Effects of Perioperative Transcutaneous Electrical Acupoint Stimulation on Monocytic HLA-DR Expression in Patients undergoing Coronary Artery Bypass Grafting with Cardiopulmonary Bypass: Study Protocol for a Double-blind Randomized Controlled Trial

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Study protocol

Keywords: CABG, TEAS, immunosuppression, mHLA-DR

Posted Date: July 31st, 2019

DOI: https://doi.org/10.21203/rs.2.84/v2

Version of Record: A version of this preprint was published on December 30th, 2019. See the published version at https://doi.org/10.1186/s13063-019-3889-z.

Abstract

Abstract Background: Cardiac surgery involving cardiopulmonary bypass (CPB) is known to be associated with a transient postoperative immunosuppression. When severe and persistent, this immune dysfunction predisposes patients to infectious complications, which contributes to a prolonged stay in the intensive care unit (ICU), even mortality. The effective prevention and treatment methods are still lacking. Recent studies revealed that acupuncture related techniques, such as electroacupuncture (EA) and transcutaneous electrical acupoint stimulation (TEAS), are able to produce effective cardioprotection and immunomodulation in adult and pediatric patients undergoing cardiac surgery with CPB, which leads to enhanced recovery. However, whether perioperative application of TEAS, a non-invasive technique, is able to improve immunosuppression of the patients with post cardiosurgical conditions is unknown. Thus, as a preliminary study, the main objective is to evaluate the effects of TEAS on the postoperative expression of monocytic human leukocyte antigen (-D related) (mHLA-DR), a standardized “global” biomarker of injury or sepsis-associated immunosuppression, in patients receiving on-pump CABG. Methods: This clinical study was a single-center clinical trial. The 88 patients scheduled to receive CABG under CPB were randomized into 2 groups: the group of TEAS, and the group of transcutaneous acupoint pseudo - electric stimulation (Sham TEAS). Monocytic HLA-DR expression serves as a primary endpoint, and other laboratory parameters (e.g. IL-6, IL-10) and clinical outcomes (e.g. postoperative infectious complications, ICU stay time, and mortality) as the secondary endpoints. In addition, some immune indicators, such as high mobility group protein 1 (HMGB1) and regulatory T cell (Treg), possibly related to the mechanism of TEAS, will also be measured. Discussion: The current study is a preliminary mono-centric clinical trial with a non-clinical primary endpoint, expression of mHLA-DR, aiming at determining whether perioperative application of TEAS has a potential to reverse CABG-associated immunosuppression. Although the immediate clinical impact of this study is limited, its results would inform further large sample clinical trial with using relevant patient-centered clinical outcomes as primary endpoints. Trial registration: This study was approved by Ethics Committee of Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine with the number 2016-455-06-01. The results of the trial will be published in an internationally peer-reviewed journal. This study was registered at ClinicalTrials with the Identifier NCT02933996 on 13 October 2016, https://www.clinicaltrials.gov/ct2/show/NCT02933996 Keywords: CABG; TEAS; immunosuppression; mHLA-DR

Background

Cardiac surgery involving cardiopulmonary bypass (CPB) is known to be associated with perioperative immune dysfunctions[1]. During CPB surgery, the exposure of blood to nonphysiological surfaces of the CPB apparatus[2], cardiac arrest and ischemia/reperfusion injury of organs[3], and translocation of endotoxin (lipopolysaccharide, LPS) across the ischemic gut wall[4–6] triggers a pronounced pro-inflammatory response, which is characterized by circulating cytokines, activation of endothelial cells and

neutrophils, complement activation, circulating arachidonic acid metabolites, platelet-activating factors, and endothelins[7, 8].

As a physiologic countermeasure to ameliorate this harmful overactivation of innate immunity, a compensatory anti-inflammatory response often (follows) occurs concurrently. It comprises secretion of anti-inflammatory cytokines such as interleukin–10 by monocytes and T cells, down-regulation of inflammatory cell surface receptors on neutrophils, impaired monocytic response to bacterial endotoxins[9, 10], reduced production of interferon (IFN)-γ, interleukin (IL)–2, and tumor necrosis factor (TNF)-α, as well as peripheral blood mononuclear cell (PBMC) proliferation in response to stimulation with phytohemagglutinin[11].

