Study Protocol and Statistical Analysis Plan

Supplement to: Li-Li Xu, Chun Wang, Chun-Mei Deng, et al. Esketamine for supplemental analgesia during elective cesarean delivery: a randomized clinical trial

This supplement contains the following items:

1. Study protocol.
2. Statistical analysis plan.

Study protocol

Esketamine for supplemental analgesia during elective cesarean delivery: a randomized clinical trial

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Department of Anesthesiology, Jiaxing Maternity and Child Health Care Hospital.

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1. Background

Epidural anesthesia is widely used for cesarean delivery, especially in China. However, due to incomplete block of the splanchnic nerve, parturients often complain discomfort or pain during exploration of the uterus, pulling peritoneum, and fetal delivery. The referred pain brings painful memories and mental stress to parturients, and also affect the conduct of surgical procedure. In some cases, change to general anesthesia is required. Therefore, supplemental analgesics during cesarean delivery under epidural anesthesia is sometimes required and necessary.[1]

Esketamine, a dextrorotatory isomer of ketamine, is two times more potent than ketamine in hypnotic and analgesic effect, with less psychiatric adverse reactions.[2,3] In a pre-clinical study, Strümper et al.[4] verified that esketamine has similar effects on uterine perfusion, and has limited effects on maternal/fetal hemodynamics and respiration when compared with racemic ketamine; it thus might be an appropriate analgesic in the obstetric setting. In clinical studies, Unlugenc et al.[5] found that adding esketamine (0.05 mg/kg) to intrathecal plain bupivacaine (10 mg) for spinal anesthesia resulted in rapid onset of sensory and motor block and enhanced segmental spread of spinal block in patients undergoing cesarean delivery. Suppa et al.[6] reported that, for women after cesarean delivery, preventive esketamine administration (0.5 mg/kg intramuscular injection at 10 minutes after childbirth, followed by a 2 μg/kg/min intravenous infusion for 12 h) has analgesic effects and was safe.

However, the efficacy and safety of intravenous esketamine before childbirth are still unclear. We therefore try to investigate the sedative and analgesic effects of intravenous esketamine before childbirth during cesarean delivery under epidural anesthesia. We will also explore the placental transfer of intravenous esketamine as well as the effects on maternal and fetal outcomes.

