TITLE PAGE

Full Title: Letrozole, Berberine or Both as Treatments for Infertility in Women with Polycystic Ovary Syndrome: A Multicenter Randomized Double-Blinded Placebo-Controlled Trial

Brief Title: Letrozole, Berberine, or Both for Infertility in PCOS

Authors: Xiao-Ke Wu, MD, PhD; Jian-Ping Liu, MD, PhD; Li-Hui Hou, MD; Ya-Qin Gao, MD; Shao-Min Du, MD; Yang Xia, MD; Jin-Feng Zhang, MD; Hui-Ying Xue, MD; Wei-Li Li, MD; Rui-Ning Liang, MD; Zhen-Yu Tan, MD; Jin-Ying Fu, MD; Hong-Xia Ma, MD; Xiao-Guang Shao, MD; Feng-Jie He, MD; Mu-er An, MD; Pei-Lin Li, MD; Cai-Fei Ding, MD; Xiao-Bin Li, MD; Xian-Ji Shen, MD; Robert M Silver, MD; Elisabet Stener-Victorin, PhD; Ernest HY Ng, MD, PhD

Setting: First Affiliated Hospital, Heilongjiang University of Chinese Medicine, Harbin, Heilongjiang Province, China (X-K W, L-H H); Centre for Evidence-Based Chinese Medicine, Beijing University of Chinese Medicine, Beijing, China (J-P L); Daqing Oilfield General Hospital, Daqing, Heilongjiang, China (Y-Q G); Daqing Longnan Hospital, Daqing, Heilongjiang, China (S-M D); First Affiliated Hospital, Tianjin University of Chinese Medicine, Tianjin, China (X Y); Second Affiliated Hospital, Tianjin University of Chinese Medicine, Tianjin, China (P-L L); Shanxi Province Hospital of Chinese Medicine, Taiyuan, Sanxi, China (J-F Z); Second Affiliated Hospital, Henan College of Chinese Medicine, Zhengzhou, Henan, China (J-Y F); Huaian Maternal and Child Health Hospital, Huaian, Jiangsu, China (F-Y X);

Affiliated Hospital of Anhui University of Chinese Medicine, Hefei, Anhui, China (W-L L); First Affiliated Hospital, Jiangxi University of Chinese Medicine, Nanchang, Jiangxi, China (R-N L); First Affiliated Hospital, Hunan University of Chinese Medicine, Changsha, Hunan, China (Z-Y T); First Affiliated Hospital, Guangzhou Medical University，Guangzhou, Guangdong, China (H-X M); Dalian Maternal and Children's Health Centre, Dalian, Liaoning, China (X-G S); Affiliated Hospital, Shanxi College of Chinese Medicine, Xianyang, Shanxi (F-J H); Zhejiang Province Hospital of Integrative Medicine, Hangzhou, Zhejing, China (C-F D); Guangdong Province Hospital of Chinese Medicine, Guangzhou, Guangdong, China (X-B L); Mudanjiang Maternal and Children's Health Hospital, Mudanjiang, Heilongjiang, China (X-J S); First Affiliated Hospital, Harbin Medical University, Harbin, Heilongjiang, China (M-A A); Department of Obstetrics and Gynecology, University of Utah Health Sciences Center, Salt Lake City, Utah (RMS); Department of Physiology and Pharmacology, Karolinska Institutet, (EHYN); Stockholm, Department of Obstetrics and Gynecology, the University of Hong Kong, Hong Kong, China (EHYN)

Correspondence: Professor Xiao-Ke Wu, MD, PhD; Department of Obstetrics and Gynecology, First Affiliated Hospital, Heilongjiang University of Chinese Medicine, Harbin 150040, China.

Email: xiaokewu2002@vip.sina.com

***Panel:* Research in context**

Evidence before this study

A recent Cochrane review comparing letrozole with clomiphene followed by timed intercourse in infertile women with PCOS showed that the birth rate was higher in the letrozole group. Another Cochrane review demonstrated that there was no evidence that metformin improved live birth rates whether used alone or in combination with clomiphene. Berberine is a major active component of the Chinese herbal medicines *Rhizoma coptidis, Cortex Phellodendri*, and *C. berberidis*, and it has been used empirically for thousands of years in its herbal form to enhance fertility. When compared to metformin, berberine showed similar metabolic effects on improving insulin sensitivity and reducing hyperandrogenemia in women with PCOS. There are no studies assessing adjunctive therapy with letrozole in hopes of improving live birth rates among infertile women with PCOS.

Added value of this study

In terms of ovulation and live births, the combination of letrozole and berberine was not superior to letrozole alone, but both were superior to berberine alone in infertile women with PCOS.

Implications of all the available evidence

Improvement in the metabolic profile by berberine did not affect rates of ovulation, conception, pregnancy, and live birth achieved with letrozole in infertile women with PCOS.

ABSTRACT

Background Letrozole leads to higher ovulation and live birth rates than clomiphene in infertile women with polycystic ovary syndrome (PCOS). Berberine, a major active component of the Chinese herbal medicines *Rhizoma coptidis*, *Cortex phellodendri* and *C. berberidis*, has even more profound metabolic effects than metformin. Our hypothesis was that a combination of letrozole and berberine would result in a higher live birth rate than letrozole or berberine alone in infertile women with PCOS.

Methods This was a multicenter randomized double-blinded placebo-controlled trial in Mainland China. A total of 644 eligible women with PCOS defined by the Rotterdam criteria were randomized into one of the following three interventions for up to six menstrual cycles: letrozole plus berberine placebo (letrozole group), berberine plus letrozole placebo (berberine group), or the combination of letrozole and berberine (combination group).

