adult women, AMH inhibits primordial follicle recruitment and antral follicle development, prevents premature follicle depletion and is an important indicator for the clinical assessment of ovarian reserve and ovarian response in ART.¹² In controlled ovarian hyperstimulation, a higher serum AMH level predicts more retrieved oocytes and more embryos; however, there is no significant correlation with oocyte quality or fertilisation rate.¹³ Moreover, recent studies have suggested that high AMH may inhibit the expression of aromatase stimulated by follicle-stimulating hormone (FSH), potentially resulting in androgen excess and inhibiting the growth of pre-antral follicles. Therefore, excessive AMH may induce ovulation disorder.¹⁴

Patients with PCOS have a significantly higher AMH level than the non-PCOS IVF population and are prone to high ovarian response or even ovarian hyperstimulation syndrome, which may preclude transferring fresh embryos.¹⁵ Several retrospective clinical studies on PCOS have found that a high serum AMH level is associated with lower clinical pregnancy and live birth rates after IVF/ICSI.^{16–19} However, other studies have reported contrasting results. After adjusting for age and body mass index (BMI), the live birth rate and pregnancy rate of PCOS patients with high AMH levels after IVF were significantly higher, especially the cumulative pregnancy rate. This may result from the fact that PCOS patients have higher antral follicle counts and more available embryos.^{20–21} Multiple retrospective studies have indicated that high AMH levels are significantly associated with preterm delivery risk in PCOS patients, especially when AMH levels are extremely high.^{22–24} However, conflicting evidence suggests the absence of an association between high AMH levels and

increased preterm delivery risk, even in PCOS patients.²⁵ Additionally, AMH levels may be related to other pregnancy complications, such as gestational hypertension and gestational diabetes mellitus.^{26–27} Although some studies have suggested that high AMH levels are associated with adverse IVF pregnancy outcomes, most of them are retrospective studies, which lack convincing evidence and draw inconsistent conclusions due to confounding factors. Moreover, the majority of AMH measurements are conducted before pregnancy. However, given that AMH levels exhibit dynamic fluctuations throughout gestation, assessing AMH levels during pregnancy could offer a more accurate reflection of its effects on both the mother and foetus.

Consequently, there is a pressing need for a well-structured prospective study to definitively establish whether high AMH levels during pregnancy are associated with IVF/ICSI pregnancy outcomes in PCOS patients. Such a study would provide more robust and conclusive insights into this complex relationship.

METHODS AND ANALYSIS

Study design

This multicentre prospective cohort study will recruit 1,320 PCOS patients (Rotterdam criteria-confirmed) and 1320 non-PCOS controls undergoing IVF/ICSI with subsequent singleton pregnancies across four reproductive medicine centres, including Peking University Third Hospital, Tongji Hospital Affiliated to Tongji Medical College of Huazhong University of Science and Technology, the Second Hospital of Hebei Medical University and Shanxi Children's Hospital (Maternal and Child Healthcare Hospital). After gathering demographic data, serum samples will be collected to detect AMH levels at approximately 6 weeks of gestation. Subsequently, pregnancy outcomes and perinatal complications will be gathered via telephone interviews or by reviewing medical records. Finally, AMH levels will be assessed, and their correlation with preterm delivery and other relevant outcome indicators will be investigated.

AMH measurement

Serum AMH will be evaluated at approximately 6 weeks of gestation. Samples will be frozen at -80°C until they will be assayed. For AMH evaluations, a sensitive GENII ELISA kit (Beckman-Coulter), following the manufacturer's instructions, will be used. The sensitivity and quality control of each assay will be assessed following the methods published by Xu *et al.*²⁸ The correlation between age and basal serum AMH levels is based on the population-based distribution patterns reported by Shebl *et al.*²⁹

Participant eligibility criteria

Inclusion criteria

1. Women with a diagnosis of PCOS undergoing IVF/ICSI and achieving a clinical pregnancy. PCOS is diagnosed if they meet two of the following three criteria according to the 2003 Rotterdam criteria,³⁰ excluding