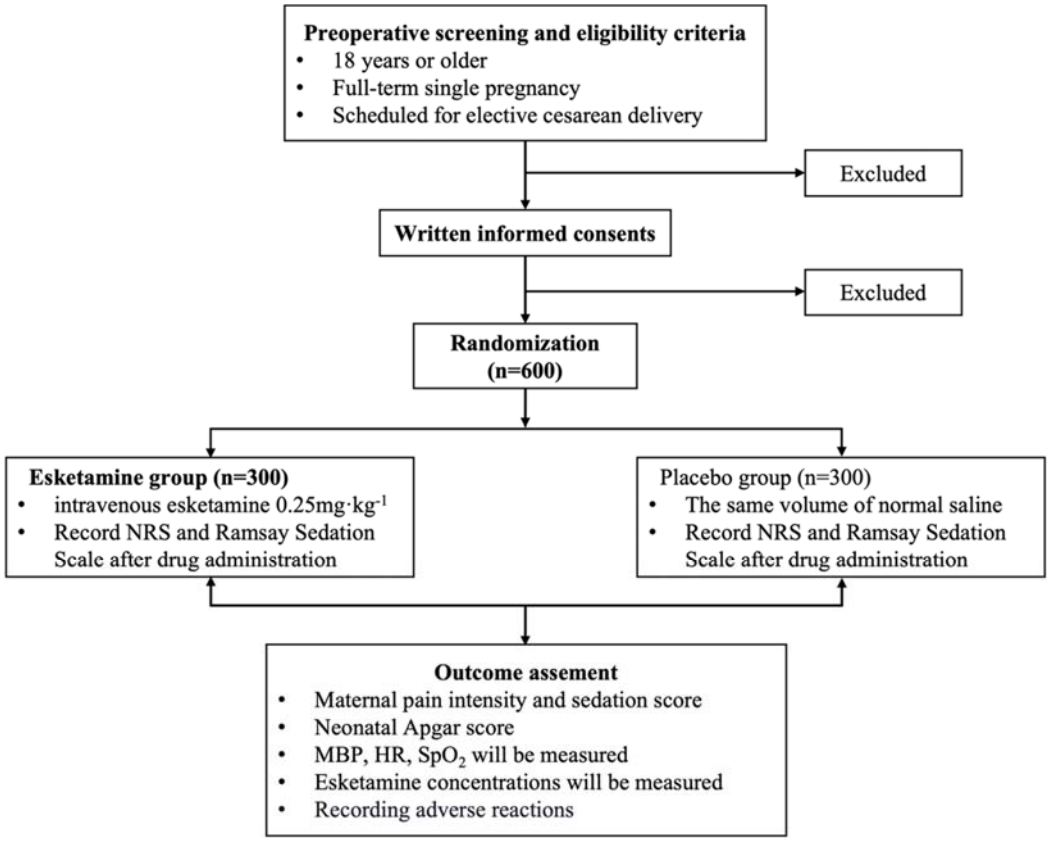
2. Purpose of the study

The purpose of the study is to investigate the sedative and analgesic effects of intravenous esketamine before childbirth during cesarean delivery under epidural anesthesia.

3. Study design

3.1 Type of the study

This is a muti-center, randomized, double blind trial with two parallel arms. The flow chart of the study is shown in Figure 1.



3.2 Sample size calculation

Based on a pilot study, we suppose that the mean difference in NRS pain score immediately after fetal delivery (10 min after administering esketamine) would be 0.3 with standard deviations (SD) of 0.85 and 1.23, respectively, between the two groups. With alpha set at 5% and power at 90%, 263 patients will be needed in each group. Considering a drop-out rate of about 10%, we plan to enroll 300 patients in each group. The sample size was estimated with the PASS 15.0 software.

3.3 Participating centers

3.3.1 This multi-center trial will be conducted in five hospitals. The five participating centers include Women's Hospital of Zhejiang University School of Medicine, Jiangxi Maternal and Child Health Hospital, Hangzhou Women's Hospital, Jiaxin Maternity and Child Health Care Hospital, and Xiaoshan Hospital.

3.3.2 The study is coordinated and monitored by the Department of Anesthesiology of Women's Hospital Zhejiang University School of Medicine; the Department of Anesthesiology and Critical Care Medicine of Peking University First Hospital is responsible for data management and data analysis.

4. Study participants

Potential participants will be screened before surgery by the qualified investigators.

4.1 Inclusion criteria

4.1.1 Women aged 18 years or older.

4.1.2 Full-term single pregnancy.

4.1.3 Scheduling for elective caesarean delivery.

4.1.4 Planning for epidural anesthesia.

4.1.5 Agree to participate, and give signed written informed consents

4.2 Exclusion criteria

Patients will be excluded if they meet any of the following criteria:

4.2.1 Body mass index (BMI) ≥27 kg/m^2.

4.2.2 Previous mental illness; central nervous system disease; liver disease, abnormal kidney function; abnormal heart and lung function; diabetes; American Society of Anesthesiologists (ASA) classification III or above.

4.2.3 Severe obstetric complications, such as preeclampsia and eclampsia, pregnancy-induced hypertension, placenta previa, or placental abruption.

4.2.4 Stillbirth, neonatal malformation.

4.2.5 Contraindications to epidural anesthesia, including abnormal coagulation function, taking anticoagulant therapy, severe hypovolemia, or hemodynamic instability.

4.3 Criteria of drop-out

4.3.1 Study intervention is not administered (change to general anesthesia) or protocol deviation occurs.

4.3.2 Intervention interrupted by the investigators/anesthesiologists (due to adverse events).

4.3.3 Use of a prohibited drugs.

Details of the above situations should be recorded and corrected when possible. The cases will be followed up according to the study protocol and included in the intention-to-treat analysis.

4.3.4 Withdraw consent after intervention started.

The situation should be recorded. The primary therapeutic effects recorded in the last time will be regarded as the final assessment results. The cases will be included in the intention-to-treat analysis.