Findings The live birth rates were comparable between the letrozole and combination groups (36.3% vs. 34.4%, OR = 0.95, 95% CI = 0.73–1.23; $p = 0.687$), and these were higher than in the berberine group (22.0%) (OR = 1.68, 95% CI = 1.23–2.28, $p = 0.001$; OR = 1.57, 95% CI = 1.15–2.14, $p = 0.004$, respectively). Conception, pregnancy, and ovulation rates were similar for the letrozole and combination

groups, and these were higher than in the berberine group. There was one twin birth in the letrozole group, three twin births in the combination group, and none in the berberine group. Berberine was associated with constipation and nausea while letrozole was associated with fatigue and hot flashes.

Interpretation For ovulation and live births, the combination of letrozole and berberine was not superior to letrozole alone, but both were superior to berberine alone.

Funding National Public Welfare Projects for Chinese Medicine of China and the "11th–Five" Supportive Program of Science and Technology of China.

INTRODUCTION

Polycystic ovary syndrome (PCOS) affects 5.6% of Chinese women 19–45 years of age, and the main clinical manifestations are oligo-anovulation and polycystic ovaries.[1] It is the most common cause of anovulatory infertility. Although less pronounced than in Caucasians, Chinese women with PCOS also suffer from hyperandrogenism and hyperinsulinemia together with insulin resistance.[2,3] Given the huge population and a lack of coverage for infertility treatment by the public health care system and insurance companies within mainland China, PCOS constitutes a considerable economic burden and source of emotional distress.[4]

The first-line medical treatment for anovulatory infertility in women with PCOS is ovulation induction by clomiphene, an anti-estrogen. The drawbacks for clomiphene include a relatively low cumulative live birth rate together with higher multiple pregnancy rates than unassisted conception.[5] Insulin-sensitizing agents such as metformin are commonly used as adjunct medication for women with PCOS. However, metformin alone is not superior to clomiphene or a combination of metformin and clomiphene.[5] Recently letrozole, an aromatase inhibitor, has been shown to be superior to clomiphene for ovulation and live birth rates in infertile women with PCOS.[6]

Berberine, a major active component of the Chinese herbal medicines *Rhizoma coptidis, Cortex Phellodendri,* and *C. berberidis*, has been prescribed empirically for the treatment of diarrhea, metabolic disorders, and infertility.[4,7,8] Berberine is commonly used in China. A total of 0.8 billion 0.1 mg tablets were consumed at

the end of 2000 and 5.9 billion were consumed in 2013, and this is projected to reach 12 billion in 2015.[9] It has been used for thousands of years in its herbal form to enhance fertility and recently as an extract combined with ovulation induction agents such as clomiphene or letrozole to enhance their effectiveness. [4]

We previously demonstrated that berberine could alleviate the insulin resistance and androgen synthesis in insulin-resistant ovaries cultured *in vitro* and when we assessed its function *in vivo*.[10,11] When compared to metformin, berberine showed similar metabolic effects on improving insulin sensitivity and reducing hyperandrogenemia, and berberine had additional effects on body composition and dyslipidemia in women with PCOS.[12] Because of its favorable effects on these metabolic factors, berberine has the potential to complement letrozole in improving live birth rates among infertile women with PCOS. To our knowledge, this is the only study to investigate ovulation and live birth rates following the use of berberine in women with PCOS.

We sought to determine the effectiveness of letrozole alone, berberine alone, and the combination of the two in achieving live births among infertile women with PCOS. Our primary hypothesis was that the combination of berberine and letrozole would result in significantly higher live birth rates than treatment with letrozole or berberine alone.[13]

METHODS

Study Oversight

This was a multicenter randomized double-blind placebo-controlled trial in Mainland China. Recruited participants were allocated randomly into one of the three groups in a ratio of 1:1:1. We previously reported details of the trial protocol,[13] which was designed by the steering committee of the National Clinical Trial Base and approved prior to initiation by the State Administration of Traditional Chinese Medicine of the People's Republic of China – the appointed scientific advisory board. The institutional review board at each participating center approved the protocol, and all participants gave written informed consent. It was first registered at http://www.chictr.org.cn/ in Chinese with identifier ChiCTR-TRC-0900 0376 on October 7, 2009 within China, and then registered at ClinicalTrials.gov with identifier NCT0111 6167 on April 27, 2010.

Participants

A total of 644 women were enrolled in 18 participating sites. Chinese women with PCOS attempting to get pregnant were eligible if they fulfilled the following criteria: (1) age between 20 and 40 years; (2) diagnosis of PCOS according to two of the three Rotterdam 2003 criteria,[14] including oligo-ovulation or anovulation, clinical and/or biochemical signs of hyperandrogenism, and/or polycystic ovaries; (3) at least one open Fallopian tube and normal uterine cavity documented by hysterosalpingogram, sonohysterogram, or diagnostic laparoscopy within the past 3 years; (4) a male partner with sperm concentration of 15 million/mL and

motility of ≥40% in at least one ejaculate; and (5) at least 1 year of infertility. Subjects were excluded if they used hormonal drugs or other medications including Chinese herbal prescriptions in the past 3 months; had known severe organ dysfunction or mental illness; were pregnant, post-miscarriage, postpartum, or breastfeeding within the past 6 weeks; or had congenital adrenal hyperplasia, clinically suspected Cushing's syndrome, or an androgen-secreting neoplasm.

Randomization and Masking

The randomization was performed through a web-based computer program (http://210.76.97.192: 8080/cjbyj) operated by an independent data center—the Institute of Basic Research in Clinical Medicine (IBRCM) of the China Academy of Chinese Medical Sciences. The randomization is stratified by the participating sites. Participants, investigators, physicians taking care of the participants, laboratory technicians, and data analyzers were blinded to the assignments.