When severe and persistent, this anti-inflammatory response has been termed immunoparalysis, which is considered to contribute to postoperative infectious complications, such as pneumonia or impaired wound healing, and therefore responsible for a prolonged stay in the intensive care unit (ICU), even motality[12–15]. Unfortunately, the counterbalancing long-lasting immunosuppressive responses still remain a clinical challenge.

Acupuncture is an ancient, non-drug treatment technology originating in China, which has been widely used in the world. Recently, more and more studies revealed that acupuncture was able to effectively regulate the function of immune system, and this technique had been clinically regarded as one of main or adjuvant therapy measures for some immune related diseases (e.g., asthma, allergic rhinitis and rheumatic arthritis, etc.)[16–18]. In recent years, acupuncture-related techniques began to be applied to cardiac surgery for better recovery. Yang et al [19] have shown that for adult patients undergoing heart valve replacements, electro-acupuncture (EA) pretreatment can alleviate cardiac ischemia-reperfusion injury indicated by reduced overall serum troponin I release and dosage of inotropic drug use after surgery. Also, ICU stay time was shortened. Subsequently, pretreatment of transcutaneous electric acupoint stimulation (TEAS), a non-invasive acupoint stimulation technique, produces similar cardioprotective effects in pediatric patients undergoing cardiac surgery. Moreover, alleviated inflammation indicated by reduced C-reactive protein level in the early postoperative period was observed[20]. TEAS has no risk of infections, needle induced contagious disease, and fear to stimulation and is more ‘user friendly’ with minimal training, which is more convenient for clinical application[21]. However, whether perioperative application of TEAS is able to improve postoperative immunosuppression of the patient receiving on-pump CABG is unknown.

In the current study, we would attempt to evaluate potential TEAS-induced reversal of CABG-associated immunosuppression. Previous clinical studies have shown that whether in conscious state (i.e. preoperative or post-operative) or in anesthetic state, TEAS or EA applied to various surgical operations can produce beneficial effects, such as prevention of hyperglycaemia[22], reduces intra-operative opioid consumption[23], relieving post-hemorrhoidectomy-associated pain[24], and improving immune and stress

responses to surgery[25]. In order to maximize possible benefit of TEAS, the perioperative administration (i.e. preoperative, intraoperative and postoperative) of TEAS will be chosen in our trial.

Considering that it is a preliminary mono-centric study, monocytic human leukocyte antigen (-D related) (mHLA-DR), a standardized “global” biomarker of injury or sepsis-associated immunosuppression, serves as a primary endpoint, and other laboratory parameters (e.g. IL–6, IL–10) and clinical outcomes (e.g. postoperative infectious complications, ICU stay time, and mortality) as the secondary endpoints. Monocytic HLA-DR is a MHC class II molecule and predominantly expressed on monocytes/macrophages[26]. Its surface expression is indispensable for antigen presentation[26]. Increased mHLA-DR expression reflects activation of immune cells, while diminished expression exhibits a phenotype with down regulation of antigen-presenting capacity and a shift from pro- to anti-inflammatory cytokine production [27, 28]. Moreover, surface expression of mHLA-DR is crucial for induction of adaptive immune responses[26, 29]. More importantly, accumulated clinical evidences indicated that its persisting decreased expression is associated with adverse clinical outcomes (e.g., secondary infection risk, mortality) in patients with trauma [30], burns [31], pancreatitis [32, 33], solid organ transplantation [34], hepatic [35] or renal injury [36], stroke [37], myocardial infarction/heart failure, and cardiac arrest [38–41], as well as sepsis [42]. The same is true of cardiac surgery with CPB. The quantification of mHLA-DR expression shows the best predictive power on outcome in pediatricand adult patients. Postoperative reduced mHLA-DR was associated with increased length of ICU stay/mechanical ventilation and development of postoperative sepsis [43–45].