4.4 Criteria of elimination

Enrolled patients will be excluded if they meet any of the following criteria:

4.4.1 Withdraw consents before intervention.

4.4.2 Surgery cancelled.

4.4.3 No assessment result of the primary outcomes.

The causes of elimination should be explained. The case will be excluded from the intention-to-treat analysis. The case report forms will be preserved for reference.

4.5 Criteria of study interruption

Study will be interrupted in the following situations:

4.5.1 Severe safety problem occurred during the study.

4.5.2 Serious mistake found in the protocol.

4.5.3 Fund or management problem of the investigators.

4.5.4 Study cancelled by the administrative authority.

Study interruption may be transient or permanent. All recorded case report forms will be preserved for reference in case of study interruption.

5. Randomization and Masking

5.1 Randomization

5.1.1 Random allocations will be generated using the SPSS 22.0 (IBM Corp, Armonk, NY, USA) in a 1:1 ratio. Assignments will be concealed in sequentially numbered opaque envelops.

5.1.2 For each participating center, a study coordinator will be designated to distribute the randomization result to the anesthesiologists according to the sequence of recruited patients, and to coordinate between investigators.

5.1.3 For each recruited patient, an anesthesiologist will be designated for anesthesia and data collection according to the result of randomization.

5.1.4 Study intervention (use esketamine or normal saline) will be provided according to the randomization results by anesthesiologists who do not participate in the outcome assessments.

5.2 Masking

5.2.1 All patients, anesthesiologists, other health care team members, and investigators who are responsible for data collection and follow-ups will be blinded to group allocation.

5.2.2 Investigators who are responsible for postoperative follow-up and outcome assessments are not involved in anesthesia and perioperative management and have no knowledge of study group assignment.

5.2.3 Statistical analysis will be performed by an independent statistician.

6. Intervention protocol

6.1 Anesthesia management

6.1.1 No premedication will be administered.

6.1.2 Intraoperative monitoring includes electrocardiogram (ECG) and oxygen saturation (SpO2) and non-invasive blood pressure.

6.1.3 All patients will undergo caesarean section under epidural anesthesia and epidural puncture will be performed in the left lateral decubitus position. A 16-G Tuohy needle will be used for puncture in the L2-3 lumbar intervertebral space. After confirming the epidural space by the loss-of-resistance-to-air method, a catheter will be inserted 3-4 cm in a cephalad direction.

6.1.4 A test dose of 1.5% lidocaine 5 ml will be given. After a 5-minute observation, 0.75% ropivacaine 10 ml will be given, followed by an infusion of 0.75% ropivacaine at 3-5 ml/h.

6.1.5 Oxygen will be provided at 5 $L \cdot min^{-1}$ via a face mask.

6.1.6 Study drugs will be administered when the targeted upper sensory block level was achieved (about 2 minutes before incision). Specifically, esketamine 0.25 $mg \cdot kg^{-1}$ will be administrated intravenously over 1 min for patients in the esketamine group; the same volume of normal saline will be injected for patients in the control group.

6.1.7 At the end of surgery, a patient-controlled epidural analgesia will be attached for postoperative analgesia, which is established with 0.2% ropivacaine and programmed to deliver a continuous infusion at 2 ml/h.

6.2 Remedial measures

6.2.1 If the epidural anesthesia is failed, spinal anesthesia or general anesthesia will be performed.

6.2.2 If the anesthesia level of the block is not sufficient, we will continue to add epidural drugs in fractions until the level of the block reaches at least T6.

6.2.3 The above measures will be recorded.

7. Data collection

7.1 Baseline data

7.1.1 Demographic parameters, pregestational comorbidity, number of gravidity and parturitions, duration of gestation, and ASA classification.

7.1.2 Pain intensity at rest will be assessed with the numeric rating scale (NRS; an 11-point scale where 0=no pain and 10=the worst pain) before anesthesia.

7.1.3 Maternal sedation level will be assessed with the Ramsay Sedation Scale (1=restlessness; 2=completely awake, quiet and cooperative; 3= drowsiness but responding to verbal commands;

4=light asleep but responding to touch or pain; 5= asleep but slowly responding to touch or pain; 6=deeply asleep and does not respond) before anesthesia.

