Study Protocol and Statistical Analysis Plan

For: Ting Yan, Xin-Quan Liang, Guo-Jun Wang, et al. Prophylactic penehyclidine inhalation for prevention of postoperative pulmonary complications in high-risk patients: A double-blind randomized controlled trial.

This supplement contains the following items:

1. Original protocol, final protocol, summary of revision.
2. Original statistical analysis plan, final statistical analysis plan, summary of revision.

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Original study protocol

Prophylactic penehyclidine inhalation for prevention of postoperative pulmonary complications in high-risk patients: a randomized controlled trial

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Study center: Peking University First Hospital
Participating departments:
Department of Anesthesiology and Critical Care Medicine, Peking University First Hospital
Department of Respiratory and Critical Care Medicine, Peking University First Hospital
Department of Thoracic Surgery, Peking University First Hospital
Department of General Surgery, Peking University First Hospital
Department of Biostatistics, Peking University First Hospital

Version of protocol: V1.0

Date of version: January 20, 2015

Contents of original protocol

1. Background

Postoperative pulmonary complications (PPCs) are major causes of morbidity, mortality, and prolonged hospital stay in patients after surgery.[1-3] The reported incidences of PPCs vary from 5% to 70% depending on the definition of PPCs, the type of surgical procedures, and the population of patients included in the study.[4,5] The ARISCAT study identified seven independent predictors, including four patient-related factors and three surgery-related factors, and built a predictive model for the risk of PPCs.[4] A subsequent study confirmed that, in high-risk patients (i.e., patients with cumulative ARISCAT risk score ≥45), the incidence of PPCs was as high as 38% (25–51%).[4,5]

Changes in pulmonary physiology after major thoracic or upper abdominal surgery include diaphragmatic dysfunction, reduced vital capacity, postoperative pain and splinting, and impaired clearance of airway secretions.[6] Affected patients are at increased risk to develop atelectasis, pneumonia, and other respiratory complications.

Prophylactic inhalation of muscarinic antagonists may be helpful in preventing PPCs by dilating bronchus and relieving airway hyperresponsiveness. In a retrospective study of patients with COPD requiring lung cancer surgery, perioperative inhalation of tiotropium was associated with decreased cardiopulmonary complications after surgery.[7] In a randomized controlled trial of gastric cancer patients with COPD, perioperative (1 week before and 2 weeks after surgery) inhalation of tiotropium decreased the incidence of postoperative complications in those with moderate COPD.[8] Theoretically, selective M1 and M3 receptors blockers may have advantages over nonselective blockers (such as tiotropium bromide) for the purpose of bronchodilation.[9]

Penehyclidine is a long-acting muscarinic antagonist which selectively blocks M1 and M3 receptors.[10,11] In a pilot randomized controlled trial of our group, 90 elderly (≥65 years) patients who were admitted to the ICU after long-duration (≥3 hours) surgery randomly received inhalation of penehyclidine, ipratropium bromide, or normal saline for 3 consecutive days. The results showed that prophylactic anticholinergic inhalation significantly reduced the incidence of postoperative bronchospasm (1/30 [3.3%] with penehyclidine hydrochloride, 1/31 [3.2%] with ipratropium bromide, and 6/ 29 [20.7%] with normal saline, respectively, P=0.025).[12]

We hypothesize that, in high-risk patients, prophylactic inhalation of penehyclidine may decrease the incidence of PPCs.

2. Purpose of the study

The purpose of this study is to investigate the impact of prophylactic penehyclidine inhalation on the incidence of PPCs in high-risk patients after major surgery.

3. Study design

3.1 Type of the study

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

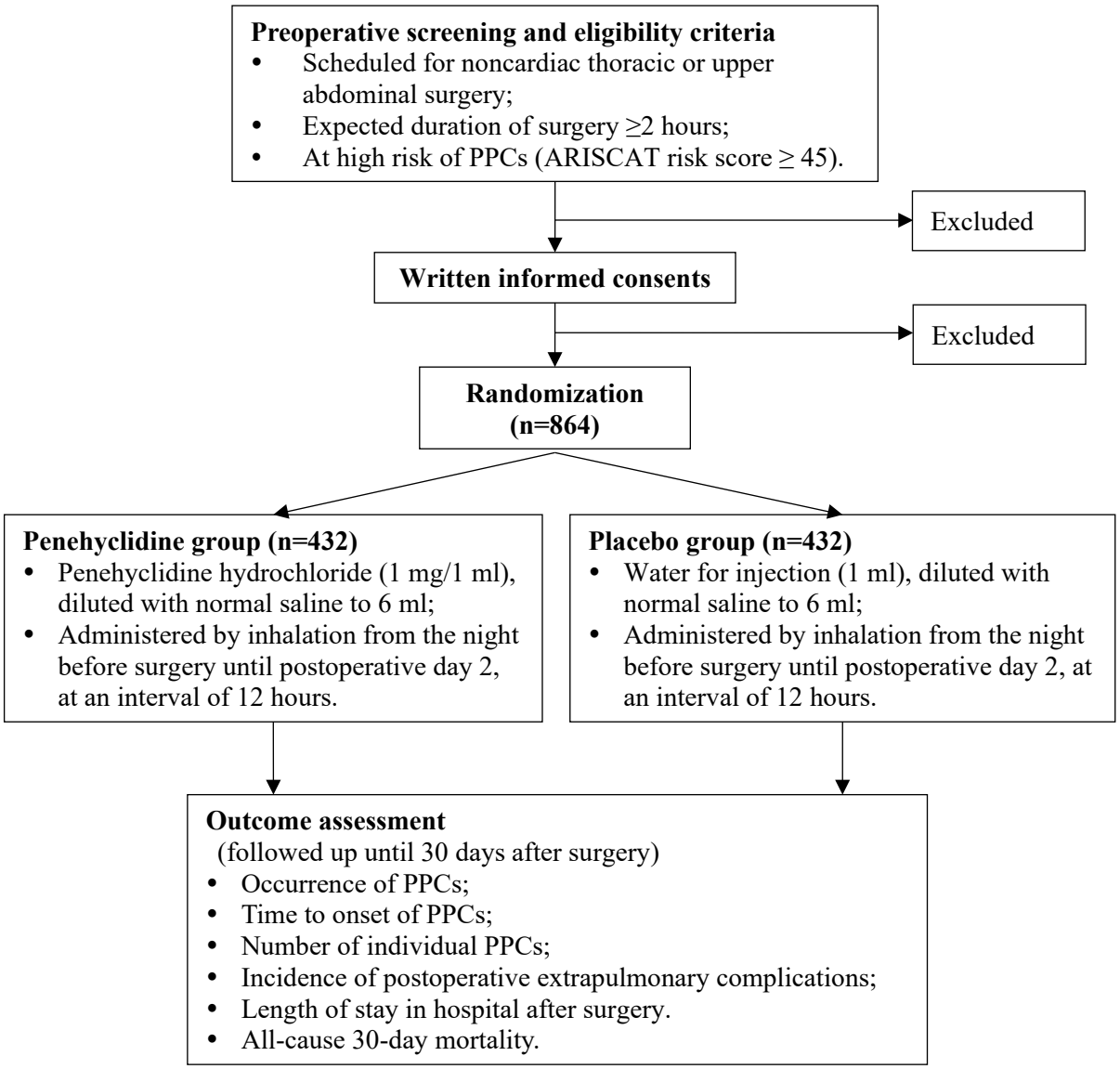

Figure 1. Flow chart of the study.

3.2 Sample size estimation

In our recent randomized controlled trial, PPCs occurred in 51.7% of patients in the placebo group.[12] Mazo et al.[5] reported incidence of PPCs from 42.1% to 44.9% in high-risk patients (ARISCAT risk score ≥45). In previous studies of COPD patients, perioperative inhalation of tiotropium reduced the incidence of PPCs by 30-62.5% (relative reduction).[7,8] In the present study, we assume that the incidence of PPCs will be 42% in high-risk patients of the placebo group, and prophylactic penehyclidine inhalation will reduce the incidence of PPCs to 32% (i.e., a 24% reduction). The calculated sample size that will provide 80% power to detect a superiority of this difference at a two-sided significance level of 0.05 is 365 patients in each group. Considering a dropout rate of about 15%, we plan to enroll 432 patients in each group. Since the safety of intervention has been confirmed by our previous pilot study, interim analysis is not planned. The sample size calculation was performed with PASS 2008 software.

3.3 Study center

This trial is conducted in Peking University First Hospital in Beijing, China.

4. Study participants

Potential participants will be screened before surgery (or Friday for those who will undergo surgery next Monday) by qualified investigators.

4.1 Inclusion criteria

4.1.1 Age >50 years.

4.1.2 Scheduled to undergo upper abdominal or thoracic surgery with expected duration ≥2 hours.

4.1.3 Judged to be at high risk of PPCs according to the ARISCAT risk score (cumulative ARISCAT risk score ≥45; Table 1).

Table 1. The ARISCAT risk score of postoperative pulmonary complications

Independent predictors of risk for PPCs	**Risk score**
Age, year	
≤50	0
51–80	3
>80	16
Preoperative SpO2, %	
≥96%	0
91–95%	8
≤90%	24
Respiratory infection in the last month	
No	0
Yes	17
Preoperative anemia (Hb ≤10 g/dl)	
No	0
Yes	11
Surgical incision	
Peripheral	0

Upper abdominal	15	
Intrathoracic	24	
Duration of surgery, hour		
≤2	0	
>2 to 3	16	
>3	23	
Emergency procedure		
No	0	
Yes	8	
Risk class	**Number of points in risk score**	**Pulmonary complications rates**
Low risk	<26 points	1.6%
Intermediate risk	26–44 points	13.3%
High risk	≥45 points	42.1%

4.2 Exclusion criteria

Patients who meet any of the following criteria will be excluded:

4.2.1 American Society of Anesthesiologists (ASA) physical classification ≥IV or expected survival duration ≤24 hours.

4.2.2 Preoperative history of moderate-to-severe symptomatic prostatic hypertrophy or narrow angle glaucoma.

4.2.3 History of myocardial infarction, severe heart dysfunction (New York Heart Association functional classification ≥3) or tachyarrhythmia within 1 year.

4.2.4 Inhalation of β2-receptor activator, M-receptor blockers and/or glucocorticoids within 1 month before surgery.

4.2.5 Severe renal dysfunction (requirement of renal replacement therapy) or severe hepatic dysfunction (Child-Pugh grade C).

4.2.6 History of acute stroke within 3 months before surgery.

4.2.7 Unable to cooperate with inhalational therapy.

4.2.8 Participation in other clinical trial during the last month or within the six half-life periods of the study drug used in the last trial.

4.2.9 Refuse to participate in the study.

4.3 Criteria of study interruption

Study will be interrupted in the following situations:

4.3.1 Severe safety problem occurred during the study.

4.3.2 Serious mistake found in the protocol.

4.3.3 Fund or management problem of the investigators.

4.3.4 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 numbers are generated in a 1:1 ratio with a block size of 6 using the SAS 9.2 software package (SAS Institute, Cary, NC, USA) by a biostatistician.