In addition, some immune indicators, such as high mobility group box–1 protein (HMGB1) and regulatory T cells (Treg), possibly related to the mechanism of TEAS would also be measured. HMGB1, originally described as a DNA-binding protein and passively released by necrotic cells and actively released by macrophages/monocytes, was discovered to be an essential cytokine that mediates the response to infection, injury, and inflammation[46]. Our previous animal study has shown that EA can inhibit excessive release of HMGB1 following myocardial ischemia and attenuate the associated inflammatory responses and myocardial injury during reperfusion[47]. Recently, HMGB1 was found to directly enhance immune inhibitory functions of Treg and limit the number and activity of conventional T cells[48, 49]. Treg, including CD4+/CD25+ regulatory T cells, are responsible for limiting tissue damage and inflammation associated with both innate and adaptive immune responses[50]. However, overactivation of Treg contributes to immunosuppression[51]. For the reason that the population of Treg in PBMCs was indeed significantly increased at 48 hours and 96 hours after CABG with CPB[52], which may contribute to CABG-associated immunosuppression, we speculate potential inhibition of excessive HMGB1 release of TEAS, consequently leading to attenuated function of Treg, may be associated with TEAS-induced reversal of immunosuppression, characterized by increased mHLA-DR.

This clinical trial was approved by Ethics Committee of Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine with the number 2016–455–06–01 and registered at ClinicalTrials with the Identifier NCT02933996 on 13 October 2016,

https://www.clinicaltrials.gov/ct2/show/NCT02933996. This study selected 88 patients scheduled for CABG under CPB as observation subjects and randomized them into 2 groups: the group of TEAS, and the group of pseudo - electric stimulation (Sham TEAS). As a primary endpoint, mHLA-DR of both groups were measured at 4 time points: One day before surgery, and Day 1 after surgery (one day after surgery), Day 3 after surgery (3 days after surgery), and Day 5 after surgery (5 days after surgery).

Methods

Study design

This study is a preliminary, mono-center, double-blind, randomized and controlled clinical trial to explore the effects of TEAS therapy on improvement of postoperative immunosuppression indicated by diminished HLA-DR expression of patients receiving CABG (Fig. 1). The trial is designed following the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 statement (see Additional file 1).

Study group

This trial will include 2 groups: the group of TEAS and the group of transcutaneous acupoint pseudo - electric stimulation (Sham TEAS). In each group, there will be 44 patients receiving CABG with cardiopulmonary bypass. The patients of TEAS group will receive TEAS therapy in perioperative period, and the Sham TEAS will not be performed to receive electrical stimulation sensation in perioperative period.

Study time

The trial will be from Dec. 1, 2017 to Dec. 31, 2019.

Patient and public involvement

The research question and outcome measures will be developed from a desire to evaluate the effect of TEAS on expression of mHLA-RD, a standardized “global” biomarker of injury or sepsis-associated immunosuppression, in patients receiving CABG. We seek to promote this clinical technique and provide theoretical basis for further improvement of this technology. Patients, however, have not been directly involved in the development of research questions, outcome measures or design of this study.

Study objective

Monocyte human leukocyte antigen (mHLA-DR)as the primary endpoint of this clinical trial, will be detected to evaluate the improvement of postoperative immunosuppression.

Related indicators of immunosuppression include interleukin–6 (IL–6), interleukin–10 (IL–10), C reactive protein (CRP), postoperative infectious complications (pneumonia, incision infection and indwelled catheter infection), ICU stay time, and mortality.

The indicators of related mechanism study include high mobility group proteins–1 (HMGB1), regulatory T-cell (Treg) and CD4+ T-cell.

Study location

A preliminary, mono-centre, randomized, double-blinded, controlled trial will be conducted in patients undergoing CABG in Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, China

Study Population

The enrolled subjects are the patients scheduled to receive CABG with cardiopulmonary bypass. Participants will be recruited voluntarily according to the inclusion and exclusion criteria below.

Inclusion criteria

1. Aged 18-75 years, male and female;
2. Patients diagnosed as coronary disease, scheduled to receive CABG;
3. BMI: $18.5kg/m^2$<BMI≤$30kg/m^2$
4. Graded as I-III by ASA;
5. Patients firstly receiving CABG under extracorporeal circulation.