7.2 Intraoperative data

7.2.1 Durations of anesthesia and surgery, fluid balance (including fluid infusion, estimated blood loss, and urine output), use of vasopressors.

7.2.2 Time intervals from study drug administration to neonatal delivery and from uterine incision to neonatal delivery.

7.2.3 Pain intensity at rest will be assessed with the NRS at the following timepoints: after anesthesia (before study drug administration), surgical incision (2 min after study drug administration), 5 min after study drug administration, immediately after fetal delivery (about 10 min after esketamine), and end of surgery.

7.2.4 Maternal sedation level will be assessed with the Ramsay Sedation Scale at the following timepoints: after anesthesia (before study drug administration), surgical incision (2 min after study drug administration), 5 min after study drug administration, immediately after fetal delivery (about 10 min after esketamine), and end of surgery.

7.2.5 Neonatal data including sex, birth weight, and Apgar scores at 1 and 5 minutes after delivery.

7.2.6 Immediately after childbirth, blood samples will be collected from artery of mothers and from umbilical artery and vein of fetuses in the esketamine group (in selected participants) and stored in a -20°C refrigerator. Esketamine concentrations were measured with the reverse phase high-performance liquid chromatography (RP-HPLC).

7.3 Postoperative data

7.3.1 Routine anesthetic follow-up after caesarean section.

7.3.2 Maternal psychiatric symptoms that may be caused by experimental drugs.

7.3.3 Pain intensity at rest will be assessed with the NRS at the following timepoints: 6 h after surgery, and 12 h after the surgery.

7.3.4 Maternal sedation level will be assessed with the Ramsay Sedation Scale at the following timepoints: 6 h after surgery, and 12 h after the surgery.

7.3.5 Admission to neonatal ward or neonatal intensive care unit.

7.3.6 Length of hospital stay after surgery.

8. Outcomes

8.1 Primary outcome

Our co-primary outcomes include maternal pain intensity and sedation score immediately after fetal delivery (about 10 min after esketamine). Pain intensity will be assessed with the NRS.

Maternal sedation level will be assessed with the Ramsay Sedation Scale.

8.2 Secondary outcomes

8.2.1 Maternal pain intensity as assessed with the NRS at the following timepoints: surgical incision (2 min after study drug administration), 5 min after study drug administration, end of surgery, 6 h after surgery, and 12 h after the surgery.

8.2.2 Maternal sedation level as assessed with the Ramsay Sedation Scale at the following timepoints: surgical incision (2 min after study drug administration), 5 min after study drug administration, end of surgery, 6 h after surgery, and 12 h after the surgery.

8.2.3 Neonatal Apgar score assessed at 1 and 5 minutes after birth.

8.2.4 Postnatal umbilical vein blood gas pH value.

8.3 Other outcomes

8.3.1 Mean blood pressure (MBP), heart rate (HR), and pulse oxygen saturation (SpO2) measured before anesthesia, immediately after anesthesia, surgical incision (2 min after study drug administration), 5 min after study drug administration, immediately after fetal delivery (10 min after study drug administration), end of surgery, and 1 h after surgery.

8.3.2 The requirement of neonatal ward admission and length of hospital stay.

8.3.3 Plasma concentrations of esketamine in maternal blood, neonatal umbilical venous blood and umbilical arterial blood after birth.

9. Adverse events

9.1 Definition

An adverse event indicates any unpredictable, unfavorable medical event that is associated with any medical intervention and occurs during the study period. It can be related to the study intervention or otherwise. It can manifest as any uncomfortable signs (including abnormal laboratory findings), symptoms, or transient morbidity.

9.2 Predicted adverse events in this study

9.2.1 Hypotension was defined as systolic pressure decrease <20% of baseline.

9.2.2 Hypertension was defined as systolic pressure increase >20% of baseline.

9.2.3 Bradycardia was defined as heart rate <60 bpm.

9.2.4 Tachycardia was defined as heart rate >100 bpm.

9.2.5 Desaturation was defined as oxygen saturation <90%.

9.2.6 Nausea and vomiting.

9.2.7 Neurological and mental signs and symptoms (such as lethargy, diplopia, dizziness, headache, nightmare, hallucination, anxiety, and irritability) and nystagmus.