Procedures

After spontaneous menses or withdrawal bleeding induced by progestin administration (medroxyprogesterone acetate [Provera, Pfizer Italia Srl Pharmaceutical Co, Ltd], 5 mg per day for 7 days), eligible patients were randomized into one of three interventions: (1) letrozole and berberine placebo (letrozole group), (2) berberine and letrozole placebo (berberine group), or (3)

letrozole and berberine (combination group). Each participant received a medication package on a monthly basis that consisted of a monthly supply of berberine capsules or placebo capsules and one or two packages of pills (letrozole or letrozole placebo, one package per month for the first three months, and two packages per month for the next three months). Berberine or berberine placebo were administrated orally at a daily dose of 1.5 g for 6 months. Patients received an initial dose of 2.5 mg (one tablet) of letrozole or one tablet of letrozole placebo on days 3–7 of the first three treatment cycles. This dose was increased to 5 mg of letrozole (two tablets) or two tablets of letrozole placebo on days 3–7 of the last three treatment cycles if not pregnant. Couples were instructed to have regular intercourse two to three times a week until becoming pregnant. Ovulation detection kits and intrauterine insemination were not used.

Berberine and berberine placebo were produced by Renhetang Pharmaceutical Co, Ltd, China. Letrozole and letrozole placebo were produced by Jiangsu Hengrui Medicine Co, Ltd, China. Neither manufacturer had a financial role in the study. Fasting blood samples for assessment of metabolic and hormonal profiles were drawn at the baseline visit and at the end of the treatment visit at menstrual cycle days 3–7 and analyzed in the core laboratory at Harbin. The intra-assay and inter-assay coefficients of variation of each assay were <10%. All baseline measures including assessment of liver and renal function were repeated at the end of the visits. Participants had monthly visits after menses to document a negative pregnancy test to avoid inadvertent exposure to letrozole and berberine during pregnancy. Once participants conceived, they were followed until a viable

intrauterine pregnancy sac was observed (fetal heart motion visualized on ultrasonography). They were then referred for antenatal care. Outcomes were tracked through regular interviews with midwives and abstractions of obstetric records.

Outcomes

The primary outcome was live birth. Secondary outcomes included (1) Ovulation: Serum progesterone level >5 ng/ml on day 22 of each treatment cycle; (2) Conception: A positive serum level of human chorionic gonadotropin; (3) Pregnancy: An intrauterine pregnancy sac with fetal heart motion as determined by ultrasonography at around 8–10 weeks gestation; (4) Multiple pregnancy; (5) Pregnancy loss: Loss before 20 completed weeks of gestation; (6) Other pregnancy complications such as stillbirth, gestational diabetes mellitus, pregnancy-induced hypertension, and small for gestational age fetus; and (7) Adverse events from the study medication. Patients were asked to record adverse events and to report them to the coordinator at each visit. Serious adverse events were defined as events that were fatal or immediately life-threatening, that were severely or permanently disabling, or that required prolonged inpatient hospitalization; overdoses (intentional or accidental); congenital anomalies; or any event deemed to be serious by the site principal investigator at the study site.

Statistical Analysis

The sample size calculation was based on anticipated live birth rate. A previous meta-analysis suggested that the live birth rate using letrozole in women with

PCOS was about 22% during a 6-month intervention [13] and our study was designed and completed prior to the more recently published large randomized trial.[6] We hypothesized that a combination of letrozole and berberine would increase the live birth rate from 22% to 30%. Accordingly, we estimated that a sample size of 220 participants per group was required considering a 20% dropout, 90% power, and an alpha error of 0.05. On the basis of these assumptions, we needed to enroll 660 subjects for the study.

All data entry, data management, and analyses were performed through a web-based system (http://210.76.97.192: 8080/cjbyj) operated by IBRCM of the China Academy of Chinese Medical Sciences.

Either a chi-square test or Fisher's exact test was used at a two-sided significance level of 0.05 for testing differences among the three study groups for categorical variables. The Kruskal-Wallis was used to test differences among the three groups for continuous variables. If significant, a Mann–Whitney U-test was used to test differences between the groups. Kaplan-Meier analysis was used to compare time to live birth according to treatment groups, [5,6] body mass index (BMI), hirsutism scores, menstrual patterns, age, and previous infertility duration. Adverse events were categorized, and the percentage of patients experiencing adverse events and serious adverse events in each treatment arm were compared using chi-square tests. All analyses were performed with the SAS software, version 9.2 (SAS Institute). Data were analyzed according to the intention-to-treat principle.

Role of the Funding Sources

The funding body of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The co-authors had full access to all the data in the study, and the corresponding author had final responsibility for the decision to submit for publication.

RESULTS

Owing to the expiration of the study drug (berberine and matching placebo), the data safety and monitoring board decided to stop enrollment after 644 patients were enrolled in November 2013. The flowchart of the study is shown in Figure 1. The number of subjects who withdrew from the study was 16 of 215 (7.4%) in the letrozole group, 25 of 214 (11.7%) in the berberine group, and 15 of 215 (7.0%) in the combination group ($p = 0.16$) (Fig. 1). Reasons for withdrawal were similar among the three groups ($p = 0.16$ for the three groups, $p = 0.19$ for lost to follow-up, $p = 0.88$ for drop-out, $p = 1.0$ for protocol violations, and $p = 0.33$ for adverse events).

There were no significant differences in the ages of the women, duration of infertility, BMI, waist/hip circumference, presence of hirsutism, menstrual pattern, ultrasound features of polycystic ovaries, or baseline hormonal profile among the three groups (Table 1).

A total of 199 live births occurred, including 195 singletons and four sets of twins. The rate of live births was comparable between the letrozole and combination groups (36.3% vs. 34.4%, OR = 0.95, 95% CI = 0.73–1.23; *p* = 0.687), and both were significantly higher than the berberine group (22%) (OR = 1.68, 95% CI = 1.23–2.28, *p*= 0.001; OR = 1.57, 95% CI = 1.15–2.14, *p* = 0.004, respectively) (Table 2 and Fig. 2A). Birth weight among live births was comparable among the three groups. There were three twin live births in the combination group, one in the letrozole group, and none in the berberine group.

Independent of treatment, subjects with age less than 33 years had significantly higher rates of live births than did women whose age was greater than 33 years (*p* = 0.018, Fig. 2D). There was no significant difference in live birth rate stratified by BMIs (*p* = 0.782, Fig. 2B), menstrual cycle pattern (*p* = 0.689,Fig. 2C), hirsutism or duration of infertility (data not shown).