Exclusion Criteria

1. Presence of surgical incision or scar at Zusanli acupoint (ST35) /Shenshu (BL23) acupoint;
2. Patients with local skin infection at acupoint;
3. Patients with nerve injury on upper or lower limbs;
4. Patients with history of spinal surgery;
5. Patients who have participated in other clinical trial in recent 4 weeks;
6. Patients using pacemaker;
7. Patients combined with pain before surgery who are using central analgesic drug or drug abuser (e.g., opioid) and dependent user;
8. Patients combined with severe central nervous system disease or severe mental disease;
9. Patients with alcoholic history;
10. Patients who have received emergent coronary bypass operation due to acute myocardial infarction.

Interventions

1. Selection of acupoints: Zusanli acupoint (ST36) – Shenshu acupoint (BL23) (Fig. 2 and 3)

1. Zusanli location: located at outside of the shank, 3 cun (10 cm) below Dubi acupoint (ST35) and a finger's width (middle finger) to tibial front edge;
2. Shenshu location: located below the spinous process of the second lumbar vertebra, 1.5 cun (5 cm) to the central line.

2. Stimulation timing: Before anesthesia + Intraoperative + Postoperative (Fig. 4)

    1. 30 mins before anesthesia: one stimulation for 30 mins;
    2. Intraoperative: stimulating in the whole course;
    3. Postoperative: 0-24h: 4 times of stimulation (30 mins per stimulation). Hour 5-6 after the surgery (the first time), Hour 11-12 after the surgery (the second time), Hour 17-18 after the surgery (the third time), Hour 23-24 after the surgery (the fourth time).

3. TEAS parameter

    1. Frequency: 2/100 Hz alternative;
    2. Intensity: 15mA;
    3. Low-frequency electronic pulse therapeutic device G6805-2 (Huayi, Shanghai, China) (Fig. 5):

4. Current intensity: main difference between the study group and control group

    1. TEAS group: the acupoints including Zusanli and Shenshu, were identified before electrical stimulation with surface electrodes (Fig 6). Selection of these acupoints was based on a consensus between the acupuncturists of the study.
    2. Sham TEAS group: No electrical stimulation sensation is performed in the Sham TEAS group. In the Sham TEAS group, pseudo-stimulation is provided by deliberately connecting the electrodes to the incorrect output socket of the electroacupuncture device, and thus there is no flow of electric current. Patients could see the output light flashing but no current was transmitted throughout the procedure. Patients would be told that the stimulation frequency selected was not perceivable by human beings.

5. Anesthesia protocol

    1. Medication before the anesthesia, Morphine: 0.1mg/kg
    2. Anesthesia induction

        1. Sufentanil 0.3–5ug/kg
        2. Propofol TCI: 2.0–5ug/ml
        3. Dextromidine 0.5–0ug/kg/hr or midazolam 0.05–0.1mg/kg
        4. Lidocaine 1mg/kg (Maximum dose not higher than 50 mg)
        5. Rocuronium bromide 0.9–2mg/kg

3. Maintenance of anesthesia

    1. Narcotic analgesics: common sufentanil by 0.2～5ug/kg by times (i.v) or remifentanil by 0.05～0.2ug/kg/min continuous intravenous pump injection, addition of sufentanil by 10～20ug before skin incision and sternum splitting.
    2. Inhaled general anesthetics: sevoflurane and isoflurane can be inhaled discontinuously as requested，with MAC 0.7～0.
    3. Muscle relaxant: common vecuronium bromide and rocuronium bromide, etc.
    4. After the completion of tracheal intubation, the anaesthesia machine is connected immediately, and $ETCO_2$ is examined, and the breathing sound of both lungs is auscultated to determine the position of endotracheal tube.
    5. Common parameters of mechanical ventilation: VT: 7～8 ml/kg, RR: 10～12 bpm; PaO_2: 200 mmHg, $PaCO_2$: 35～45 mmHg, FiO_2: 80%.

Primary outcome measures

With mHLA-DR as primary outcome of this clinical trial, the improvement of postoperative immunosuppression is evaluated.

Peripheral blood is collected from the patient to test this indicator at the following time points: One day before surgery, Day 1 after surgery (one day after surgery), Day 3 after surgery (3 days after surgery), and Day 5 after surgery (5 days after surgery).

The percentage of $HLA\text{-}DR^+/CD14^+$ cell in all $CD14^+$ cells will be determined by flow cytometry (Becton-Dickinson, New Jersey, U.S.) in central lab of Shuguang hospital affiliated to Shanghai university of traditional Chinese medicine.