5.1.2 Study drugs (penehyclidine hydrochloride 1 mg/1 ml, or water for injection 1 ml) are provided as clear aqueous solution in the same 1 ml ampoules (manufactured by Chengdu List Pharmaceutical Co., Ltd., Sichuan, China) and labelled according to the randomization results by a biostatistician and a pharmacist who are independent of patient recruitment and data collection.

5.1.3 The results of randomization are sealed in opaque envelopes and stored at the site of investigation until the end of the study.

5.2 Masking

5.2.1 For investigators and healthcare team members:

5.2.1.1 A study coordinator is designated to preserve and distribute randomization results, prepare study drugs, and coordinate among investigators and physicians/surgeons.

5.2.1.2 An investigator is designated to administer study drugs.

5.2.1.3 Another investigator (anesthesiologist) who has been specially trained and qualified before the study period is designated to follow up patients for outcome assessment.

5.2.1.4 All study coordinator, investigators and healthcare team members are blinded to study group assignment; they are not allowed to communicate with each other regarding patients' management and follow-up results.

5.2.1.5 Data analysis will be performed by the Department of Biostatistics Peking University First Hospital.

5.2.2 For participants:

5.2.2.1 All participants use same inhalation device.

5.2.2.2 All drugs are diluted with normal saline to 6 ml.

5.2.2.3 All participants are blinded to study group assignment throughout the study period.

5.3 Unmasking

5.3.1 The randomization envelopes will be unblinded after (1) patient recruitment and follow-up are completed, (2) data collection, input and double-checked is performed without errors, (3) all data queries have been solved, and (4) database is locked.

6. Intervention protocol

6.1 Distribution of study drugs

6.1.1 During the study period, each recruited patient will be assigned a serial number according to the sequence of recruitment.

6.1.2 The study coordinator will select study drugs according to the serial number and distribute the selected drugs to the investigators for inhalation. As such, the recruited patients are randomly divided into two groups, i.e., the penehyclidine group and the placebo group.

6.1.3 The number of labelled study drugs and the preparation of study drug mixtures are recorded in a study drug management record form and the case report forms (CRFs).

6.2 Study drug inhalation

6.2.1 Before inhalation, the study drugs (penehyclidine hydrochloride 1.0 mg/1.0 ml for patients in the penehyclidine group, or water for injection 1.0 ml for patients in the placebo group) are diluted with normal saline to 6 ml.

6.2.2 The study drug mixtures are administered by inhalation in the night (7 pm) before surgery, in the morning (7 am) of surgery, and then every 12 hours until postoperative day 2, resulting in a total number of 7 inhalations.

6.2.3 Study drug inhalation is performed with a nebulizer for extubated patients or a vibrating mash nebulizer for patients with endotracheal intubation (mechanical ventilation).

6.3 Follow-up of patients

6.3.1 Patients will be followed up until 30 days after surgery. Discharged patients will be contacted by telephone.

6.3.2 Investigator will assess the development of adverse events, PPCs, and postoperative extrapulmonary complications according to clinical features, laboratory tests, and instrumental examination results. Data in the Anesthesia Information System and the Electronic Medical Record System will be achieved.

6.4 Interruption of study drug administration

6.4.1 For patients who develop adverse events or clinical deterioration, the dose of study drugs can be decreased or the study drug administration can be discontinued on request of participants, attending surgeons or investigators.

6.4.2 These situations will be recorded in the CRFs.

6.5 Allowed and prohibited medications

6.5.1 Anticholinergics are prohibited unless being used for the treatment of bradycardia, in which case atropine is administered.

6.5.2 During the study period, inhalations of any respiratory medications for prophylactic purpose other than the study drugs are prohibited.

6.5.3 For patients who develop PPCs, inhalation of respiratory medications is allowed.

7. Data collection

7.1 Baseline data

7.1.1 Demographic data, including date of birth, sex, body mass index, and education level.

7.1.2 Diagnosis and medical history, including surgical diagnosis, comorbidities, medical therapy, drinking and smoking history, food and drug allergy, and previous history of surgery and anesthesia.

7.1.3 Main results of laboratory tests and instrumental examinations, including blood gas and chest X-ray.

7.1.4 General status, including Charlson Comorbidity Index, American Society of Anesthesiologists classification, and New York Heart Association classification.

7.1.5 Medication history, especially for the respiratory system.

7.2 Intraoperative data

7.2.1 Location, type, name, and duration of surgery.

7.2.2 Type and duration of anesthesia, types and doses of anesthetics and other medications used during anesthesia.

7.2.3 Parameters of mechanical ventilation.

7.2.4 Fluid balance (including fluid infusion, estimated blood loss, and urine output) and transfusion of blood products.

7.2.5 The final cumulative ARISCAT risk score will be calculated just after the surgery.

7.3 Postoperative data

7.3.1 The intensity of pain both at rest and with coughing are assessed daily (7:00-8:00 am) during the first 3 postoperative days with the numeric rating scale (NRS; an 11-point scale where 0=no pain and 10=the worst pain). The final status of patient-controlled analgesia pump use (completed use, dose reduction, early termination, change to other analgesic method) is recorded. The use of supplemental analgesics and other medications (including antiemetics) are recorded.

7.3.2 Respiratory management, such as prophylactic chest physiotherapy, and intravenous and/or oral respiratory medications. Prophylactic aerosol inhalation is prohibited but, if used, will be documented.

7.3.3 Gastrointestinal tract management, such as placement of nasogastric tube, administration of parenteral nutrition, and use of proton pump inhibitors.

7.3.4 ICU admission after surgery. For patients who are admitted to ICU after surgery, the percentage with endotracheal intubation, the duration of mechanical ventilation, and the length of ICU stay are recorded.

7.3.5 Occurrence of PPCs, which is generally defined as any condition that affects the respiratory system, may adversely influence patients' outcome and requires therapeutic intervention.[1,2] The diagnostic criteria of each individual PPC are listed in Table 2.[4] If a PPC occurs during the follow-up period, the date of earliest diagnosis and the evidences according to which the diagnosis is made will be documented.

Table 2. Definition of postoperative pulmonary complications.

Complication	Definition
Respiratory infections	Receiving antibiotics for a suspected respiratory infection and meet at least one of the following criteria: new or changed sputum, new or changed lung opacities, fever, leukocyte count >12×10^9/L.
Respiratory failure	PaO_2 <60 mmHg on room air, a ratio of PaO_2 to inspired oxygen fraction <300, or arterial oxyhemoglobin saturation measured with pulse oximetry <90% and requiring oxygen therapy.
Pleural effusion	Chest X-ray demonstrating blunting of the costophrenic angle, loss of the sharp silhouette of the ipsilateral hemidiaphragm in upright position, evidence of displacement of adjacent anatomical structures,

	or (in supine position) a hazy opacity in one hemithorax with preserved vascular shadows.
Atelectasis	Lung opacification with a shift of the mediastinum, hilum, or hemidiaphragm toward the affected area, and compensatory overinflation in the adjacent nonatelectatic lung.
Pneumothorax	Air in the pleural space with no vascular bed surrounding the visceral pleura.
Bronchospasm	Newly detected expiratory wheezing treated with bronchodilators.
Aspiration pneumonitis	Acute lung injury after inhalation of regurgitated intragastric contents.

7.3.6 Occurrence of extrapulmonary complications. Postoperative extrapulmonary complications are defined as complications other than PPCs that occur within 30 days after surgery and require therapeutic intervention. For each diagnosed extrapulmonary complication, the date of earliest diagnosis and the evidences according to which the diagnosis is made will also be documented.

7.3.7 Length of hospital stay after surgery.

7.3.8 All-cause 30-day mortality after surgery.

8. Outcomes

8.1 Primary outcome

The incidence of PPCs within 30 days after surgery.

8.2 Secondary outcomes

8.2.1 Time to onset of PPCs, i.e., from end of surgery to first diagnosis of PPCs.

8.2.2 Occurrence and number of individual PPCs.

8.2.3 Incidence of postoperative extrapulmonary complications within 30 days after surgery.

8.2.4 Length of stay in hospital after surgery.

8.2.5 All-cause 30-day mortality.

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 Exceptional adverse events that may occur during anesthesia/surgery include (but not limited to) the following: hypertension (systolic blood pressure >180 mmHg or an increase of more than 30% from baseline), hypotension (systolic blood pressure <90 mmHg or a decrease of more than 30% from baseline), tachycardia (heart rate >100 beats per minute), bradycardia (heart rate <40 beats per minute), desaturation (PaO_2 <60 mmHg or SpO_2 <90%), high airway pressure (peak airway pressure >40 cm H_2O), hypercapnia ($PaCO_2$ >50 mmHg), difficult airway (failed intubation attempt >3 times), massive bleeding (estimated blood loss >1000 ml), other new-onset arrhythmia (atrial fibrillation, supraventricular tachycardia, ventricular premature beats, atrioventricular block, and cardiac arrest), etc.

9.2.2 Other adverse events that may occur during the perioperative period include (but not limited to) the following: nausea and vomiting, dry mouth, flushing, dizziness, palpitation, somnolence, cough, sneeze, delirium, etc. These adverse events are diagnosed according to patient's complains and symptoms (sought by nondirective questioning), physical and mental signs, as well as results of laboratory tests and instrumental examinations. Delirium is diagnosed according to the Confusion Assessment Method for the Intensive Care Unit.

9.3 Management

9.3.1 Any developed adverse events will be managed according to patients' condition and routine practice.

9.3.2 The study intervention can be stopped temporarily or permanently if considered necessarily by the attending anesthesiologists or surgeons. The time and reasons of intervention interruption will be recorded.

9.4 Record

9.5.1 The information including the diagnosis, date(s) of onset and resolution (if applicable), severity of influence, relationship with intervention, treatment, and outcomes (sequelae) are recorded.

9.5.2 Any adverse event should be followed up until it is completely resolved or therapy terminated.

9.5.3 The occurrence of adverse events will be reported to the ethics committee in the final report.

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 stay in hospital, persistent disability or organ 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 according to routine practice.

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 Clinical Research Ethics Committee of Peking University First Hospital will be informed within 24 hours in report form.

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 the treatment is terminated.

10.3.4 Harmful consequence resulted directly from study participation will be compensated according to the corresponding legal provisions.

11. Data management

11.1 Investigators are trained to record data promptly, completely, and correctly in the CRFs according to original observation.

11.2 Data entry will be performed with EpiData software (Version 3.1). Dataset will be locked when the following tasks have been completed: (1) patient recruitment and follow-up are completed, (2) data collection, entry and double-checked is performed without errors, and (3) all data queries have been solved.