The rates of ovulation, conception, and pregnancy also were similar in the combination and letrozole groups, and these were significantly higher than those in the berberine group. As for fecundity among subjects who ovulated, the letrozole and combination groups were superior to the berberine group with regard to conception rates and/or pregnancy rates. The three groups had comparable rates of pregnancy loss after conception.

No serious adverse events occurred during the intervention in any of the three groups. Berberine was associated with a significantly higher incidence of

constipation and nausea while letrozole was associated with a significantly higher incidence of fatigue and hot flashes. There were no significant differences among the three groups with regard to rates of total cases of adverse events (37.0%, 48.0%, and 45.5% for the letrozole, berberine, and combination groups, respectively) or for cases of serious adverse events including ectopic pregnancies, pregnancy loss during the second trimester, or preterm labor (21.4%, 20.8%, and 23.5%, respectively) (Table 3).

During pregnancy, the most common complication was pregnancy-induced hypertension, followed by gestational diabetes, threatened abortion, and premature rupture of membranes. There were no significant differences among the three treatment groups for these events. Two major congenital anomalies were reported, but no autopsies were performed. One fetal abnormality in the letrozole group was found during ultrasound examination at gestational week 16, resulting in termination of the pregnancy. Another abnormality resulted in an infant death on day 31 after birth in the combination group, but the cause of death was unknown.

DISCUSSION

Our findings do not support the hypothesis that a combination of letrozole and berberine is superior to letrozole alone for achieving live birth in infertile women with PCOS. The rates of ovulation, conception, pregnancy, and live birth were comparable between the letrozole and combination groups. These endpoints

were significantly higher in both groups with letrozole than those in the berberine alone group. We found that the higher ovulation rate per cycle account for the superiority of this cumulative live birth rate for letrozole or combination treatment compared to berberine alone.

The use of insulin-sensitizing agents such as metformin in women with PCOS undergoing ovulation induction has been widely studied. A Cochrane review [15] showed that there was no evidence that metformin improved live birth rates, whether used alone or in combination with clomiphene. Therefore, the role of metformin in improving reproductive outcomes in women with PCOS appears to be limited. Berberine, an active ingredient from Chinese medicinal herbs, has multiple biological activities and pharmacological effects in several metabolic diseases such as type 2 diabetes mellitus, hyperlipidemia, and nonalcoholic fatty liver disease.[7,8,16] A systemic review and meta-analysis for berberine in the treatment of type 2 diabetes mellitus demonstrated comparable glycemic control with other oral hypoglycemic agents. However, berberine also showed an anti-dyslipidemic effect.[7] The relevant targets of berberine might link to the insulin pathway, adenosine monophosphate-activated protein kinase signaling, the gut environment, and hepatic lipid transportation. [16]

In a study [12] comparing berberine and metformin, 89 Chinese women with PCOS and insulin resistance were randomized into one of three treatment groups: berberine and cyproterone acetate, metformin and cyproterone acetate, or

placebo and cyproterone acetate for three months. Berberine showed similar restoration of insulin sensitivity and reduction of hyperandrogenemia when compared with metformin. Berberine also appeared to have a greater effect on changes in body composition and dyslipidemia in PCOS patients.

Our results showed that ovulation and live birth rates for a combination of letrozole and berberine were comparable to letrozole alone. This implied that improvement in the metabolic profile by berberine did not affect the ovulation or live birth rates achieved with letrozole. We did not measure insulin sensitivity before and after exposure to the study medication in the present study. We did not find a significant difference in live birth rate stratified by BMI, hirsutism score, menstrual patterns, or duration of infertility. Our results contrasted with those from a study conducted in the Netherlands,[17] where BMI, age, free androgen index, and cycle history were all associated with live birth in women with PCOS receiving clomiphene. In the population-based Northern Finland Birth Cohort 1966 study,[18] previous oligo-amenorrhea and/or hirsutism and obesity were both found to be independently associated with decreased fecundity. These differences might be related to the distinct phenotypes of PCOS in which Chinese women are

more likely to have irregular menstrual cycles and less hyperandrogenism than Caucasians women. [1,2]

Letrozole has a shorter half-life than clomiphene leading to a shorter exposure during implantation and early fetal development, and, therefore, a low theoretical risk of congenital anomalies in the offspring.[6,19] There has been significant concern for congenital anomalies since the introduction of letrozole for use in inducing ovulation. In this trial, we reported one fetal abnormality in the letrozole group, one neonatal death in the combination group, and no abnormalities in the berberine group. However, our study was underpowered to detect a significant difference for rare but potentially serious adverse events. In the PCOS II trial,[6] Legro et al found four major congenital anomalies in the letrozole group and one in the clomiphene group. There was no pattern to the four major anomalies with letrozole, implying they were random events. The rate of congenital anomalies in the present study is below what has been reported in other studies. [20]

In the present study, the use of berberine alone achieved a 36% ovulation rate, similar to metformin, and a 22% live birth rate similar to clomiphene after 6 months of use.[5] To the best of our knowledge, these is the first study to show the

effect of berberine alone on the ovulation and live birth rates in women with PCOS. However, we cannot make conclusions regarding efficacy because berberine was not compared to a placebo or to no treatment. Studies comparing clomiphene and berberine for ovulation induction have not yet been performed.

One of the limitations of this study was its early termination because of the expiration of the study drug (berberine and its placebo). However, the absolute difference (12–14%) in live-birth rates for the original two primary comparisons of letrozole to berberine in this trial was greater than the projected 8%; hence, the unexpected reduction to 644 participants still provided adequate power (≥90%) for the study. Other limitations included not all subjects having pelvic sonograms to assess for polycystic ovaries and the inclusion of subjects with regular cycles. The major strength of this study is that it was a large multi-center double blind trial with close monitoring of adverse events and serious adverse events and tracking of live birth in line with our recent Harbin consensus. [21]

In summary, a combination of letrozole and berberine was not superior to letrozole alone in terms of live birth and ovulation, but both were superior to berberine alone in infertile women with PCOS.