Secondary outcome measures

1. Related indicators of immunosuppression include interleukin-6 (IL-6), interleukin-10 (IL-10), C-reactive protein (CRP), postoperative infectious complications (pneumonia, incision infection and indwelled catheter infection), ICU stay time, and mortality. The examination methods and time points are as follows:

    1. IL-6 and IL-10: Plasma levels of IL-6 and IL-10 will be determined using the ELISA(enzyme-linked immuno sorbent assay) with high sensitivity kits (ABCAM, Shanghai, China), one day before surgery, Day 1, 3 and 5 after surgery.
    2. CRP: CRP expression level in blood samples, automatic biochemical analyzer (Beckman Coulter, Georgia, U.S.), one day before surgery, Day 1, 3 and 5 after surgery;
    3. Postoperative infectious complications:

- **Pneumonia**: Pneumonia was defined according to the Centre of Disease Control and Prevention (CDC) guidelines. Postoperative healthcare-associated pneumonia will be proven throughout adiograph suspicious for pneumonia, clinical signs and symptoms suspicious for pneumonia and positive culture from broncho-alveolar lavage, a positive blood culture not related to another infection, or a positive sputum culture.
- Guidelines[Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of healthcare-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control 2008; 36:309–332.
- **Postoperative infection other than pneumonia:** observe the incidence of incision infection, indwelled catheter infection after the surgery (fever, bacterial culture).

4. ICU stay time: We will record the length of ICU stay after surgery(days (d), mean ± standard deviation (SD)).
5. Mortality: We will record a 30-day mortality rate after surgery.

2. The indicators of related mechanism study include high mobility group proteins-1 (HMGB1), regulatory T-cell (Treg) and $CD4^+$ T-cell. The examination methods and time points are as follows:

    1. HMGB1: HMGB1expression level in blood samples, ELISA kit (ABCAM, Shanghai, China), one day before surgery, Day 1, 3 and 5 after surgery;
    2. Treg: Percentage of $CD4^+/CD25^+$ T-cell in $CD4^+$ T-cell, flow cytometry(Becton-Dickinson, New Jersey, U.S.)[one day before surgery, Day 1, 3 and 5 after surgery;
    3. CD4+ T-cell: CD4+ T-cell number /ml blood, flow cytometry(Becton-Dickinson, New Jersey, U.S.)[one day before surgery, Day 1, 3 and 5 after surgery;

All the related indicators and the indicators of related mechanism will be tested in central lab of Shuguang hospital affiliated to Shanghai university of traditional Chinese medicine.

Randomization and blinding

Computer generates random group numbers. It will be printed and placed in separate sealed envelopes. When receiving a subject who meets the inclusion criteria, the anaesthesiologist will assign the newly recruited subject to a group according to the number in the envelope. Nurse anaesthetists will perform TEAS or Sham TEAS for the patients. Both anaesthesiologists and patients will be blinded to the regimen. The anaesthesiologist will be notified of the study group by the nurse anaesthetist in case of emergency.

Current Sample size justification

We calculate the sample size based on the primary outcome Human leukocyte antigen of monocyte (mHLA-DR). To compare two groups across four timepoints, we calculate the sample size based on the repeated measures ANOVA. With the assumptions of 5 percent Type I error rate, 80 percent power and medium effect size of 0.5, we will need 39 observations in each group to finish the study. Assuming that the dropout rate to be 10%, we will need to enroll 88 observations (44 observations per group).

Statistical analysis

We will describe normally distributed continuous data and skewed continuous data using mean [standard deviation] or median (percentile 25 to percentile 75). Categorical variables are expressed as number and fraction (%). The statistical methods will include descriptive statistics, Student's t-test, Mann-Whitney U-test, chi-squared test, Fisher's exact test and repeated measures ANOVAs. All statistical analysis will be completed with SPSS 19.0 or other statistical software packages as needed, and the significance level will be set at 5%.

Data collection and management

The data will be collected as primary and secondary outcome measures, with above described method. All data will be saved safely in internal server of Shuguang Hospital, with complete confidentiality. The participants of this study will be cited with a code different from their real names. The data management program will be approved by the trial manager and other clinicians before the registration of the first participant.