12. Statistical analysis

12.1 General principles

12.1.1 All statistical analyses will be performed with SPSS 14.0 software package (SPSS, Chicago, IL, USA) and SAS 9.2 software (SAS Institute, Cary, NC, USA) by statisticians in the Department of Biostatistics of Peking University First Hospital.

12.1.2 Since the safety of intervention has been confirmed by our previous pilot study, interim analysis is not planned.

12.1.3 Analyses will be performed on an intention-to-treat basis, that all subjects will be analyzed in the group to which they were assigned. For the primary outcome (the incidence of PPCs within 30 days after surgery), per protocol analysis will also be performed.

12.2 Baseline data

12.2.1 Statistical description will be provided for baseline data such as demographic variables, medical history, preoperative medications, and perioperative management.

12.3 Outcome analysis

12.3.1 Primary outcome (the incidence of PPCs within 30 days after surgery) will be compared with chi-square test. The difference between groups and the 95% confidence interval of the difference will be calculated.

12.3.2 For secondary outcomes, continuous variables with normal distribution will be analyzed using an independent sample t-test; continuous variables with non-normal distribution or ranked data will be analyzed using Mann-Whitney U test; categorical variables will be analyzed using the chi-square test, continuity correction chi-square test or Fisher exact test; time-to-event results will be analyzed using the Kaplan-Meier estimator, and the differences between groups will be tested by the log-rank test. Subgroup analyses will be performed according to the type of the surgery (upper abdominal or thoracic).

13. Quality control and quality assurance

13.1 The principle of randomization and blinding must be strictly followed.

13.2 The storage and return of study drugs must follow relevant storage regulations.

13.3 The instruments and equipment used in the study should have strict quality standards. Investigators should ensure proper operation of these instruments and equipment.

13.4 The investigator training must be performed before initiating the study. The study protocol is thoroughly explained during the training process. Investigators are trained to record data promptly, completely, and correctly in the CRFs according to original observation. If there are any abnormal findings, investigators should reconfirm and record properly to ensure the reliability of the data.

13.5 All statistical analyses will be performed by statisticians.

14. Ethics requirements

14.1 Ethics Committee

The study protocol must be approved by the Clinical Research Ethics Committee of Peking University First Hospital 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 Ethics Committee.

14.2 Written informed consent

Investigators responsible for recruiting participants must have been trained and qualified by the principal investigator. 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.

14.3 Privacy and confidentiality

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

14.3.2 All personal information of the participants will be kept confidential. The filing cabinets storing the study documents will be locked. Apart from the study investigators, only authorized inspectors from the Peking University Clinical Research Institute or members from the Clinical Research Ethics Committee of Peking University First Hospital are allowed to access the information after obtaining consents from the participants.

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

15. Study termination

15.1 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.

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

16. 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 28 months.

17. Declaration of interests

Study drugs were manufactured and supplied by Chengdu List Pharmaceutical Co, Ltd, Sichuan, China. The investigators declare no conflict of interests.

18. References

1. Shander A, Fleisher LA, Barie PS, Bigatello LM, Sladen RN, Watson CB: Clinical and economic burden of postoperative pulmonary complications: patient safety summit on definition, risk-reducing interventions, and preventive strategies. Crit Care Med 2011; 39: 2163-72

2. Ghaferi A, Birkmeyer J, Dimick J: Variation in Hospital Mortality Associated with Inpatient Surgery. N Engl J Med. 2009; 361: 1368-75

3. Molena D, Mungo B, Stem M, Lidor AO: Incidence and risk factors for respiratory complications in patients undergoing esophagectomy for malignancy: a NSQIP analysis. Semin Thorac Cardiovasc Surg 2014; 26: 287-94

4. Canet J, Gallart L, Gomar C, Paluzie G, Vallès J, Castillo J, Sabaté S, Mazo V, Briones Z, Sanchis J, Group， A: Prediction of postoperative pulmonary complications in a population-based surgical cohort. Anesthesiology 2010; 113: 1338-50

5. Mazo V, Sabaté S, Canet J, Gallart L, de Abreu M, Belda J, Langeron O, Hoeft A, Pelosi P: Prospective external validation of a predictive score for postoperative pulmonary complications. Anesthesiology 2014; 121: 219-31

6. Duggan M, Kavanagh B: Pulmonary atelectasis: a pathogenic perioperative entity. Anesthesiology. 2005; 102: 838-54

7. Nojiri T, Inoue M, Yamamoto K, Maeda H, Takeuchi Y, Nakagiri T, Shintani Y, Minami M, Sawabata N, Okumura M: Inhaled tiotropium to prevent postoperative cardiopulmonary complications in patients with newly diagnosed chronic obstructive pulmonary disease requiring lung cancer surgery. Surg Today 2014; 44: 285-90

8. Fushida S, Oyama K, Kaji M, Hirono Y, Kinoshita J, Tsukada T, Nezuka H, Nakano T, Noto M, Nishijima K, Fujimura T, Ohta T: A randomized multicenter Phase II study of perioperative tiotropium intervention in gastric cancer patients with chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis 2015; 10: 2177-83

9. Barnes P: Muscarinic receptor subtypes in airways. Life Sci 1993; 52: 521-7

10. Xiao H, Liao Z, Tong R: Penehyclidine hydrochloride: a potential drug for treating COPD by attenuating Toll-like receptors. Drug Des Devel Ther 2012; 6: 317-22

11. Xiao H, Liao Z, Meng X, Yan X, SJ. C, Mo Z: Effects of the selective muscarinic receptor antagonist penehyclidine hydrochloride on the respiratory tract. Pharmazie 2009; 64: 337-41

12. Yan T, Wang D: Effects of penehyclidine inhalation on postoperative pulmonary complications of elderly patients after long-duration surgery. Zhonghua Yi Xue Za Zhi. 2014; 94: 122-6.

Final study protocol

Prophylactic penehyclidine inhalation for prevention of postoperative pulmonary complications in high-risk patients: a randomized controlled trial

Principal investigator: Prof. Dong-Xin Wang, MD, PhD, Department of Anesthesiology and Critical Care Medicine, Peking University First Hospital

Study center: Peking University First Hospital
Participating departments:
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Department of Respiratory and Critical Care Medicine, Peking University First Hospital
Department of Thoracic Surgery, Peking University First Hospital
Department of General Surgery, Peking University First Hospital
Department of Biostatistics, Peking University First Hospital

Version of updated protocol: V5.1

Version of version: December 4, 2017

Contents of final protocol

1. Background

Postoperative pulmonary complications (PPCs) are major causes of morbidity, mortality, and prolonged hospital stay in patients after surgery.[1-3] The reported incidences of PPCs vary from 5% to 70% depending on the definition of PPCs, the type of surgical procedures, and the population of patients included in the study.[4,5] The ARISCAT study identified seven independent predictors, including four patient-related factors and three surgery-related factors, and built a predictive model for the risk of PPCs.[4] A subsequent study confirmed that, in high-risk patients (i.e., patients with cumulative ARISCAT risk score ≥45), the incidence of PPCs was as high as 38% (25–51%).[4,5]

Changes in pulmonary physiology after major thoracic or upper abdominal surgery include diaphragmatic dysfunction, reduced vital capacity, postoperative pain and splinting, and impaired clearance of airway secretions.[6] Affected patients are at increased risk to develop atelectasis, pneumonia, and other respiratory complications.

Prophylactic inhalation of muscarinic antagonists may be helpful in preventing PPCs by dilating bronchus and relieving airway hyperresponsiveness. In a retrospective study of patients with COPD requiring lung cancer surgery, perioperative inhalation of tiotropium was associated with decreased cardiopulmonary complications after surgery.[7] In a randomized controlled trial of gastric cancer patients with COPD, perioperative (1 week before and 2 weeks after surgery) inhalation of tiotropium decreased the incidence of postoperative complications in those with moderate COPD.[8] Theoretically, selective M1 and M3 receptors blockers may have advantages over nonselective blockers (such as tiotropium bromide) for the purpose of bronchodilation.[9]

Penehyclidine is a long-acting muscarinic antagonist which selectively blocks M1 and M3 receptors.[10,11] In a pilot randomized controlled trial of our group, 90 elderly (≥65 years) patients who were admitted to the ICU after long-duration (≥3 hours) surgery randomly received inhalation of penehyclidine, ipratropium bromide, or normal saline for 3 consecutive days. The results showed that prophylactic anticholinergic inhalation significantly reduced the incidence of postoperative bronchospasm (1/30 [3.3%] with penehyclidine hydrochloride, 1/31 [3.2%] with ipratropium bromide, and 6/ 29 [20.7%] with normal saline, respectively, P=0.025).[12]

We hypothesize that, in high-risk patients, prophylactic inhalation of penehyclidine may decrease the incidence of PPCs.

2. Purpose of the study

The purpose of this study is to investigate the impact of prophylactic penehyclidine inhalation on the incidence of PPCs in high-risk patients after major surgery.

3. Study design

3.1 Type of the study

This is a single-center, randomized, double-blind, placebo-controlled trial with two parallel arms. The flow chart of the study is shown in Figure 1. The protocol is reported according to the Standard Protocol Items: recommendations for randomized controlled trials.