Contributors: X-KW and EHYN contributed to the study conception and design. X-KW, and L-HH obtained funding. X-KW, L-HH, Y-QG, S-MD, YX, J-FZ, H-YX, W-LL, R-NL, Z-YT, J-YF, H-XM, X-GS, F-JH, M-eA, P-LL, C-FD, X-BL, and X-JS contributed to the acquisition of the data. X-KW, X-YM, RMS, ES-V, EHYN, and JPL analyzed and interpreted the data. X-KW and EHYN drafted the report, and all authors contributed to revision of the report.

Declaration of interests: All authors declare financial support to their institutions for the submitted work from the National Public Welfare Projects for Chinese Medicine (200807021) of China and The "11th–Five" Supportive Program for Science and Technology of China (2007BAI20B015). X-KW has received other research grants for this trial from the Heilongjiang Province Foundation for Outstanding Youths (JC200804) and the Intervention for Polycystic Ovary Syndrome Based on Traditional Chinese Medicine Theory—'TianGui Shi Xu' (2011TD006). ES-V has received a travel grant from Longjiang Scholarship from Heilongjiang Province. X-KW, EHYN, J-PL, ES-V, Zhaoling You, and Chengzong Xiao are members of the Steering Committee and received conference travel expenses and lecture fees from National Key Discipline of China. Drs. Sulun Sun, Weiliang Weng, Yanming Xie, Yixun Liu, Yongyan Wang are members of the Scientific

Advisory Board and have received payment from National Key Discipline and TCM Trial Base of China.

Acknowledgments: This trial was funded by the National Public Welfare Projects for Chinese Medicine (200807021) of China, the "11th –Five" Supportive Program for Science and Technology of China (2007BAI20B015), the Heilongjiang Province Foundation for Outstanding Youths (JC200804), and the Intervention for Polycystic Ovary Syndrome Based on Traditional Chinese Medicine Theory—'TianGui Shi Xu' (2011TD006). We thank the participants and the staff of participating centers for their contribution to the trial.

References

1. Li R, Zhang Q, Yang D, et al. Prevalence of polycystic ovary syndrome in women in China: a large community-based study. *Hum Reprod* 2013; **28:** 2562-9.
2. Li R, Qiao J, Yang D, et al. Epidemiology of hirsutism among women of reproductive age in the community: a simplified scoring system. *Eur J Obstet Gynecol Reprod Biol* 2012; **163**: 165-9.

3. Li R, Yu G, Yang D, et al. Prevalence and predictors of metabolic abnormalities in Chinese women with PCOS: a cross- sectional study. *BMC Endocr Disord* 2014; **14:** 76.

4. Wu XK. Polycystic ovary syndrome. In: Hou LH, Wang YT and Wu XK, eds. Today Gynecology in Traditional Chinese Medicine. Beijing: People's Medical Publishing House, 2010: 118-145.

5. Legro RS, Barnhart HX, Schlaff WD, et al. Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome. *N Engl J Med* 2007; **356:** 551–66

6. Legro RS, Brzyski RG, Diamond MP, et al. Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. *N Engl J Med* 2014; **371**: 119-29

7. Dong H, Wang N, Zhao L, Lu F. Berberine in the treatment of type 2 diabetes mellitus: a systemic review and meta-analysis. *Evid Based Complement Alternat Med* 2012; **2012:** 591654.

8. Dong H, Zhao Y, Zhao L, Lu F. The effects of berberine on blood lipids: a systemic review and meta-analysis of randomized controlled trials. *Planta Med* 2013; **79:** 437-46.

9. Xu H. Analysis of market outlook in 2014' Cortex Phellodendri high-quality stocks, March 20, 2014. http:// www. kmzyw.com.cn /pages/ channel_467/ 1140320 467.13952937140 00.2579.shtml

10. Zhao L, Li W, Han F, et al. Berberine reduces insulin resistance induced by dexamethasone in theca cells in vitro. *Fertil Steril* 2011; **95**: 461-3.

11. Li Y, Ma H, Zhang Y, et al. Effect of berberine on insulin resistance in women with polycystic ovary syndrome: study protocol for a randomized multicenter controlled trial. *Trials* 2013; **14:** 226.

12. Wei W, Zhao H, Wang A, et al. A clinical study on the short-term effect of berberine in comparison to metformin on the metabolic characteristics of women with polycystic ovary syndrome. *Eur J Endocrinol* 2012; **166:** 99-105.

13. Li Y, Kuang H, Shen W, et al. Letrozole, berberine, or their combination for anovulatory infertility in women with polycystic ovary syndrome: study design of a double-blind randomised controlled trial. *BMJ Open* 2013; **3:** e003934.

14. Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril* 2004; **81:** 19-25.

15. Tang T, Lord JM, Norman RJ, et al. Insulin-sensitising drugs(metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. *Cochrane Database Syst Rev* 2012; **5:** CD003053..

16. Liu Y, Zhang L, Song H, Ji G. Update on berberine in nonalcoholic Fatty liver disease. *Evid Based Complement Alternat Med* 2013; **2013:** 308134.

17. Imani B, Eijkemans MJ, te Velde ER, Habbema JD, Fauser BC. A nomogram to predict the probability of live birth after clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. *Fertil Steril* 2002; **77:** 91-7

18. Koivunen R, Pouta A, Franks S, et al. Fecundability and spontaneous abortions in women with self-reported oligo-amenorrhea and/or hirsutism: Northern Finland Birth Cohort 1966 Study. *Hum Reprod* 2008; **23:** 2134-9.

19. Franik S, Kremer JA, Nelen WL, Farquhar C. Aromatase inhibitors for subfertile women with polycystic ovary syndrome. *Cochrane Database Syst Rev* 2014; **2:** CD010287.

20. Davies MJ, Moore VM, Willson KJ, et al. Reproductive technologies and the risk of birth defects. *N Engl J Med* 2012; **366:** 1803-13.