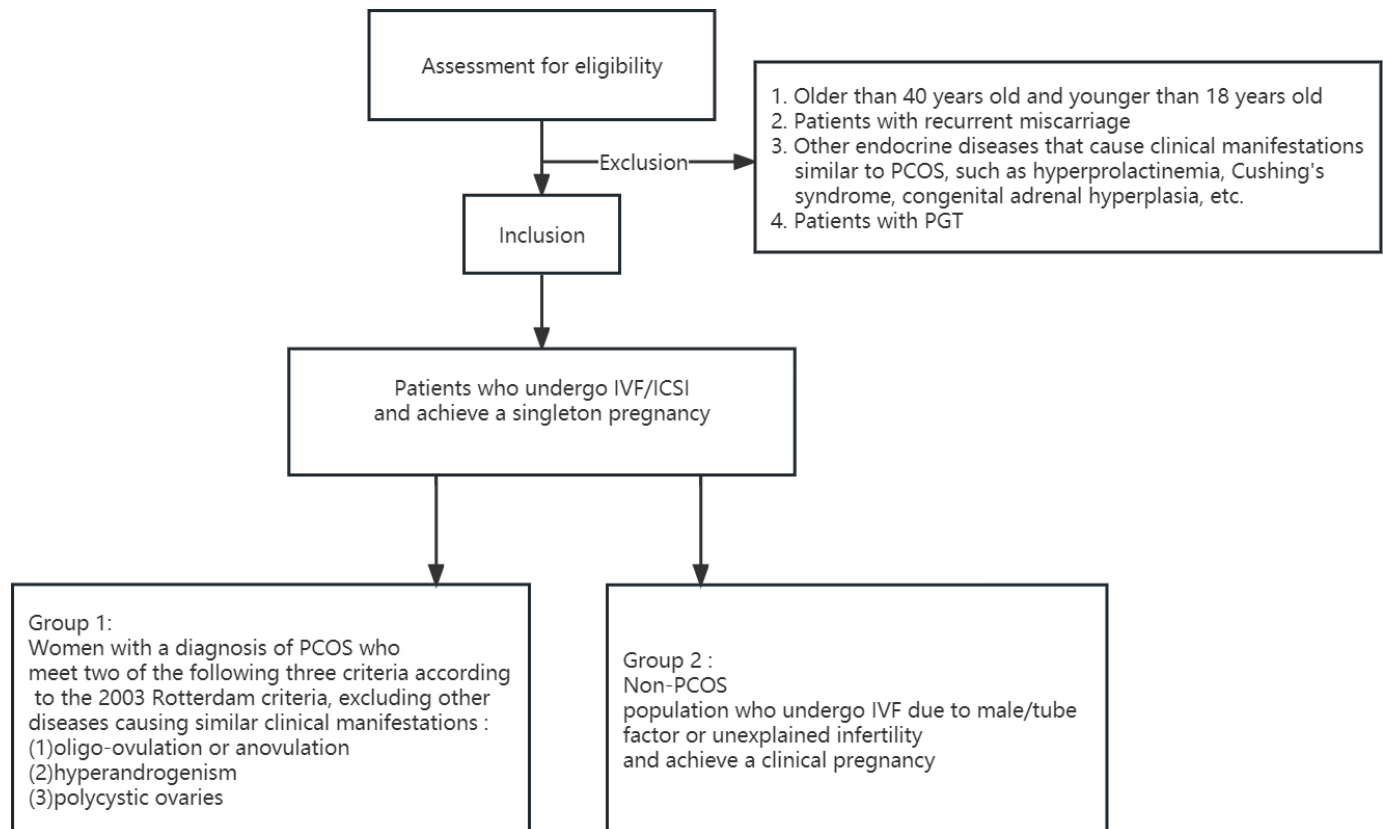


Figure 1 Flowchart of the inclusion and exclusion criteria. IVF/ICSI, in vitro fertilisation/intracytoplasmic sperm injection; PGT, pre-implantation genetic testing; PCOS, polycystic ovary syndrome

other diseases causing similar clinical manifestations: (1) oligo-ovulatory or anovulatory, (2) hyperandrogenism (clinical and/or biochemical) and (3) polycystic ovaries: unilateral ovarian volume increased by >10 mL (excluding cysts and dominant follicles) and/or ≥12 follicles with a diameter of 2–9 mm in unilateral ovary.