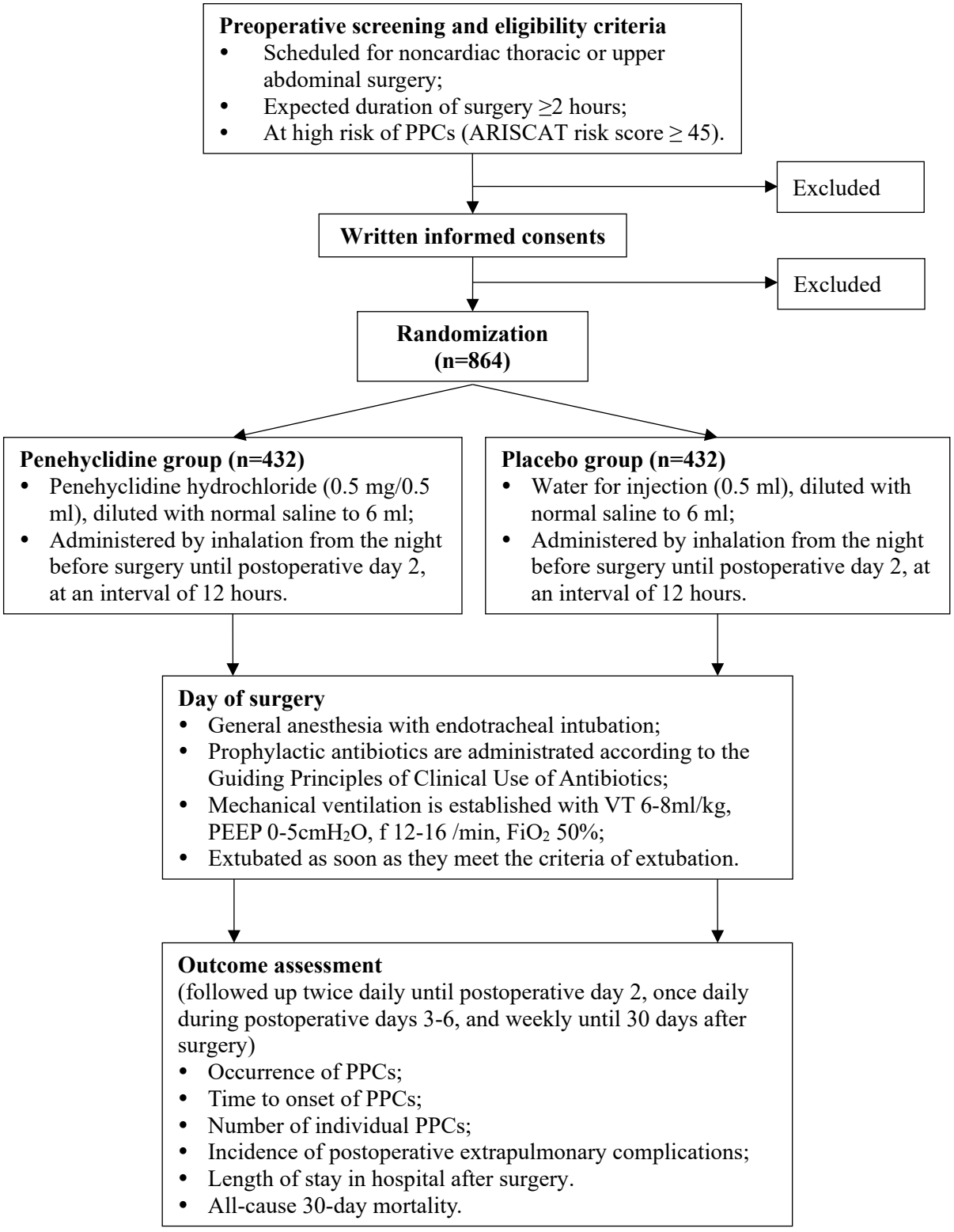

Figure 1. Flow chart of the study.

3.2 Sample size estimation

In our recent randomized controlled trial, PPCs occurred in 51.7% of patients in the placebo group.[12] Mazo et al.[5] reported incidence of PPCs from 42.1% to 44.9% in high-risk patients (ARISCAT risk score ≥45). In previous studies of COPD patients, perioperative inhalation of tiotropium reduced the incidence of PPCs by 30-62.5% (relative reduction).[7,8] In the present study, we assume that the incidence of PPCs will be 42% in high-risk patients of the placebo group, and prophylactic penehyclidine inhalation will reduce the incidence of PPCs to 32% (i.e., a 24% reduction). The calculated sample size that will provide 80% power to detect a superiority of this difference at a two-sided significance level of 0.05 is 365 patients in each group. Considering a dropout rate of about 15%, we plan to enroll 432 patients in each group. Since the safety of intervention has been confirmed by our previous pilot study, interim analysis is not planned. The sample size calculation was performed with PASS 2008 software.

3.3 Study center

This trial is conducted in Peking University First Hospital in Beijing, China.

4. Study participants

Potential participants will be screened the day before surgery (or Friday for those who will undergo surgery next Monday) by qualified investigators.

4.1 Inclusion criteria

4.1.1 Age >50 years.

4.1.2 Scheduled to undergo upper abdominal or noncardiac thoracic surgery with expected duration ≥2 hours. For those who undergo thoracoscopic or laparoscopic surgery, the expected length of incision must be 5 cm or more.

4.1.3 Judged to be at high risk of PPCs according to the ARISCAT risk score (cumulative ARISCAT risk score ≥45; Table 1).

Table 1. The ARISCAT risk score of postoperative pulmonary complications

Independent predictors of risk for PPCs	**Risk score**
Age, year	
≤50	0
51–80	3
>80	16
Preoperative SpO2, %	
≥96%	0
91–95%	8
≤90%	24
Respiratory infection in the last month	
No	0
Yes	17
Preoperative anemia (Hb ≤10 g/dl)	
No	0
Yes	11

Surgical incision		
Peripheral	0	
Upper abdominal	15	
Intrathoracic	24	
Duration of surgery, hour		
≤2	0	
>2 to 3	16	
>3	23	
Emergency procedure		
No	0	
Yes	8	
Risk class	**Number of points in risk score**	**Pulmonary complications rates**
Low risk	<26 points	1.6%
Intermediate risk	26–44 points	13.3%
High risk	≥45 points	42.1%

4.2 Exclusion criteria

Patients who meet any of the following criteria will be excluded:

4.2.1 American Society of Anesthesiologists (ASA) physical classification ≥IV or expected survival duration ≤24 hours.

4.2.2 Preoperative history of moderate-to-severe symptomatic prostatic hypertrophy or narrow angle glaucoma.

4.2.3 History of myocardial infarction, severe heart dysfunction (New York Heart Association functional classification ≥3) or tachyarrhythmia within 1 year.

4.2.4 Inhalation of β2-receptor activator, M-receptor blockers and/or glucocorticoids within 1 month before surgery.

4.2.5 Severe renal dysfunction (requirement of renal replacement therapy) or severe hepatic dysfunction (Child-Pugh grade C).

4.2.6 History of acute stroke within 3 months before surgery.

4.2.7 Unable to cooperate with inhalational therapy.

4.2.8 Participation in other clinical trial during the last month or within the six half-life periods of the study drug used in the last trial.

4.2.9 Refuse to participate in the study.

4.3 Criteria of drop-out

Enrolled patients will be included in the intention-to-treat analysis but excluded from the per-protocol analysis if they meet any of the following criteria:

4.3.1 Consents withdrawn by participants themselves (at least one dose of study drug has been administered).

4.3.2 Lost to follow-up.

4.3.3 Ordered to exit by the investigators or attending physicians (poor compliance, occurrence of severe complications, or occurrence of severe adverse events).

4.3.4 Prophylactic inhalation of any prohibited drugs (muscarinic antagonists or any medications other than the study drugs) from study recruitment to postoperative day 2.

4.3.5 Blindness unmasked.

4.4 Criteria of elimination

Enrolled patients will be excluded from analysis of therapeutic effects if they meet any of the following criteria:

4.4.1 Consents withdrawn by the participants themselves (study drug is not administered).

4.4.2 No study record.

4.4.3 Surgery delayed or cancelled, unable to assess the occurrence of PPCs.

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 numbers are generated in a 1:1 ratio with a block size of 6 using the SAS 9.2 software package (SAS Institute, Cary, NC, USA) by a biostatistician.

5.1.2 Study drugs (penehyclidine hydrochloride 1 mg/1 ml, or water for injection 1 ml) are provided as clear aqueous solution in the same 1 ml ampoules (manufactured by Chengdu List Pharmaceutical Co., Ltd., Sichuan, China) and labelled according to the randomization results by a biostatistician and a pharmacist who are independent of patient recruitment and data collection.

5.1.3 The results of randomization are sealed in opaque envelopes and stored at the site of investigation until the end of the study.

5.2 Masking

5.2.1 For investigators and healthcare team members:

5.2.1.1 A study coordinator is designated to preserve and distribute randomization results, prepare study drugs, and coordinate among investigators and physicians/surgeons.

5.2.1.2 An investigator is designated to administer study drugs.

5.2.1.3 Another investigator (anesthesiologist) who has been specially trained and qualified before the study period is designated to follow up patients for outcome assessment.

5.2.1.4 All study coordinator, investigators and healthcare team members are blinded to study group assignment; they are not allowed to communicate with each other regarding patients' management and follow-up results.

5.2.1.5 Data analysis will be performed by the Department of Biostatistics Peking University First Hospital.

5.2.2 For participants:

5.2.2.1 All participants use same inhalation device.

5.2.2.2 All drugs are diluted with normal saline to 6 ml.

5.2.2.3 All participants are blinded to study group assignment throughout the study period.

5.3 Unmasking

5.3.1 The randomization envelopes will be unblinded after (1) patient recruitment and follow-up are completed, (2) data collection, input and double-checked is performed without errors, (3) all data queries have been solved, and (4) database is locked.

5.3.2 In case of an emergency (such as development of a severe adverse event), attending physicians or surgeons can request unmasking of the treatment allocation and manage patients according to study group assignment. These situations will be documented; but analyses will be performed in the intention-to-treat population.

6. Intervention protocol

6.1 Study drugs

6.1.1 Active drug

Drug name: Penehyclidine hydrochloride for injection

Specification: 1 mg/1 ml

Batch number: 141106

Manufacturer: Chengdu List Pharmaceutical Co., Ltd, Sichuan, China

6.1.2 Placebo

Drug name: Water for injection

Specification: 1 ml/1 ml

Batch number: 150301

Manufacturer: Chengdu List Pharmaceutical Co., Ltd, Sichuan, China

6.1.3 The study drugs will be store in confined conditions, i.e., room temperature, light avoidance, and locked.

6.2 Distribution of study drugs

6.2.1 During the study period, each recruited patient will be assigned a serial number according to the sequence of recruitment.

6.2.2 The study coordinator will select study drugs according to the serial number and distribute the selected drugs to the investigators for inhalation. As such, the recruited patients are randomly divided into two groups, i.e., the penehyclidine group and the placebo group.

6.2.3 The number of labelled study drugs and the preparation of study drug mixtures are recorded in a study drug management record form and the case report forms (CRFs).

6.3 Study drug inhalation

6.3.1 Before inhalation, the study drugs (penehyclidine hydrochloride 0.5 mg/0.5 ml for patients in the penehyclidine group, or water for injection 0.5 ml for patients in the placebo group) are diluted with normal saline to 6 ml.

6.3.2 The study drug mixtures are administered by inhalation in the night (7 pm) before surgery, in the morning (7 am) of surgery, and then every 12 hours until postoperative day 2, resulting in a total number of 7 inhalations.

6.3.3 Study drug inhalation is performed with a jet nebulizer (DNA100, manufactured by Congren Medical Equipment Co., Ltd., Xiamen, China) for extubated patients or a vibrating mash nebulizer (Agrogen Professional Nebulizer System, AG-AP6000CH, Aerogen Ltd, Ireland) for patients with endotracheal intubation (mechanical ventilation).

6.4 Follow-up of patients

6.4.1 Patients will be followed up twice daily until postoperative day 2, once daily from postoperative days 3 to 6, and then weekly until 30 days after surgery. Discharged patients will be contacted by telephone.

6.4.2 Investigator will assess the development of adverse events, PPCs, and postoperative extrapulmonary complications according to clinical features, laboratory tests, and instrumental examination results. Data in the Anesthesia Information System and the Electronic Medical Record System will be achieved.

6.5 Interruption of study drug administration

6.5.1 For patients who develop adverse events or clinical deterioration, the dose of study drugs can be decreased or the study drug administration can be discontinued on request of participants, attending surgeons or investigators.