21. Harbin Consensus Conference Workshop Group. Improving the Reporting of Clinical Trials of Infertility Treatments (IMPRINT): modifying the CONSORT statement. *Hum Reprod* 2014; **29:** 2075-82.

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	4
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	6
	2b	Specific objectives or hypotheses	7
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	8
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	no
Participants	4a	Eligibility criteria for participants	8
	4b	Settings and locations where the data were collected	9
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	10
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	11
	6b	Any changes to trial outcomes after the trial commenced, with reasons	no
Sample size	7a	How sample size was determined	12
	7b	When applicable, explanation of any interim analyses and stopping guidelines	no
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	9
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	9
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	9
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	9
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	9

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	12
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	12
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	13 and Figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	13
Recruitment	14a	Dates defining the periods of recruitment and follow-up	13
	14b	Why the trial ended or was stopped	13
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	14, Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	14 and Table 1
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	14
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	14
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	14
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	15
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	19
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	20
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	20
Other information			
Registration	23	Registration number and name of trial registry	8, China clinical trial registry and clinicaltrials.gov
Protocol	24	Where the full trial protocol can be accessed, if available	7,publication
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	13,22

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

Figure 1: Enrollment and outcomes

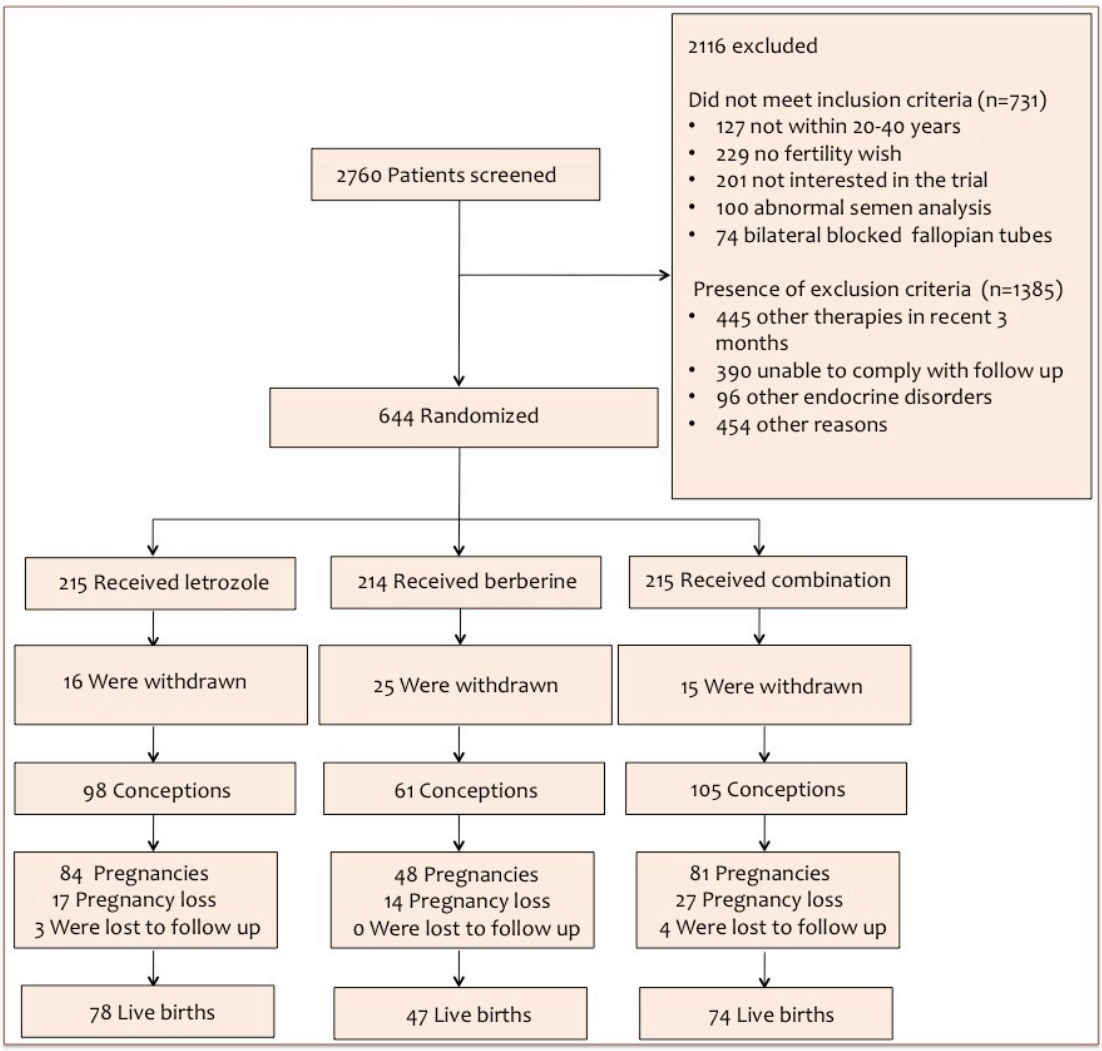

Figure 2: Kaplan–Meier curves for live births according to treatment group (Panel A), body-mass Index (BMI) (Panel B), menstrual pattern (Panel C) and age (Panel D).