9.2.8 Adverse events will be managed according to routine practice.

10. Severe adverse events

10.1 Definition

A severe adverse event indicates any unpredictable medical events that lead to death, threat of life, prolonged length of hospital stays, persistent disability or dysfunction, or other severe event.

10.2 Management

In case of any severe adverse events, the study intervention will be stopped and treatment will be initiated immediately.

10.3 Record and report

10.3.1 In case of any severe adverse event, apart from active treatment and record as above, the principal investigator and the Ethics Committee will be informed within 24 hours in written report.

10.3.2 In case of study intervention related death, immediately stop the clinical trial, report the event to the Ethics Committee as soon as possible, record in detail and carefully preserve the related documents.

10.3.3 Any severe adverse event must be followed up until it is completely resolved or when therapy is ended.

11. The rule of unmasking

11.1 After the follow-up of all cases have been completed, the data of case report forms have been checked as correct, and the data entry have been finished, a database inspection report will be written by the data manager.

11.2 After the database is locked, unmasking will be conducted. And the database will be sent to the statisticians for statistical analysis.

12. Data management

12.1 The investigators should record data timely, completely and correctly according to the original observations and assessments.

12.2 The completed case report forms, after signed by the supervisors, will be sent to a clinical data custodian.

12.3 After the data in the case report forms have been input and checked, the case report forms will be stored in sequence order.

12.4 Data management will be inspected by Clinical Research Institute of Women's Hospital School of Medicine Zhejiang University.

13. Statistical analysis

Statistical analyses will be performed using IBM SPSS for Windows version 22.0 (IBM Corp, Armonk, NY, USA) and GraphPad Prism version 5.0 (GraphPad Software Inc, San Diego, CA, USA).

13.1 General principles

13.1.1 The primary and secondary outcomes will be analyzed in an intention-to-treat population, i.e., all patients are analyzed in the group to which they are randomized. Also, we will do per-protocol analysis for the primary endpoints after excluding patients with major protocol deviation.

13.1.2 For continuous variables, the Kolmogorov-Smirnov test will be applied to evaluate the distribution. Variables with normal distribution were presented as mean ± standard deviation (SD). Variables with non-normal distribution will be presented as median and interquartile range (IQR).

13.1.3 Categorical variables will be presented as number of cases (%).

13.1.4 For the primary outcomes, $p<0.025$ (0.05/2) will be considered statistically significant. For other outcomes, two-tailed tests will be used and $p<0.05$ will be considered to be statistically significant unless otherwise indicated after Bonferroni correction.

13.2 Patient recruitment and drop-out status

The status of patient recruitment and drop-out will be summarized and listed. Comparison of the overall elimination/drop-out rate between the two groups will be performed with Chi-Square test.

13.3 Demographics and baseline characteristics

13.3.1 Demographics and baseline data will be presented.

13.3.2 For between-group differences, numeric variables will be analyzed using independent-samples t test or Mann-Whitney U test; categorical variables will be analyzed using the chi-square test, continuity correction chi-square test or Fisher exact test.

13.4 Intra- and postoperative variables

Numeric variables will be analyzed using the independent-samples t tests or Mann-Whitney U tests; categorical variables will be analyzed using the chi-square tests, continuity correction chi-square tests or Fisher exact tests. Missing data will not be replaced.

13.5 Efficacy analysis

13.5.1 Evaluation of primary endpoints

For the primary outcomes (maternal pain intensity and sedation score immediately after fetal delivery), the differences between group will be analyzed using Mann-Whitney U test. Median differences and 95% CIs will be calculated with the Hodges-Lehmann estimators.

13.5.2 Evaluation of secondary and other endpoints

13.5.2.1 Discrete variables (maternal pain intensity and sedation score during the perinatal period, neonatal Apgar scores, and umbilical vein pH) will be analyzed with the Mann-Whitney U tests. Median differences and 95% CIs will be calculated with the Hodges-Lehmann estimators. Missing data will not be replaced.