6.5.2 These situations will be recorded in the CRFs.

7. Anesthesia and perioperative management

7.1 Premedication and prophylactic antibiotics

7.1.1 Anesthetic premedication is not administered.

7.1.2 Prophylactic antibiotics (the first- or second-generation cephalosporin, with or without metronidazole, or cephamycins) are administered 30 minutes to 1 hour before surgical incision. The duration of prophylactic antibiotic treatment is less than 48 hours. The choice of antibiotics and the duration of prophylactic antibiotic therapy are decided according to the Guiding Principles of Clinical Use of Antibiotics (published by Chinese National Health and Family Planning Commission in 2015; http://www.nhc.gov.cn/ewebeditor/uploadfile/2015/09/20150928170007470.pdf). Therapeutic antibiotic agents are only indicated in patients who are diagnosed with infection.

7.2 Intraoperative monitoring

7.2.1 Routine monitoring includes electrocardiogram, pulse oxygen saturation, non-invasive blood pressure, airway pressure, end-tidal carbon dioxide concentration, inhaled and expired concentrations of anesthetics, nasopharyngeal temperature, urine output, and bispectral index (BIS) value.

7.2.2 Intra-arterial pressure (and derivative parameters such as stroke volume variation by FloTrac system) and central venous pressure are monitored when considered necessary.

7.3 Anesthesia care

7.3.1 General anesthesia with endotracheal intubation is performed for all patients.

7.3.1.1 General anesthesia is induced with intravenous propofol or etomidate, sufentanil, and rocuronium or cisatracurium, and maintained with intravenous propofol, remifentanil and/or sufentanil, and rocuronium or cisatracurium, with or without inhalational sevoflurane and/or nitrous oxide.

7.3.1.2 Mechanical ventilation is established with a tidal volume from 6 to 8 ml/kg during two-lung ventilation or from 5 to 7 ml/kg during one-lung ventilation, a positive end-expiratory pressure from 0 to 5 cm H_2O, a frequency from 12 to 16 breaths per minute, and ≤50% oxygen (mixed with nitrous oxide or air). Higher oxygen concentration is administered during one-lung ventilation.

7.3.1.3 BIS value is maintained between 40 and 60. Hemodynamic management and fluid infusion are performed according to routine practice. Packed red blood cells are transfused to maintain a hemoglobin level above 7 g/dl.

7.3.2 Neuraxial or peripheral nerve block is performed depending on the decision of attending anesthesiologists.

7.4 Postoperative care

7.4.1 At the end of surgery, patients are transferred to the postoperative care unit (PACU) after extubation and monitored for at least 30 minutes before being sent back to the general wards.

7.4.2 For patients who are admitted to the ICU with endotracheal intubation:

7.4.2.1 Propofol and/or dexmedetomidine sedation are provided in addition to opioid analgesia.

7.4.2.2 Mechanical ventilation is performed with bilevel ventilation mode (established with a low level between 3 and 5 cm H_2O, a high level between 13 and 16 cm H_2O, and a trigger flow between 2 and 3 l/min; PB 840 ventilator, Puritan-Bennett Corp., Mervue, Ireland).

7.4.2.3 Patients are extubated as soon as possible when they meet the criteria of extubation (regain consciousness and airway protective reflex, normal circulatory status and temperature, no residual neuromuscular blockade).

7.4.2.4 For patients requiring mechanical ventilation of more than 24 hours, a ventilator weaning protocol is executed in order to extubate patients early.[13]

7.4.2.5 For patients with acute respiratory failure, non-invasive positive pressure ventilation is used to promote early extubation or to avoid reintubation.

7.4.3 For all patients after extubation, low-flow supplemental oxygen is routinely provided during the day of surgery.

7.4.4 Postoperative analgesia

7.4.4.1 Patient-controlled analgesia (either intravenous or epidural) is provided for up to 3 days after surgery.

7.4.4.2 Nonsteroidal anti-inflammatory drugs are administered when considered necessary and without contraindications.

7.4.4.3 The target of analgesia is to maintain a Numeric Pain Rating Scale (NPRS, an 11-point scale where 0=no pain and 10=the worst pain) pain score of ≤3.

7.4.5 Other treatments including chest physiotherapy, early mobilization, removal of nasogastric tube, parenteral or enteral nutrition, and intravenous respiratory medications (such as theoclears and expectorants, and glucocorticoids) are administered according to routine practice.

7.5 Management of PPCs

7.5.1 The occurrence of PPCs will be carefully monitored by observing vital signs and lung conditions. Auxiliary examinations such as arterial blood gas analysis, blood routine test and chest X-ray will be performed when considered necessary.

7.5.2 Any PPCs will be actively treated once diagnosed. Treatment of PPCs will be conducted according to routine practice and clinical guidelines, including chest physiotherapy, use of bronchodilators and theoclears/expectorants, administration of antibiotics, and others.

7.5.3 Consultation of pulmonary physicians or other specialists will be asked when considered necessary.

7.6 Allowed and prohibited medications

7.6.1 Anticholinergics are prohibited unless being used for the treatment of bradycardia, in which case atropine is administered.

7.6.2 During the study period, inhalations of any respiratory medications other than the study drugs for prophylactic purpose are prohibited.

7.6.3 For patients who develop PPCs, inhalation of respiratory medications is allowed.

8. Data collection

8.1 Baseline data

8.1.1 Demographic data, including date of birth, sex, body mass index, and education level.

8.1.2 Diagnosis and medical history, including surgical diagnosis, comorbidities, medical therapy, drinking and smoking history, food and drug allergy, and previous history of surgery and anesthesia.

8.1.3 Main results of laboratory tests and instrumental examinations, including blood gas and chest X-ray.

8.1.4 General status, including Charlson Comorbidity Index, American Society of Anesthesiologists classification, and New York Heart Association classification.

8.1.5 Medication history, especially for the respiratory system.

8.2 Intraoperative data

8.2.1 Location, type, name, and duration of surgery.

8.2.2 Type and duration of anesthesia, types and doses of anesthetics and other medications used during anesthesia.

8.2.3 Parameters of mechanical ventilation.

8.2.4 Fluid balance (including fluid infusion, estimated blood loss, and urine output) and transfusion of blood products.

8.3 Postoperative data

8.3.1 The intensity of pain both at rest and with coughing are assessed daily (7:00-8:00 am) during the first 3 postoperative days with the numeric rating scale (NRS; an 11-point scale where 0=no pain and 10=the worst pain). The final status of patient-controlled analgesia pump use (completed use, dose reduction, early termination, change to other analgesic method) is recorded. The use of supplemental analgesics and other medications (including antiemetics) are recorded.

8.3.2 Respiratory management, such as prophylactic chest physiotherapy, and intravenous and/or oral respiratory medications. Prophylactic aerosol inhalation is prohibited but, if used, will be documented.

8.3.3 Gastrointestinal tract management, such as placement of nasogastric tube, administration of parenteral nutrition, and use of proton pump inhibitors.

8.3.4 ICU admission after surgery. For patients who are admitted to ICU after surgery, the percentage with endotracheal intubation, the duration of mechanical ventilation, and the length of ICU stay are recorded.

8.3.5 Occurrence of PPCs, which is generally defined as any condition that affects the respiratory system, may adversely influence patients' outcome and requires therapeutic intervention.[1,2] The diagnostic criteria of each individual PPC are listed in Table 2.[4] We adopt the Clavien-Dindo Classification to categorize PPCs into five major grades, and PPCs of grade II or above will be used to calculate the incidence of PPCs (Table 3).[14,15] If a PPC occurs during the follow-up period, the date of earliest diagnosis and the evidences according to which the diagnosis is made will be documented.

Table 2. Definition of postoperative pulmonary complications.

Complication	Definition
Respiratory infection	Receiving antibiotics for a suspected respiratory infection and meet at least one of the following criteria: new or changed sputum, new or changed lung opacities, fever, leukocyte count >12×10^9/L.
Respiratory failure	PaO_2 <60 mmHg on room air, a ratio of PaO_2 to inspired oxygen fraction <300, or arterial oxyhemoglobin saturation measured with pulse oximetry <90% and requiring oxygen therapy.
Pleural effusion	Chest X-ray demonstrating blunting of the costophrenic angle, loss of the sharp silhouette of the ipsilateral hemidiaphragm in upright position, evidence of displacement of adjacent anatomical structures, or (in supine position) a hazy opacity in one hemithorax with preserved vascular shadows.
Atelectasis	Lung opacification with a shift of the mediastinum, hilum, or hemidiaphragm toward the affected area, and compensatory overinflation in the adjacent nonatelectatic lung.
Pneumothorax	Air in the pleural space with no vascular bed surrounding the visceral pleura.
Bronchospasm	Newly detected expiratory wheezing treated with bronchodilators.
Aspiration pneumonitis	Acute lung injury after inhalation of regurgitated intragastric contents.

Table 3. Criteria of Clavien-Dindo classification of postoperative pulmonary complications.

Complications	I	II	IIIa	IIIb	IVa	IVb	V
Respiratory infections	Clinical observation or diagnostic evaluation only; intervention not indicated except for nebulizers, expectorants, or lung physiotherapy (e.g., postural drainage)	Medical management indicated (e.g., antibiotics)	Intervention not under general anesthesia (e.g., bronchoscopic aspiration, tracheal puncture)	Intervention under general anesthesia (e.g., tracheostomy under general anesthesia or sedation)	Mechanical ventilation indicated	Sepsis or multiple organ failure	Death
Respiratory failure	_	_	_	_	Mechanical ventilation indicated	Sepsis or multiple organ failure	Death
Pleural effusion	Clinical observation or diagnostic evaluation only; intervention not indicated (drainage only through existing drainage tube)	Medical management indicated (e.g., diuretics)	Intervention not under general anesthesia (e.g., image-guided drain placement or thoracentesis including drain replacement indicated)	Intervention under general anesthesia indicated	Mechanical ventilation indicated	Multiple organ failure	Death
Atelectasis	Clinical observation or diagnostic evaluation only; intervention not indicated, except for nebulizers, expectorants, or lung physiotherapy (e.g., postural drainage)	Medical management indicated (e.g., antibiotics)	Intervention not under general anesthesia (e.g., bronchoscopic aspiration, tracheal puncture)	Intervention under general anesthesia (e.g., tracheostomy under general anesthesia or sedation)	Mechanical ventilation indicated	Sepsis or multiple organ failure	Death
Pneumothorax	Clinical observation or diagnostic evaluation only;	_	Intervention not under general anesthesia (e.g.,	Intervention under general anesthesia	Mechanical ventilation	Multiple organ	Death

	intervention not indicated (drainage only through existing drainage tube)		closed drainage of thoracic cavity or thoracentesis including drain replacement indicated)	indicated	indicated	failure	
Bronchospasm	Clinical observation or diagnostic evaluation only; intervention not indicated except for nebulizers (bronchodilators not included), expectorants, or lung physiotherapy (e.g., postural drainage)	Medical management indicated (e.g., bronchodilators)	_	_	Mechanical ventilation indicated	Multiple organ failure	Death
Aspiration pneumonitis	Clinical observation or diagnostic evaluation only; intervention not indicated except for nebulizers, expectorants, or lung physiotherapy (e.g., postural drainage)	Medical management indicated (e.g., antibiotics, or bronchodilators, or glucocorticoids)	Intervention not under general anesthesia (e.g., bronchoscopic aspiration)	Intervention under general anesthesia (e.g., tracheostomy under general anesthesia or sedation)	Mechanical ventilation indicated	Sepsis or multiple organ failure	Death

8.3.6 Occurrence of extrapulmonary complications. Postoperative extrapulmonary complications are defined as complications other than PPCs that occur within 30 days after surgery and require therapeutic intervention, i.e., grade II or above on the Clavien-Dindo Classification.[14,15] For each diagnosed extrapulmonary complication, the date of earliest diagnosis and the evidences according to which the diagnosis is made will also be documented.