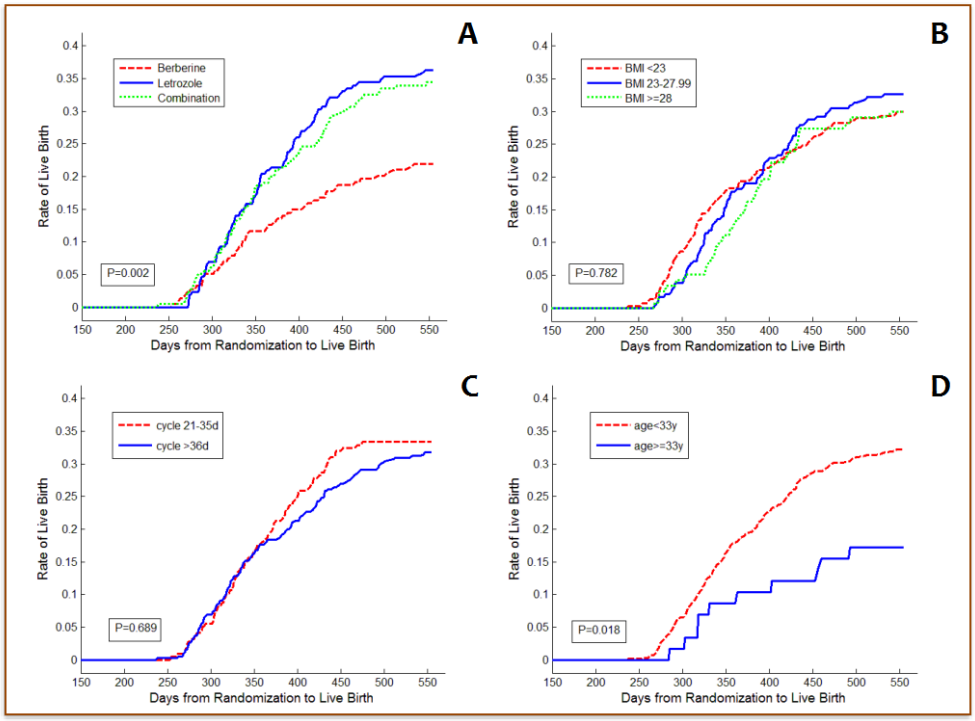

Table 1. Baseline Characteristics

Biometric features	Letrozole Group (N = 215)	Berberine Group (N = 214)	Combination Group (N = 215)
Age of women (yr)[a]	27.8 ± 3.6*	27.8 ± 3.7	27.8 ± 3.6
Body mass index (kg/m^2)[a]	24.8 ± 4.5	24.5 ± 4.1	25.1 ± 5.0
Waist circumference (cm)[a]	83.5 ± 10.9	82.7 ± 11.8	83.1 ± 11.8
Hip circumference (cm)[a]	98.1 ± 11.4	97.7 ± 11.8	97.9 ± 10.9
WHR[a]	0.85 ± 0.08	0.85 ± 0.08	0.85 ± 0.07
Hirsutism (Ferriman-Gallwey ≥5)[b]	78/202 (38.6)	76/201 (37.8)	60/209 (28.7)
Menstrual pattern[b,c]			
Oligomenorrhea	126/201 (62.7)	116/193 (60.1)	121/198 (61.1)
Regular menses	75/201 (37.3)	77/193 (39.9)	77/198 (38.9)
Duration of infertility (months)[a]	32.7 ± 24.0	28.5 ± 21.6	29.8 ± 21.3
Previous infertility therapy[b]	178 (82.8)	180 (84.1)	185 (86.0)
Traditional Chinese Medicine	107/178 (60.1)	112/180 (62.2)	110/185 (59.5)
Ovulation drugs	121/178 (68.0)	109/180 (60.6)	107/185 (57.8)
Assisted Reproductive Technology	9/178 (5.1)	11/180 (6.1)	12/185 (6.5)
Other therapies	8/178 (4.5)	8/180 (4.4)	6/185 (3.2)
Previous pregnancy[b]			
Conception	67 (31.2)	62 (29.0)	80 (37.2)
Live birth	9 (4.2)	7 (3.3)	7 (3.3)
Miscarriage	13 (6.1)	16 (7.5)	26 (12.1)
Termination of pregnancy	48 (22.3)	43 (20.1)	64 (29.8)
Ultrasonographic findings			
Polycystic ovary morphology — no. (%)[#]	104/151 (68.9)	113/153 (73.9)	98/155 (63.2)
Ovarian volume (cm^3)[a]			
Left ovary	10.3 ± 6.8	9.5 ± 7.9	11.0 ± 6.8
Right ovary	11.0 ± 6.3	10.2 ± 6.4	11.6 ± 7.4
Fasting serum levels[a]			
Testosterone (nmol/L)	1.6 ± 0.8	1.4 ± 0.7	1.4 ± 0.7
Estradiol (pmol/L)	213.9 ± 206.3	244.0 ± 252.4	228.8 ± 233.0
Free androgen index[d]	5.0 ± 4.8	5.3 ± 6.3	5.1 ± 5.8
FSH (mIU/L)	5.7 ± 2.1	5.3 ± 1.8	5.5 ± 1.9
LH (mIU/L)	10.9 ± 6.7	10.0 ± 6.5	10.4 ± 6.1
LH/FSH	2.0 ± 1.1	1.9 ± 1.2	1.9 ± 1.1
SHBG (nmol/L)	48.1 ± 31.1	47.6 ± 32.9	42.6 ± 28.3
Prolactin (mIU/L)	318.0 ± 160.9	311.3 ± 168.4	296.7 ± 155.8

[a]Data are given in means ± standard deviation

[b]Data are given in number (%)

[c]Missing information in some subjects

[d]The free androgen index was calculated according to the following formula: (total testosterone [nanomoles per liter] /SHBG [nanomoles per liter]) × 100.