13.5.2.2 Categorical variables (neonatal ward admission proportion) will be analyzed using the chi-square tests, continuity correction chi-square tests or Fisher exact tests. The rate differences and 95% CIs will be provided. Missing data will not be replaced.

13.5.2.3 Time-to-event variables (length of hospital stay after surgery) will be analyzed with the Kaplan-Meier estimators with differences between groups assessed by the log-rank test; Cox proportional hazards models will be used to calculate HRs and 95% CIs.

13.6 Safety analysis

13.5.1 Describe the occurrence of adverse events in each group.

13.5.2 Describe the management of adverse events when appropriate.

13.5.3 Describe the occurrence of severe adverse events.

13.5.4 The rates of adverse events and/or managements between the two groups will be compared with Chi-Square test, continuity correction Chi-Square test or Fisher exact test.

13.5.5 Missing data will not be replaced.

14. Quality control and quality assurance

14.1 Training for investigators

14.1.1 An investigator training program will be designed by the principal investigator. A study coordinator will be designated to organize and implement the training program, and to record and preserve the related documents.

14.1.2 Investigator training will be performed during the month before starting the study.

14.1.3 The training program will be repeated 1-2 times a year throughout the study period, or will be performed whenever necessary.

14.2 Monitoring of study conduct

14.2.1 The study will be monitored by the Women's Hospital School of Medicine Zhejiang University Clinical Research Institute.

14.2.2 A project specialist will be designated by the Women's Hospital School of Medicine Zhejiang University Clinical Research Institute and will verify that the conduct of the study,

the record of data and the analysis are in accord with the study protocol and related regulations. Investigators should cooperate with the project specialist.

14.2.3 Before and during the study period, the project specialist will go to the study centers for initiation inspection, regular inspection, and end of study inspection.

14.2.4 The contents of inspection include the following:

14.2.4.1 To verify that investigators are designated and completed the training program.

14.2.4.2 To verify the authenticity of participants, and the process to obtain written informed consents.

14.2.4.3 To verify the eligibility of participants.

14.2.4.4 To verify the correctness of the randomization procedure.

14.2.4.5 To verify that the follow-ups and assessments are performed according to the study protocol.

14.2.4.6 Original data will be inspected in at least 5% of the recruited participants. Original data of the primary outcome will be inspected in 100% of the recruited participants.

14.2.4.7 To verify that all severe adverse events are reported to the Ethics Committee according to the study protocol. The original data of all severe adverse events will be inspected.

14.2.4.8 To verify the transport, dissemination and retrieve of study drugs, and the records of storage and return of study drugs.

14.2.4.9 To verify that the blood samples are collected and stored according to the study protocol and the standard operating procedures.

14.2.4.10 To verify that the revised study protocol, participant-related documents, report of severe adverse events, and annual summary report are submitted to the Ethics Committee timely by the investigators for approval or record.

14.2.4.11 To verify the preservation of study-related documents and original data.

14.2.4.12 To verify the trial management in the study centers, the progress of participant recruitment and the study conduct, the accomplishment of recruited cases, and the situation of case drop-out.

14.2.5 A written report will be provided after each inspection.

14.3 Inspection of data quality

14.3.1 The project specialist will check and verify the completeness and correctness of the data recorded in the case report forms.

14.3.2 All data queries must be solved before the database can be locked for statistical analysis.

15. Ethics requirements

15.1 Ethics Committee

The study protocol must be approved by the Women's Hospital School of Medicine Zhejiang University Institutional Review Board before the study can be started. The investigators must strictly follow the Helsinki Declaration and China's relevant clinical trial management regulations. The principal investigator is responsible to report the status and the progress of the study to the Institutional Review Board.

15.2 Written informed consent

For each potential participant, investigators are responsible to fully explain the purpose, procedures and possible risks of this study in a written form manner. The investigators must let every potential participant know that he/she has the right to withdraw consent from the study at any time. Every potential participant must be given a written informed consent. Every participant or the authorized surrogate of the participant must sign the consent before he/she can be enrolled in the study. The written informed consents will be kept as a part of the clinical trial documents.

15.3 Privacy and confidentiality

15.3.1 During the study period, the collected data from participants are labelled with special recruitment numbers and acronyms of names.