2. Non-PCOS population undergoing IVF/ICSI for male factor infertility, tubal factor infertility or unexplained infertility and achieving a clinical pregnancy.
3. Older than 18 years old.
4. Patients achieving a singleton pregnancy.

Eligibility requires fulfilment of criteria 1, 3 and 4 or criteria 2, 3 and 4.

Exclusion criteria

1. Over 40 years old.
2. Individuals experiencing recurrent abortion, defined as the occurrence of two or more consecutive miscarriages before the 20th week of gestation.
3. Patients with other endocrine disorders presenting clinical symptoms resembling PCOS, including hyperprolactinaemia, Cushing's syndrome, congenital adrenal hyperplasia, etc.
4. Patients undergoing pre-implantation genetic testing.

The flowchart of the inclusion and exclusion criteria is presented in figure 1.

Outcome assessment

Primary outcome indicator

Preterm delivery defined as delivery before 37 weeks of gestation is the primary outcome.

Secondary outcome indicators

These include early miscarriage (before 12 weeks of gestation), late miscarriage (at or after 12 weeks of gestation), clinical pregnancy, live birth, maternal complications, birth weight, birth defects and other adverse events. Clinical pregnancy is defined as the detection of a gestational sac in the uterine cavity, along with a foetal heartbeat on transvaginal ultrasonography 30 days after ET.

Sample size calculation

The preterm delivery rate among PCOS patients who achieve singleton births at Peking University Third Hospital stands at 8%.²³ To demonstrate that the preterm delivery rate of PCOS patients with AMH concentration above the median is 5% higher than that of patients with AMH concentration below the median (10.5% vs 5.5%) with a power of 80% and a two-tailed $\alpha=0.05$, a total of 922 patients are needed. However, considering 20% dropout and 10% loss of follow-up from clinical pregnancy to live birth (a total of 30% shedding), 1320 patients with PCOS who achieve clinical pregnancy are required to be included. A control group of 1320 cases of infertility due