8.3.7 Length of hospital stay after surgery.

8.3.8 All-cause 30-day mortality after surgery.

9. Outcomes

9.1 Primary outcome

The incidence of PPCs within 30 days after surgery.

9.2 Secondary outcomes

9.2.1 Time to onset of PPCs, i.e., from end of surgery to first diagnosis of PPCs.

9.2.2 Occurrence and number of individual PPCs.

9.2.3 Incidence of postoperative extrapulmonary complications within 30 days after surgery.

9.2.4 Length of stay in hospital after surgery.

9.2.5 All-cause 30-day mortality.

10. Adverse events

10.1 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.

10.2 Monitoring adverse events

In the present study, adverse events will be monitored from initiation of study drug administration (the day before surgery) until postoperative day 3. Any developed adverse event will be monitored until it is completely resolved or the treatment is terminated.

10.3 Predicted adverse events in this study

10.3.1 Exceptional adverse events that may occur during anesthesia/surgery include (but not limited to) the following: hypertension (systolic blood pressure >180 mmHg or an increase of more than 30% from baseline), hypotension (systolic blood pressure <90 mmHg or a decrease of more than 30% from baseline), tachycardia (heart rate >100 beats per minute), bradycardia (heart rate <40 beats per minute), desaturation (PaO_2 <60 mmHg or SpO_2 <90%), high airway pressure (peak airway pressure >40 cm H_2O), hypercapnia ($PaCO_2$ >50 mmHg), difficult airway (failed intubation attempt >3 times), massive bleeding (estimated blood loss >1000 ml), other new-onset arrhythmia (atrial fibrillation, supraventricular tachycardia, ventricular premature beats, atrioventricular block, and cardiac arrest), etc.

10.3.2 Other adverse events that may occur during the perioperative period include (but not limited to) the following: nausea and vomiting, dry mouth, flushing, dizziness, palpitation, somnolence, cough, sneeze, delirium, etc. These adverse events are diagnosed according to patient's complains and symptoms (sought by nondirective questioning), physical and mental signs, as well as results of

laboratory tests and instrumental examinations. Delirium is diagnosed according to the Confusion Assessment Method for the Intensive Care Unit.

10.4 Management

10.4.1 Any developed adverse events will be managed according to patients' condition and routine practice.

10.4.2 The study intervention can be stopped temporarily or permanently if considered necessarily by the attending anesthesiologists or surgeons. The time and reasons of intervention interruption will be recorded.

10.5 Record

10.5.1 The information including the diagnosis, date(s) of onset and resolution (if applicable), severity of influence, relationship with intervention, treatment, and outcomes (sequelae) are recorded.

10.5.2 Any adverse event should be followed up until it is completely resolved or therapy terminated.

10.5.3 The occurrence of adverse events will be reported to the ethics committee in the final report.

11. Severe adverse events

11.1 Definition

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

11.2 Monitoring severe adverse events

In the present study, severe adverse events will be monitored from initiation of study drug administration (the day before surgery) until postoperative day 3. Any developed severe adverse event will be monitored up until it is completely resolved or the treatment is terminated.

11.3 Management

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

11.4 Record and report

11.4.1 In case of any severe adverse event, apart from active treatment and record as above, the principal investigator and the Clinical Research Ethics Committee of Peking University First Hospital will be informed within 24 hours in report form.

11.4.2 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.

11.4.3 Any severe adverse event must be followed up until it is completely resolved or the treatment is terminated.

11.4.4 Harmful consequence resulted directly from study participation will be compensated according to the corresponding legal provisions.

12. Data management

12.1 Investigators are trained to record data promptly, completely, and correctly in the CRFs according to original observation.

12.2 On-site auditing will be performed by Peking University Clinical Research Institute for at least three times during the study period, with reports and feedbacks provided in written form.

12.3 Data entry will be performed with EpiData software (Version 3.1). Dataset will be locked when the following tasks have been completed: (1) patient recruitment and follow-up are completed, (2) data collection, entry and double-checked is performed without errors, and (3) all data queries have been solved.

12.4 Data management will be performed by Peking University Clinical Research Institute who is independent from the investigators and sponsors.

13. Statistical analysis

13.1 General principles

13.1.1 Baseline/perioperative data and outcomes will be described according the data type and distribution. Continuous variables with normal or approximate normal distribution will be expressed as mean ± standard deviation (SD); continuous variables with non-normal distribution will be expressed as median (minimum, maximum; or interquartile range, IQR). Categorical variables will be presented as number of cases (percentage). Time-to-events variables will be presented as mean/median time (95% CI).

13.1.2 For each hypothesis, two-tailed tests will be used in all statistical analysis, and $p<0.05$ will be considered statistically significant. For the treatment-by-covariate interaction in predefined subgroup analyses, a $p<0.10$ will be defined as statistically significant.

13.1.3 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, receive at least part of study intervention, and undergo surgery. Also, we will do per-protocol analysis for the primary outcome, in this case, drop-out patients will be excluded. Safety outcomes are analyzed in the safety population, i.e., all patients are analyzed in the group to which they are randomized and receive at least part of study intervention.

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 Between-group differences are compared using the absolute standardized differences (ASDs), which are defined as the absolute difference in means, mean ranks or proportions divided by the pooled standard deviation and calculated with the formula published by Austin.[16] Baseline variables with an ASD ≥0.196 ($ASD = 1.96 \times \sqrt{(n1+n2)/(n1 \times n2)}$) will be considered imbalanced between the two groups and adjusted for in all analyses if considered necessary.

13.4 Intra- and postoperative variables

Numeric variables will be analyzed using the 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. Ordinal variables will be compared with Wilcoxon test. Missing data will not be replaced.

13.5 Effectiveness analysis

13.5.1 Primary outcome

13.5.1.1 The incidence of PPCs within 30 days after surgery will be calculated. Comparison between groups will be performed using chi-square test, with difference between groups expressed as relative risk (95% CI). The upper limit of the 95% CI of less than 1 is regarded as statistical superiority. Missing data will not be replaced. The number-needed-to-treat is calculated as the reciprocal of the absolute risk reduction.

13.5.1.2 The relative risks and corresponding 95% CIs are calculated to assess the treatment effect in predefined subgroups. The interactions between treatment effect and predefined factors will be assessed separately with logistic regression models. The predefined factors include age, sex, body mass index, respiratory disease, respiratory symptoms in last month, cigarette smoking, type of anesthesia, location of surgery, and type of surgery. The relative risk (and 95% CI) for each subgroup and the p values of treat-by-covariate interactions will be displayed in a forest plot.

13.5.2 Secondary/exploratory outcomes

13.5.2.1 Categorical variables (percentages of ICU admission and ICU admission with endotracheal intubation, incidence of individual PPCs within 30 days, incidence of extrapulmonary complications within 30 days, and all-cause 30-day mortality) will be compared using the chi-square test, continuity correction chi-square test or Fisher exact test. The estimated relative risk (RR) and 95% CI will be provided. Missing data will not be replaced.

13.5.2.2 Time-to-event variables (time to onset of PPCs, duration of mechanical ventilation, length of stay in ICU, and length of stay in hospital after surgery) will be analyzed using Kaplan-Meier survival analyses, with differences between groups tested using the log-rank method; univariable Cox proportional hazards models will be used to calculate hazard ratios (HRs) and 95% CIs. Patients who die within 30 days after surgery will be censored at the time of death.

13.5.2.3 Discrete variables (number of individual PPCs) will be analyzed using the Mann-Whitney U test, with median difference and 95% CI calculated with the Hodges-Lehmann estimator, or using the chi-square test, continuity correction chi-square test or Fisher exact test. Missing data will not be replaced.

13.6 Safety analysis

13.6.1 Describe the occurrence of adverse events in each group.

13.6.2 Describe the management of adverse events when appropriate.

13.6.3 Describe the occurrence of severe adverse events.

13.6.4 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. The estimated risks ratio (RR) and 95% CI will be provided.

13.6.5 Missing data will not be replaced.

14. Quality control and quality assurance

14.1 General principles

14.1.1 The principle of randomization and blinding must be strictly followed.

14.1.2 The storage and return of study drugs must follow relevant storage regulations.

14.1.3 The instruments and equipment used in the study should have strict quality standards. Investigators should ensure proper operation of these instruments and equipment.

14.1.4 The investigator training must be performed before starting the study. The study protocol is thoroughly explained during the training process. Investigators are trained to record data promptly, completely, and correctly in the CRFs according to original observation. If there are any abnormal findings, investigators should reconfirm and record properly to ensure the reliability of the data.

14.1.5 All statistical analyses will be performed by statisticians.

14.2 Inspection of study conduct

14.2.1 The study will be audited by the Peking University Clinical Research Institute.

14.2.2 A project auditor will be designated by the Peking 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 auditor.

14.2.3 Before and during the study period, the project auditor will visit the study center for initiation inspection, regular inspection, and end of study inspection.

14.2.4 The contents of audit 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. For the first three participants recruited in the study, 100% of the original data of will be checked and verified.

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. Investigators will be asked to correct or replenish data when necessary.

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 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.

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

14.2.4.11 To verify the trial management in the study center, 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. The report should include date, time, name of inspector, and the problems found during inspection. The project inspector will inform the principal investigator about the identified problems and will discuss the approaches to solve these problems. In case of important problems, such as those regarding participant safety, adherence to the study protocol or Good Clinical Practice principles, the project inspector should report to the management office of the Clinical Research Ethics Committee of Peking University First Hospital.

14.3 Inspection of data quality

14.3.1 Data manager from the Peking University Clinical Research Institute will recheck data according to the logical relations and to identify the existence of protocol deviation and out of normal limit. For the drop-out or missing data or data with logical contradictions, query forms will be sent to the investigators. The investigators are responsible to reply queries, and to verify or correct data.