Table 2. Live birth and other fecundity outcomes

Outcomes number (%)	Letrozole Group (N = 215)	Berberine Group (N = 214)	Combination Group (N = 215)	Odds Ratio Between Combination and Letrozole	*p*-value	Odds Ratio Between Combination and Berberine	*p*-value	Odds Ratio Between Letrozole and Berberine	*p*-value
		no. /total no. (%)		*OR (95% CI)*		*OR (95% CI)*		*OR (95% CI)*	
Primary outcomes									
Live birth	78/215 (36.3)	47/214 (22.0)	74/215 (34.4)	0.95 (0.73–1.23)	0.687	1.57 (1.15–2.14)	0.004	1.68 (1.23–2.28)	0.001
Singleton live birth	77/78 (98.7)	47/47 (100)	71/74 (95.9)	0.97 (0.92–1.03)	0.357	0.96 (0.92–1.01)	0.28	0.99 (0.96–1.01)	1.000
Twin live birth	1/78 (0.1)	0/47 (0)	3/74 (0.4)	3.16 (0.34–29.72)	0.357	4.48 (0.24–84.82)	0.28	1.82 (0.08–43.85)	1.000
Birth weight (gram)	3463 ± 575	3542 ± 399	3484 ± 504	21.20 (−177.16–219.57)	0.845	−58.23 (−248.84–132.38)	0.246	−79.43 (−282.07–123.20)	0.216
Secondary outcomes									
Ovulation	473/796 (59.4)	302/831 (36.3)	486/797 (61.0)	1.03 (0.95–1.11)	0.526	1.68 (1.51–1.87)	<0.0001	1.64 (1.47–1.82)	<0.0001
Conception	98/215 (45.6)	61/214 (28.5)	105/215 (48.8)	1.07 (0.88–1.31)	0.499	1.71 (1.33–2.21)	<0.0001	1.60 (1.24–2.07)	0.0003
Immediate loss to follow up	3/98（3.1）	0	4/105（3.8）	1.24 (0.29 - 5.42)	1.000	-	-	-	-
Pregnancy	84/215 (39.1)	48/214 (22.4)	81/215 (37.7)	0.96 (0.76–1.23)	0.766	1.68 (1.24–2.27)	0.0006	1.741 (1.29–2.35)	0.0002
Singleton	83/84 (98.8)	48/48 (100.0)	78/81 (96.3)	0.97 (0.93–1.02)	0.361	0.96 (0.92–1.01)	0.294	0.990.97–1.01)	1.000
Twins	1/84 (1.2)	0	3/81 (3.7)	3.11 (0.33–29.30)	0.361	-	-	-	-
Pregnancy loss	17/98 (17.4)	14/61 (23.0)	27/105 (25.7)	1.48 (0.86–2.55)	0.148	1.12 (0.64–1.97)	0.691	0.76 (0.40–1.42)	0.386
In the first trimester	13/98 (13.2)	14/61 (23.0)	24/105 (22.9)	1.72 (0.93–3.19)	0.077	0.996 (0.56–1.78)	0.989	0.58 (0.29–1.15)	0.114
In the second trimester	4/98 (4.1)	0	3/105 (2.9)	0.70 (0.16–3.05)	0.714	-	-	-	-
Fecundity among ovulated cycles									
Conception	98/473 (20.7)	61/302 (20.2)	105/486 (21.6)	1.05 (0.77-1.44)	0.737	1.09 (0.76-1.55)	0.638	1.03 (0.72-1.48)	0.861
Pregnancy	84/473 (17.5)	48/302 (15.9)	81/486 (16.1)	0.94 (0.71-1.24)	0.654	1.05 (0.76- 1.45)	0.776	1.13 (0.76-1.66)	0.549
Live birth	78/473 (16.3)	47/302 (15.6)	74/486 (14.6)	0.92 (0.69-1.24)	0.592	0.98 (0.70-1.37)	0.899	1.06 (0.71-1.57)	0.791
Fecundity among subjects who ovulated									
Conception	98/188 (52.1)	61/147 (41.5)	105/184 (57.1)	1.09 (0.91–1.32)	0.339	1.38 (1.09–1.73)	0.005	1.26 (0.99–1.59)	0.053
Pregnancy	84/188 (44.2)	48/147 (32.7)	81/184 (42.4)	0.99 (0.78–1.24)	0.898	1.35 (1.02–1.79)	0.035	1.37 (1.03–1.81)	0.025
Live birth	78/188 (41.0)	47/147 (32.0)	74/184 (38.6)	0.97 (0.76–1.24)	0.803	1.26 (0.94–1.69)	0.122	1.30 (0.97–1.74)	0.074

Ovulation was defined as a serum progesterone level according to the standard of the local lab (minimum value of luteal phase) or more than 5 ng/ml. Conception was defined as any positive serum level of human chorionic gonadotropin. Pregnancy was defined as an intrauterine pregnancy sac with fetal heart motion as determined by ultrasonography. Live birth was defined as the delivery of a viable infant.; [a] **means ± standard deviation**

Table 3: Adverse events during study medication, pregnancy, and neonatal period			
Adverse Events	Letrozole Group (N = 215)	Berberine Group (N = 214)	Combination Group (N = 215)
Serious adverse event from study medication	0/215 (0)	0/214 (0)	0/215 (0)
Other adverse events			
Constipation	10/215 (4.7)	26/214 (12.1)*	12/215 (5.6)
Nausea	13/215 (6.0)	34/214 (15.9)*	26/215 (12.1)
Diarrhea	13/215 (6.0)	5/214 (2.3)	5/215 (2.3)
Hot flashes	21/215 (9.8)	6/214 (2.8)*	19/215 (8.8)
Fatigue	17/215 (7.9)	5/214 (2.3)*	18/215 (8.4)
Serious events during pregnancy			
First trimester			
Ectopic pregnancy	2/84 (2.4)	2/48 (4.2)	2/81 (2.5)
Second and third trimesters			
Pregnancy loss after 12 weeks	4/84 (4.8)	0/48 (0)	3/81 (3.7)
Pre-eclampsia	4/84 (4.8)	6/48 (12.5)	7/81 (8.6)
Gestational diabetes	3/84 (3.6)	3/48 (6.3)	7/81 (8.6)
Preterm labor	2/84 (2.4)	0/48 (0)	2/81 (2.5)
Premature rupture of membranes	5/84 (6.0)	1/48 (2.1)	2/81 (2.5)
Serious event in fetus and infant			
Fetal abnormality	1/84 (1.2)	0/48 (0)	0/48 (0)
Neonatal death	0/78 (0)	0/47 (0)	1/74 (1.4)

Data given in number (%)
*$p < 0.05$ vs. other two groups.