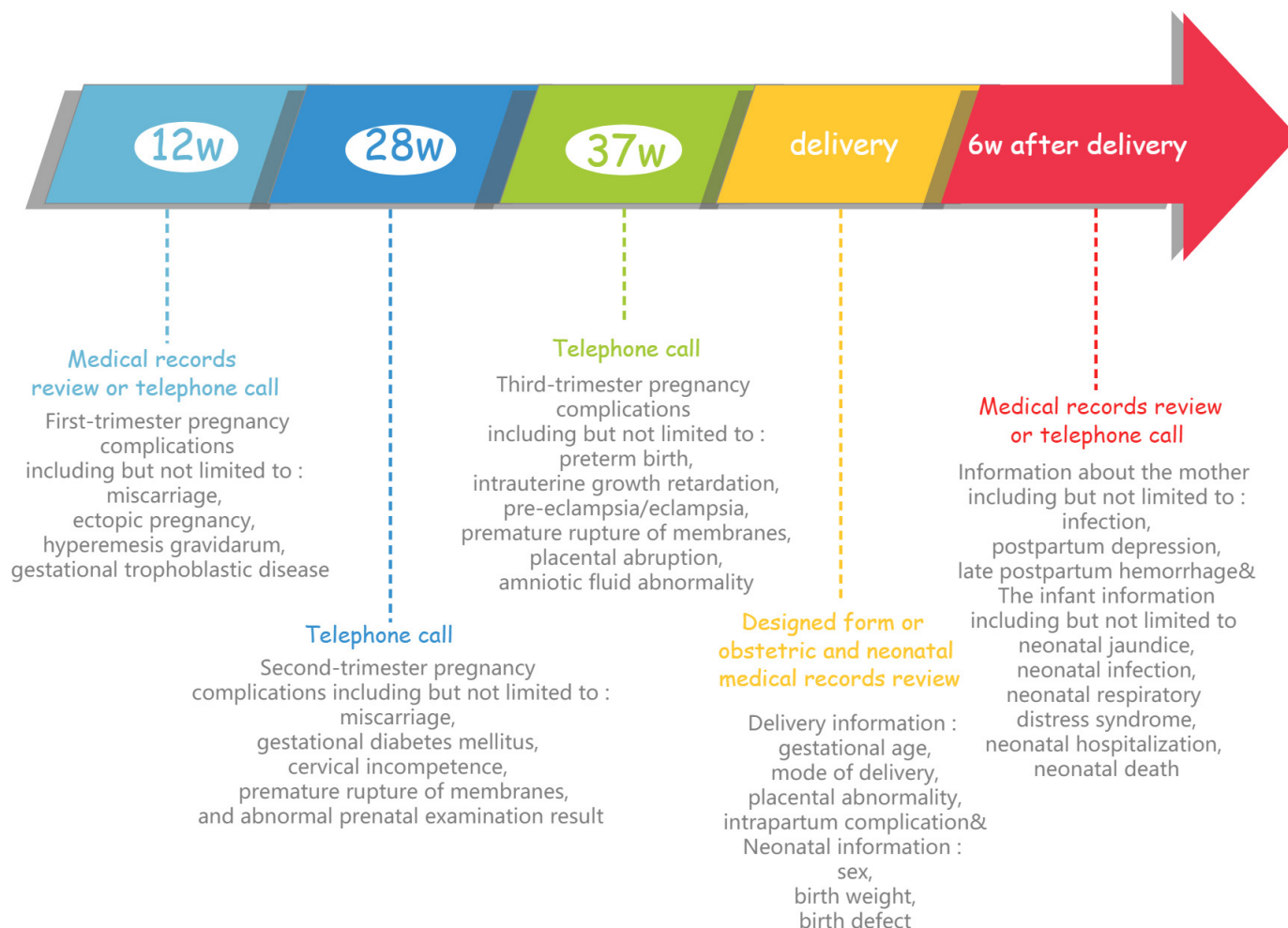


Figure 2 Follow-up protocol.

to male/tubular factor or unexplained infertility, totaling 2640 cases, will be included in this study on a 1:1 basis.

Demographic information data collection

The following data will be collected: age, AMH level before pregnancy and at approximately 6 weeks of gestation, basic sex hormone levels (prolactin, FSH, luteinising hormone, testosterone, progesterone and estradiol) on days 2–4 of the menstrual cycle within 1 year, BMI, causes of infertility (including male factor, tubal factor, ovulation disorder, and unknown reasons), infertility duration, previous pregnancy and delivery history, whether complicated with medical and/or surgical diseases, number of embryos transferred, embryo quality, date of ET, fresh ET or frozen-thawed ET, number of oocytes retrieved, number of fertilised eggs, stimulation protocol, gonadotrophin dose and endometrial preparation protocol (if frozen-thawed embryos are transferred).

Follow-up protocol

First follow-up visit during pregnancy (at 12 weeks of gestation)

First-trimester pregnancy complications, including but not limited to miscarriage, ectopic pregnancy, hyperemesis gravidarum and gestational trophoblastic disease, will be assessed. The information will be systematically

collected through electronic medical records, standardised case report forms or telephone interviews.

Second follow-up visit during pregnancy (at 28 weeks of gestation)

During the second trimester, pregnancy complications such as gestational diabetes mellitus, cervical incompetence, premature rupture of membranes and abnormal prenatal examination results will be monitored. The information above will be collected through telephone.

Third follow-up visit during pregnancy (at 37 weeks of gestation)

The third trimester will focus on complications including preterm delivery, intrauterine growth restriction, pre-eclampsia/eclampsia, premature rupture of membranes, placental abruption and amniotic fluid abnormality. Data will be gathered via telephone.