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 Clinical Research Ethics Committee of Peking University First Hospital 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 Ethics Committee. The study has

been *a priori* registered with Chinese Clinical Trial Registry (ChiCTR-IPC-15006603) and ClinicalTrial.gov (NCT02644876).

15.2 Written informed consent

Investigators responsible for recruiting participants must have been trained and qualified by the principal investigator. 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. The filing cabinets storing the study documents will be locked. Apart from the study investigators, only authorized inspectors from the Peking University Clinical Research Institute or members from the Clinical Research Ethics Committee of Peking University First Hospital are allowed to access the information after obtaining consents from the participants.

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 study is supported by National Key R&D Program of China (2018YFC2001800), a grant from Chinese Society of Cardiothoracic and Vascular Anesthesiology (2017-01), and a scientific research fund from Peking University First Hospital (2015QN025). Study drugs are manufactured and supplied by Chengdu List Pharmaceutical Co, Ltd, Sichuan, China. The investigators declare no conflict of interests.

19. References

1. Shander A, Fleisher LA, Barie PS, Bigatello LM, Sladen RN, Watson CB: Clinical and economic burden of postoperative pulmonary complications: patient safety summit on definition, risk-reducing interventions, and preventive strategies. Crit Care Med 2011; 39: 2163-72

2. Ghaferi A, Birkmeyer J, Dimick J: Variation in Hospital Mortality Associated with Inpatient Surgery. N Engl J Med. 2009; 361: 1368-75

3. Molena D, Mungo B, Stem M, Lidor AO: Incidence and risk factors for respiratory complications in patients undergoing esophagectomy for malignancy: a NSQIP analysis. Semin Thorac Cardiovasc Surg 2014; 26: 287-94

4. Canet J, Gallart L, Gomar C, Paluzie G, Vallès J, Castillo J, Sabaté S, Mazo V, Briones Z, Sanchis J, Group， A: Prediction of postoperative pulmonary complications in a population-based surgical cohort. Anesthesiology 2010; 113: 1338-50

5. Mazo V, Sabaté S, Canet J, Gallart L, de Abreu M, Belda J, Langeron O, Hoeft A, Pelosi P: Prospective external validation of a predictive score for postoperative pulmonary complications. Anesthesiology 2014; 121: 219-31

6. Duggan M, Kavanagh B: Pulmonary atelectasis: a pathogenic perioperative entity. Anesthesiology. 2005; 102: 838-54

7. Nojiri T, Inoue M, Yamamoto K, Maeda H, Takeuchi Y, Nakagiri T, Shintani Y, Minami M, Sawabata N, Okumura M: Inhaled tiotropium to prevent postoperative cardiopulmonary complications in patients with newly diagnosed chronic obstructive pulmonary disease requiring lung cancer surgery. Surg Today 2014; 44: 285-90

8. Fushida S, Oyama K, Kaji M, Hirono Y, Kinoshita J, Tsukada T, Nezuka H, Nakano T, Noto M, Nishijima K, Fujimura T, Ohta T: A randomized multicenter Phase II study of perioperative tiotropium intervention in gastric cancer patients with chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis 2015; 10: 2177-83

9. Barnes P: Muscarinic receptor subtypes in airways. Life Sci 1993; 52: 521-7

10. Xiao H, Liao Z, Tong R: Penehyclidine hydrochloride: a potential drug for treating COPD by attenuating Toll-like receptors. Drug Des Devel Ther 2012; 6: 317-22

11. Xiao H, Liao Z, Meng X, Yan X, SJ. C, Mo Z: Effects of the selective muscarinic receptor antagonist penehyclidine hydrochloride on the respiratory tract. Pharmazie 2009; 64: 337-41

12. Yan T, Wang D: Effects of penehyclidine inhalation on postoperative pulmonary complications of elderly patients after long-duration surgery. Zhonghua Yi Xue Za Zhi. 2014; 94: 122-6.

13. Haas CF, Loik PS: Ventilator discontinuation protocols. Respir Care 2012; 57: 1649-62

14. Katayama H, Kurokawa Y, Nakamura K, Ito H, Kanemitsu Y, Masuda N, Tsubosa Y, Satoh T, Yokomizo A, Fukuda H, Sasako M: Extended Clavien-Dindo classification of surgical complications: Japan Clinical Oncology Group postoperative complications criteria. Surg Today 2016; 46: 668-85

15. Dindo D, Demartines N, Clavien PA: Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. Ann Surg 2004; 240: 205-13

16. Austin PC: An Introduction to Propensity Score Methods for Reducing the Effects of Confounding in Observational Studies. Multivariate Behavioral Research 2011; 46: 399-424

Summary of changes from the original study protocol approved by the ethics committee

Number	Version	Date of version	Drafter, Reviser/ Chairman	Contents of revision	Reasons for revision
1	V 1.0	Apr. 8, 2015	YT, D-XW/ WDX	(This was the first version approved by the Ethics Committee).	---
2	V 2.0	May 7, 2015	YT, D-XW/ WDX	Reduced the dose of study drugs from 1 ml (penehyclidine 1 mg) every 12 h to 0.5 ml (penehyclidine 0.5 mg) every 12 h (Figure 1 and clause 6.3.1) before initiating the trial.	According to existing evidence, inhalation of 0.5 mg penehyclidine is effective for bronchodilation.
3	V 3.0	Mar. 16, 2016	YT, D-XW/ WDX	Revised inclusion criteria, i.e., added "For those who undergo thoracoscopic or laparoscopic surgery, the expected length of incision must be 5 cm or more" (clause 4.1.2).	To clarify the inclusion criteria for high-risk patients undergoing thoracoscopic or laparoscopic surgery.
4	V 4.0	Feb. 13, 2017	YT, D-XW/ WDX	Added Clavien-Dindo Classification to categorize PPCs ("…, PPCs of grade II or above will be used to calculate the incidence of PPCs" [clause 8.3.5]) and extrapulmonary complications (clause 8.3.6).	To clarify the classification of PPCs and extrapulmonary complications.
5	V 5.1	Dec. 4, 2017	YT, D-XW/ WDX	1. Revised inclusion criteria, i.e., limited to "noncardiac thoracic surgery" (clause 4.1.2). 2. Added criteria of drop-out (clause 4.3). 3. Added criteria of elimination (clause 4.4). 4. Added method of emergency unblinding: "In case of an emergency (such as development of a severe adverse event), attending physicians or surgeons can request unmasking of the treatment allocation …" (clause 5.3.2). 5. Added description and preservation of study drugs (clause 6.1). 6. Added description of inhalation device: "Study drug inhalation is performed with a jet nebulizer (…) for extubated patients or a vibrating mash nebulizer (…) for patients with endotracheal intubation …" (clause 6.3.3). 7. Clarified the frequency of follow-up: "Patients will be followed up twice daily until postoperative day 2, once daily from	1. To clarify the inclusion criteria. 2. To clarify criteria of drop-out. 3. To clarify criteria of elimination. 4. To clarify the method of unblinding in case of an emergency. 5. To clarify the specification and preservation of study drugs. 6. To clarify the device used for inhalation. 7. To clarify the frequency of postoperative follow-up.

				postoperative days 3 to 6, and then weekly until 30 days after surgery. …" (Figure 1 and clause 6.4.1). 8. Added description of anesthesia and perioperative management (Figure 1 and clauses 7.1 to 7.5). 9. Clarified the duration of monitoring for adverse events (clause 10.2) and severe adverse events (clause 11.2). 10. Add description of study auditing and data management (clauses 12.2 and 12.4; clauses 14.2 and 14.3).	8. To clarify methods of anesthesia and perioperative management. 9. To clarify the duration for monitoring (severe) adverse events. 10. To clarify the contents of study auditing and data management.

Original statistical analysis plan

Original statistical analysis plan as reported in the original trial protocol.

1. Sample size estimation

In our recent randomized controlled trial, PPCs occurred in 51.7% of patients in the placebo group.[1] Mazo et al.[2] reported incidence of PPCs from 42.1% to 44.9% in high-risk patients (ARISCAT risk score ≥45). In previous studies of COPD patients, perioperative inhalation of tiotropium reduced the incidence of PPCs by 30-62.5% (relative reduction).[3,4] In the present study, we assume that the incidence of PPCs will be 42% in high-risk patients of the placebo group, and prophylactic penehyclidine inhalation will reduce the incidence of PPCs to 32% (i.e., a 24% reduction). The calculated sample size that will provide 80% power to detect a superiority of this difference at a two-sided significance level of 0.05 is 365 patients in each group. Considering a dropout rate of about 15%, we plan to enroll 432 patients in each group. Since the safety of intervention has been confirmed by our previous pilot study, interim analysis is not planned. The sample size calculation was performed with PASS 2008 software.

2. Outcomes

2.1 Primary outcome

The incidence of PPCs within 30 days after surgery.

2.2 Secondary outcomes

2.2.1 Time to onset of PPCs, i.e., from end of surgery to first diagnosis of PPCs.

2.2.2 Occurrence and number of individual PPCs.

2.2.3 Incidence of postoperative extrapulmonary complications within 30 days after surgery.

2.2.4 Length of stay in hospital after surgery.

2.2.5 All-cause 30-day mortality.

3. Statistical analysis

3.1 General principles

3.1.1 All statistical analyses will be performed with SPSS 14.0 software package (SPSS, Chicago, IL, USA) and SAS 9.2 software (SAS Institute, Cary, NC, USA) by statisticians in the Department of Biostatistics of Peking University First Hospital.

3.1.2 Since the safety of intervention has been confirmed by our previous pilot study, interim analysis is not planned.

3.1.3 Analyses will be performed on an intention-to-treat basis, that all subjects will be analyzed in the group to which they were assigned. For the primary outcome (the incidence of PPCs within 30 days after surgery), per protocol analysis will also be performed.

3.2 Baseline data

3.2.1 Statistical description will be provided for baseline data such as demographic variables, medical history, preoperative medications, and perioperative management.

3.3 Outcome analysis

3.3.1 Primary outcome (the incidence of PPCs within 30 days after surgery) will be compared with chi-square test. The difference between groups and the 95% confidence interval of the difference will be calculated.

3.3.2 For secondary outcomes, continuous variables with normal distribution will be analyzed using an independent sample t-test; continuous variables with non-normal distribution or ranked data will be analyzed using Mann-Whitney U test; categorical variables will be analyzed using the chi-square test, continuity correction chi-square test or Fisher exact test; time-to-event results will be analyzed using the Kaplan-Meier estimator, and the differences between groups will be tested by the log-rank test. Subgroup analyses will be performed according to the type of the surgery (upper abdominal or thoracic).