15.3.2 All personal information of the participants will be kept confidential.

15.3.3 Results of the study will be published as scientific articles. But all personal data (including name and age, etc.) are strictly confidential.

16. Study termination

16.1 In case that severe adverse events or serious quality problem occur during the study period, the study will be stopped. A report will be sent to the Ethics Committee. Restart of the study will need an approval from the Ethics Committee.

16.2 The study will be terminated after accomplishment of required patient recruitment and data collection. Decision will be made by the principal investigator.

17. Preservation of documents

Investigators will carefully preserve all documents and data of the clinical trial according to the requirements of Good Clinic Practice for a period of 5 years.

18. Declaration of interests

This work will be supported by the National Natural Science Foundation of China (82271287), Zhejiang Medical and Health Science and Technology Project (WKJ-ZJ-2319), the Exploration Project of Zhejiang Natural Science Foundation (LY21H090006), the Zhejiang Health Science and Technology Planning Project (2021KY768) and the 4+X Clinical Research Project of

Women's Hospital, School of Medicine, Zhejiang University (ZDFY2022-4XA102).

19. References

1. Morgan PJ, Halpern S, Lam-McCulloch J. Comparison of maternal satisfaction between epidural and spinal anesthesia for elective Cesarean section. Can J Anaesth. 2000;47(10):956-61.
2. White PF, Ham J, Way WL, Trevor AJ. Pharmacology of ketamine isomers in surgical patients. Anesthesiology. 1980;52(3):231-9.
3. White PF, Schüttler J, Shafer A, Stanski DR, Horai Y, Trevor AJ. Comparative pharmacology of the ketamine isomers. Studies in volunteers. Br J Anaesth. 1985;57(2):197-203.
4. Strümper D, Gogarten W, Durieux ME, Hartleb K, Van Aken H, Marcus MAE. The effects of S+-ketamine and racemic ketamine on uterine blood flow in chronically instrumented pregnant sheep. Anesth Analg. 2004;98(2):497-502.
5. Unlugenc H, Ozalevli M, Gunes Y, Olguner S, Evrüke C, Ozcengiz D, Akman H. A double-blind comparison of intrathecal S(+) ketamine and fentanyl combined with bupivacaine 0.5% for Caesarean delivery. Eur J Anaesthesiol. 2006;23(12):1018-24.
6. Suppa E, Valente A, Catarci S, Zanfini BA, Draisci G. A study of low-dose esketamine infusion as "preventive" pain treatment for cesarean section with spinal anesthesia: benefits and side effects. Minerva Anestesiol. 2012;78(7):774-81.

Statistical analysis plan

This is a copy from the study protocol.

1. Study design and objectives

This is a muti-center, randomized, double blind trial with two parallel arms. The purpose of the study is to investigate the sedative and analgesic effects of intravenous esketamine before childbirth during cesarean delivery under epidural anesthesia.

2. Sample size estimation

Based on a pilot study, we suppose that the mean difference in NRS pain score immediately after fetal delivery (10 min after administering esketamine) would be 0.3 with standard deviations (SD) of 0.85 and 1.23, respectively, between the two groups. With alpha set at 5% and power at 90%, 263 patients will be needed in each group. Considering a drop-out rate of about 10%, we plan to enroll 300 patients in each group. The sample size was estimated with the PASS 15.0 software.

3. Efficacy outcomes

3.1 Primary outcome

Our co-primary outcomes include maternal pain intensity and sedation score immediately after fetal delivery (about 10 min after esketamine). Pain intensity will be assessed with the numeric rating scale (NRS; an 11-point scale where 0=no pain and 10=the worst pain). Maternal sedation level will be assessed with the Ramsay Sedation Scale (1=restlessness; 2=completely awake, quiet and cooperative; 3= drowsiness but responding to verbal commands; 4=light asleep but responding to touch or pain; 5= asleep but slowly responding to touch or pain; 6=deeply asleep and does not respond).

3.2 Secondary outcomes

3.2.1 Maternal pain intensity as assessed with the NRS at the following timepoints: surgical incision (2 min after study drug administration), 5 min after study drug administration, end of surgery, 6 h after surgery, and 12 h after the surgery.