Fourth follow-up visit (at delivery)

Participants will notify us of their delivery date. Information regarding gestational week of delivery, delivery mode, placental abnormality and any intrapartum complications will be collected. Additionally, neonatal information, including the infant's sex, birth weight, and any birth defects, will be recorded. The information will

be obtained through designed forms and by reviewing obstetric and neonatal medical records.

Fifth and final follow-up visit (6 weeks after delivery)

We will inquire about the health status of both the mother and the infant. For mothers, this may include information related to puerperal infection, postpartum depression and late postpartum haemorrhage. For infants, issues such as neonatal jaundice, neonatal infection, neonatal respiratory distress syndrome, neonatal hospitalisation and neonatal mortality will be assessed. Information will be collected via telephone interviews or by reviewing medical records.

Throughout these follow-up visits, any adverse events and concomitant medications used by the participants will also be gathered. Any cases of dropout or loss to follow-up visits will be duly documented. An overview of the follow-up visit protocol is presented in figure 2.

Statistical analysis plan

IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA) will be used for data analysis. Normally distributed continuous variables will be presented as mean \pm SD, and differences between groups will be assessed using Student's t-test. Non-normally distributed continuous variables will be expressed as median and range, with differences between groups analysed via the Wilcoxon rank sum test. Categorical data will be reported as frequency and percentage. Differences between groups will be evaluated using Pearson's χ^2 test or Fisher's exact test when the expected frequency is <5 . Significance is defined as a P value <0.05 .

For outcome indicators such as the preterm delivery rate, multivariate and stratified analyses will be conducted. Multivariable logistic regression models will be constructed adjusting for age, BMI, type and number of embryos transferred, endometrial preparation protocols (if frozen-thawed ET) and other parameters that might affect the outcome. Subgroup analysis will be conducted by ET type (fresh vs frozen-thawed) using logistic regression models. Coincidence analysis will be conducted based on the participants who complete the entire study. As part of the secondary analysis, generalised linear mixed-effect models will be fitted with logistic links, accounting for random stratification factors, embryo staging adjustments and other explanatory variables. Random intercepts will be incorporated to adjust for correlations among patients within the research centres.

Patient and public involvement

None.

ETHICS AND DISSEMINATION

The study was approved by the Medical Research Ethics Committee of Peking University Third Hospital (M2022618) and registered with the Chinese Clinical Trial Register (ChiCTR2300068554). Informed consent

will be obtained from all patients. The results of this clinical study will be presented at scientific conferences and submitted to a peer-reviewed journal.

Author affiliations

- ¹Department of Obstetrics and Gynecology, Center for Reproductive Medicine, Peking University Third Hospital, Beijing, China
- ²National Clinical Research Center for Obstetrics and Gynecology, Peking University Third Hospital, Beijing, China
- ³Beijing Key Laboratory of Reproductive Endocrinology and Assisted Reproductive Technology, Peking University Third Hospital, Beijing, China
- ⁴Department of Pharmacy, Peking University Third Hospital, Beijing, China
- ⁵Center for Reproductive Medicine, Huazhong University of Science and Technology, Tongji Medical College, Tongji Hospital, Wuhan, Hubei, China
- ⁶Center for Reproductive Medicine, Children's Hospital of Shanxi, Taiyuan, Shanxi, China
- ⁷Center for Reproductive Medicine, The Second Hospital of Hebei Medical University, Shijiazhuang, Hebei, China
- ⁸Key Laboratory of Assisted Reproduction (Peking University), Ministry of Education, Beijing, China
- ⁹State Key Laboratory of Female Fertility Promotion, Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, China

Contributors QL and K-LH will take part in the design, planning, conduct, data analysis, and manuscript writing. JS, SY, YC, HW, YZ, JY, GH and XW will participate in the conduct of the study. RL and JZ will take part in the design, planning and conduct of the study. QL and K-LH are co-first authors. RL and JZ are co-corresponding authors. All authors meet the ICMJE criteria for authorship. QL is the guarantor.

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ORCID iDs

Qiong Liu <http://orcid.org/0000-0002-4260-2240>

Kai-Lun Hu <http://orcid.org/0000-0002-2788-4614>

Rong Li <http://orcid.org/0000-0003-0305-5579>

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