4. References

1. Yan T, Wang D: Effects of penehyclidine inhalation on postoperative pulmonary complications of elderly patients after long-duration surgery. Zhonghua Yi Xue Za Zhi 2014; 94: 122-6

2. Mazo V, Sabaté S, Canet J, Gallart L, de Abreu M, Belda J, Langeron O, Hoeft A, Pelosi P: Prospective external validation of a predictive score for postoperative pulmonary complications. Anesthesiology 2014; 121: 219-31

3. Nojiri T, Inoue M, Yamamoto K, Maeda H, Takeuchi Y, Nakagiri T, Shintani Y, Minami M, Sawabata N, Okumura M: Inhaled tiotropium to prevent postoperative cardiopulmonary complications in patients with newly diagnosed chronic obstructive pulmonary disease requiring lung cancer surgery. Surg Today 2014; 44: 285-90

4. Fushida S, Oyama K, Kaji M, Hirono Y, Kinoshita J, Tsukada T, Nezuka H, Nakano T, Noto M, Nishijima K, Fujimura T, Ohta T: A randomized multicenter Phase II study of perioperative tiotropium intervention in gastric cancer patients with chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis 2015; 10: 2177-83

Final statistical analysis plan

Final statistical analysis plan, except exploratory analysis, was completed prior to unblinding group assignment and outcome analysis.

1. Sample size estimation

In our recent randomized controlled trial, PPCs occurred in 51.7% of patients in the placebo group.[1] Mazo et al.[2] reported incidence of PPCs from 42.1% to 44.9% in high-risk patients (ARISCAT risk score ≥45). In previous studies of COPD patients, perioperative inhalation of tiotropium reduced the incidence of PPCs by 30-62.5% (relative reduction).[3,4] In the present study, we assume that the incidence of PPCs will be 42% in high-risk patients of the placebo group, and prophylactic penehyclidine inhalation will reduce the incidence of PPCs to 32% (i.e., a 24% reduction). The calculated sample size that will provide 80% power to detect a superiority of this difference at a two-sided significance level of 0.05 is 365 patients in each group. Considering a dropout rate of about 15%, we plan to enroll 432 patients in each group. Since the safety of intervention has been confirmed by our previous pilot study, interim analysis is not planned. The sample size calculation was performed with PASS 2008 software.

2. Outcomes

2.1 Primary outcome

The incidence of PPCs within 30 days after surgery.

2.2 Secondary outcomes

2.2.1 Time to onset of PPCs, i.e., from end of surgery to first diagnosis of PPCs.

2.2.2 Occurrence and number of individual PPCs.

2.2.3 Incidence of postoperative extrapulmonary complications within 30 days after surgery.

2.2.4 Length of stay in hospital after surgery.

2.2.5 All-cause 30-day mortality.

3. Statistical analysis

3.1 General principles

3.1.1 Baseline/perioperative data and outcomes will be described according the data type and distribution. Continuous variables with normal or approximate normal distribution will be expressed as mean ± standard deviation (SD), while continuous variables with non-normal distribution will be expressed as median (minimum, maximum; or interquartile range, IQR). Categorical variables will be presented as number of cases (percentage). Time-to-events variables will be presented as mean/median time (95% CI).

3.1.2 For each hypothesis, two-tailed tests will be used in all statistical analysis, and $p < 0.05$ will be considered statistically significant. For the treatment-by-covariate interaction in predefined subgroup analyses, a $p < 0.10$ will be defined as statistically significant.

3.1.3 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, receive at least part of study

intervention, and undergo surgery. Also, we will do per-protocol analysis for the primary outcome, in this case, drop-out patients will be excluded. Safety outcomes are analyzed in the safety population, i.e., all patients are analyzed in the group to which they are randomized and receive at least part of study intervention.

3.2 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.

3.3 Demographics and baseline characteristics

3.3.1 Demographics and baseline data will be presented.

3.3.2 Between-group differences are compared using the absolute standardized differences (ASDs), which are defined as the absolute difference in means, mean ranks or proportions divided by the pooled standard deviation and calculated with the formula published by Austin.[5] Baseline variables with an ASD $\geq$0.196 (ASD $=1.96\times\sqrt{(n1+n2)/(n1\times n2)}$) will be considered imbalanced between the two groups and adjusted for in all analyses if considered necessary.

3.4 Intra- and postoperative variables

Continuous variables will be analyzed using the 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; ordinal variables will be compared with Wilcoxon test. Missing data will not be replaced.

3.5 Effectiveness analysis

3.5.1 Primary outcome

3.5.1.1 The incidence of PPCs within 30 days after surgery will be calculated. Comparison between groups will be performed using chi-square test, with difference between groups expressed as relative risk (95% CI). The upper limit of the 95% CI of less than 1 is regarded as statistical superiority. Missing data will not be replaced. The number-needed-to-treat is calculated as the reciprocal of the absolute risk reduction.

3.5.1.2 The relative risks and corresponding 95% CIs are calculated to assess the treatment effect in predefined subgroups. The interactions between treatment effect and predefined factors will be assessed separately with logistic regression models. The predefined factors include age, sex, body mass index, respiratory disease, respiratory symptoms in last month, cigarette smoking, type of anesthesia, location of surgery, and type of surgery. The relative risk (and 95% CI) for each subgroup and the p values of treat-by-covariate interactions will be displayed in a forest plot.

3.5.2 Secondary/exploratory outcomes

3.5.2.1 Categorical variables (percentages of ICU admission and ICU admission with endotracheal intubation, incidence of individual PPCs within 30 days, incidence of extrapulmonary complications within 30 days, and all-cause 30-day mortality) will be compared using the chi-square test, continuity correction chi-square test or Fisher exact test. The estimated relative risk (RR) and 95% CI will be provided. Missing data will not be replaced.

3.5.2.2 Time-to-event variables (time to onset of PPCs, duration of mechanical ventilation, length of stay in ICU, and length of stay in hospital after surgery) will be analyzed using Kaplan-Meier survival analyses, with differences between groups tested using the log-rank method; univariable Cox proportional hazards models will be used to calculated hazard ratios (HRs) and 95% CIs. Patients who die within 30 days after surgery will be censored at the time of death.

3.5.2.3 Discrete variables (number of individual PPCs) will be analyzed using the Mann-Whitney U test, with median difference and 95% CI calculated with the Hodges-Lehmann estimator, or using the

chi-square test, continuity correction chi-square test or Fisher exact test. Missing data will not be replaced.

3.6 Safety analysis

3.6.1 Describe the occurrence of adverse events in each group.

3.6.2 Describe the management of adverse events when appropriate.

3.6.3 Describe the occurrence of severe adverse events.

3.6.4 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. The estimated risks ratio (RR) and 95% CI will be provided.

3.6.5 Missing data will not be replaced.

4. References

1. Yan T, Wang D: Effects of penehyclidine inhalation on postoperative pulmonary complications of elderly patients after long-duration surgery. Zhonghua Yi Xue Za Zhi 2014; 94: 122-6

2. Mazo V, Sabaté S, Canet J, Gallart L, de Abreu M, Belda J, Langeron O, Hoeft A, Pelosi P: Prospective external validation of a predictive score for postoperative pulmonary complications. Anesthesiology 2014; 121: 219-31

3. Nojiri T, Inoue M, Yamamoto K, Maeda H, Takeuchi Y, Nakagiri T, Shintani Y, Minami M, Sawabata N, Okumura M: Inhaled tiotropium to prevent postoperative cardiopulmonary complications in patients with newly diagnosed chronic obstructive pulmonary disease requiring lung cancer surgery. Surg Today 2014; 44: 285-90

4. Fushida S, Oyama K, Kaji M, Hirono Y, Kinoshita J, Tsukada T, Nezuka H, Nakano T, Noto M, Nishijima K, Fujimura T, Ohta T: A randomized multicenter Phase II study of perioperative tiotropium intervention in gastric cancer patients with chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis 2015; 10: 2177-83

5. Austin PC: An Introduction to Propensity Score Methods for Reducing the Effects of Confounding in Observational Studies. Multivariate Behav Res. 2011; 46: 399-424

Summary of revision from the original statistical analysis plan

In "General principles" section, we clarified the description of baseline/perioperative data and outcomes (clause 3.1.1) and the population of analyses (clause 3.1.3); we added the significant level for hypotheses and treat-by-covariate interaction: "For each hypothesis, two-tailed tests will be used in all statistical analysis, and $p < 0.05$ will be considered statistically significant. For the treatment-by-covariate interaction in predefined subgroup analyses, a $p<0.10$ will be defined as statistically significant" (clause 3.1.2).

We added a section of "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" (clause 3.2).

In "Demographics and baseline characteristics" section, we clarified the method to compare between-group differences: "Between-group differences are compared using the absolute standardized differences (ASDs), which are defined as the absolute difference in means, mean ranks or proportions divided by the pooled standard deviation and calculated with the formula published by Austin.[1] Baseline variables with an ASD ≥ 0.196 ($ASD = 1.96 \times \sqrt{(n1+n2)/(n1 \times n2)}$) will be considered imbalanced between the two groups and adjusted for in all analyses if considered necessary" (clause 3.3.2).

In "Intra- and postoperative variables" section, we added methods to analyze intra- and postoperative variables: "Continuous variables will be analyzed using the 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; ordinal variables will be compared with Wilcoxon test. Missing data will not be replaced" (clause 3.4).

In "Primary endpoint" section, we clarified the methods to express difference between groups: "…, with difference between groups expressed as relative risk (95% CI). The upper limit of the 95% CI of less than 1 is regarded as statistical superiority. Missing data will not be replaced" (clause 3.5.1.1). We added the method to calculate the number-need-to-treat: "The number-needed-to-treat is calculated as the reciprocal of the absolute risk reduction" (clause 3.5.1.1). We added method of analysis for the treatment-by-covariate interactions: "The relative risks and corresponding 95% CIs are calculated to assess the treatment effect in predefined subgroups. The interactions between treatment effect and predefined factors will be assessed separately with logistic regression models. The predefined factors include age, sex, body mass index, respiratory disease, respiratory symptoms in last month, cigarette smoking, type of anesthesia, location of surgery, and type of surgery. The relative risk (and 95% CI) for each subgroup and the p values of treat-by-covariate interactions will be displayed in a forest plot" (clause 3.5.1.2).

In "Secondary/exploratory outcomes" section, we clarified methods to analyze categorical and time-to-event variables (clauses 3.5.2.1 and 3.5.2.2). We added method to analyze discrete variables: "Discrete variables (number of individual PPCs) will be analyzed using the Mann-Whitney U test, with median difference and 95% CI calculated with the Hodges-Lehmann estimator, or using the chi-square test, continuity correction chi-square test or Fisher exact test. Missing data will not be replaced (clause 3.5.2.3)."

We added a section of "Safety analysis": "Describe the occurrence of adverse events in each group. Describe the management of adverse events when appropriate. Describe the occurrence of severe adverse events. 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. The estimated risks ratio (RR) and 95% CI will be provided. Missing data will not be replaced" (clauses 3.6.1 to 3.6.4).

References

1. Austin PC: An Introduction to Propensity Score Methods for Reducing the Effects of Confounding in Observational Studies. Multivariate Behav Res. 2011; 46: 399-424