3.2.2 Maternal sedation level as assessed with the Ramsay Sedation Scale at the following timepoints: surgical incision (2 min after study drug administration), 5 min after study drug administration, end of surgery, 6 h after surgery, and 12 h after the surgery.

3.2.3 Neonatal Apgar score assessed at 1 and 5 minutes after birth.

3.2.4 Postnatal umbilical vein blood gas pH value.

3.3 Other outcomes

3.3.1 Mean blood pressure (MBP), heart rate (HR), and pulse oxygen saturation (SpO2) measured before anesthesia, immediately after anesthesia, surgical incision (2 min after study drug administration), 5 min after study drug administration, immediately after fetal delivery (10 min after study drug administration), end of surgery, and 1 h after surgery.

3.3.2 The requirement of neonatal ward admission and length of hospital stay.

3.3.3 Plasma concentrations of eskatemine in maternal blood, neonatal umbilical venous blood and umbilical arterial blood after birth.

4. Statistical analysis

4.1 General principles

4.1.1 The primary and secondary outcomes will be analyzed in an intention-to-treat population, i.e., all patients are analyzed in the group to which they are randomized. Also, we will do per-protocol analysis for the primary endpoints after patients with major protocol deviation.

4.1.2 For continuous variables, the Kolmogorov-Smirnov test will be applied to evaluate the distribution. Variables with normal distribution were presented as mean ± standard deviation (SD). Variables with non-normal distribution will be presented as median and interquartile range (IQR).

4.1.3 Categorical variables will be presented as number of cases (%).

4.1.4 For the primary outcomes, $p<0.025$ (0.05/2) will be considered statistically significant. For other outcomes, two-tailed tests will be used and $p<0.05$ will be considered to be statistically significant unless otherwise indicated after Bonferroni correction.

4.2 Patient recruitment and drop-out status

The status of patient recruitment and drop-out will be summarized and listed. Comparison of the overall elimination/drop-out rate between the two groups will be performed with Chi-Square test.

4.3 Demographics and baseline characteristics

4.3.1 Demographics and baseline data will be presented.

4.3.2 For between-group differences, numeric variables will be analyzed using independent-samples t test or Mann-Whitney U test; categorical variables will be analyzed using the chi-square test, continuity correction chi-square test or Fisher exact test.

4.4 Intra- and postoperative variables

Numeric variables will be analyzed using the independent-samples t tests or Mann-Whitney U tests; categorical variables will be analyzed using the chi-square tests, continuity correction

chi-square tests or Fisher exact tests. Missing data will not be replaced.

4.5 Efficacy analysis

4.5.1 Evaluation of primary endpoints

For the primary outcomes (maternal pain intensity and sedation score immediately after fetal delivery), the differences between group will be analyzed using Mann-Whitney U test. Median differences and 95% CIs will be calculated with the Hodges-Lehmann estimators.

4.5.2 Evaluation of secondary and other endpoints

4.5.1 Discrete variables (maternal pain intensity and sedation score during the perinatal period, neonatal Apgar scores, and umbilical vein pH) will be analyzed with the Mann-Whitney U tests. Median differences and 95% CIs will be calculated with the Hodges-Lehmann estimators. Missing data will not be replaced.

4.5.2 Categorical variables (neonatal ward admission proportion) will be analyzed using the chi-square tests, continuity correction chi-square tests or Fisher exact tests. The rate differences and 95% CIs will be provided. Missing data will not be replaced.

4.5.3 Time-to-event variables (length of hospital stay after surgery) will be analyzed with the Kaplan-Meier estimators with differences between groups assessed by the log-rank test; Cox proportional hazards models will be used to calculate HRs and 95% CIs.

4.6 Safety analysis

4.6.1 Describe the occurrence of adverse events in each group.

4.6.2 Describe the management of adverse events when appropriate.

4.6.3 Describe the occurrence of severe adverse events.

4.6.4 The rates of adverse events and/or managements between the two groups will be compared with Chi-Square test, continuity correction Chi-Square test or Fisher exact test.

4.6.5 Missing data will not be replaced.