Adverse events

The status related to adverse events is acquired according to the self-report of the patient or direct observation of clinicians or by non-induced query of the patient, and his/her clinical safety is evaluated (see Table 1).

Table 1 Clinical safety evaluation in perioperative period

Quality control

The chief surgeon of thoracic surgery department, anesthetist to implement anesthetic management, nurse of anesthesiology department to carry out TEAS (having received specialized acupuncture training) as well as blood sampling personnel of clinical lab and data recording personnel are fixed to avoid bias from human operations. Specialized acupuncture training mainly includes selection of acupoints, TEAS operation standard and procedure (see Table 2).

Table 2 TEAS operation standard and procedure

Discussion

The patients undergoing CABG with CPB are more prone to have immunosuppression which may lead to postoperative infectious complications and a prolonged ICU stay, even mortality. Previous studies have proved that the acupuncture was able to improve the patient's immune function. TEAS have the similar efficacy as electroacupuncture, but it is easier to operate, non-invasive and easy to accept by patients. The current study is aiming at evaluating potential TEAS-induced reversal of decreased mHLA-DR□a

standardized “global” biomarker of immunosuppression and exploring the possible underlying mechanism related to TEAS.

Although the immediate clinical impact of this study is limited, its results will inform further research. Following demonstration of possible immunological efficiency, biomarker-guided immunological interventions of TEAS for immunosuppression should be performed in populations with sufficiently large sample size using relevant patient-centered clinical outcomes (e.g., mortality or infectious complications). Only in this way can we finally determine the clinical value of TEAS for counterbalancing CABG-associated immunosuppression and promote its application.

List Of Abbreviations

TEAS: Transcutanclus electrical acupoint stimulation

GA: General anesthesia

CABG: Coronary artery bypass grafting

CPB: Cardiopulmonary bypass

mHLA-DR: Monocytic human leukocyte antigen DR

CRP: C-reaction protein

IL−6: Interleukin−6

HMGB1: High mobility group box 1

Treg: Regulatory T cell

$CD4^+$ T cell: CD4 positive thymocyte cell

BMI: Body mass index

VT: Tidal volume

PCO_2: Partial pressure of carbon dioxide

PO_2: Oxygen partial pressure

RR: Respiratory rate

FiO_2: Fraction of inspiration O_2

Declarations

Additional file Additional file 1: The SPIRIT recommendations for clinical trials protocols.

Ethics approval and consent to participate This study has been approved by Ethics Committee of Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine with the number 2016–455–06–01 and registered at ClinicalTrials with the Identifier NCT02933996 on 13 October 2016, https://www.clinicaltrials.gov/ct2/show/NCT02933996. Informed consent will be obtained from each of the participants. Patient consent was obtained.

Consent for publication Figure 2, 3, 4 and 5 are all produced by our institution and all rights are reserved. The patient photographed in Figure 6 is aware of this publication and has signed the patient consent form.

Availability of data and material The final trial dataset will only be accessible to the study investigators.

Competing interests The authors declare that they have no competing interests. The Ethics Committee of Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine is independent from the sponsor and competing interests.

Funding The present study is supported by the project of the Shanghai Municipal Commission of Health and Family Planning (No. 20154Y0111) and the project of the National Natural Science Foundation of China (No. 81603450).

Authors' contributions W. T. Chen and J. F. Wei drafted the manuscript, and contributed greatly to study conception and design. L. Yuan and D. W. Zhang participated in study design, and is responsible for the formulation of TEAS operation standard and procedure. G. Q. Fu, J. Wang and Y. L. Zhou participated in study design, and is responsible for the contact of all surgeons. W. Tang is a local investigator in an involved center, and helped in the revision of the manuscript. Y. Yong participated in this study, and is responsible for blood specimen collection, determination and recording. L. Wang participated in the study, and is responsible for anesthetic management of the patients in perioperative period, and contributed greatly to the successful implementation of the study. J. G. Song and S. Wang drafted the manuscript, and contributed greatly to study conception and design. All authors strictly reviewed and approved final manuscript.

Acknowledgements We would like to express gratitude to the Acupuncture and Anesthesia Research Institute of Shuguang Hospital affiliated to Shanghai University of Traditional Chinese Medicine, for help in study's organization.

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Tables

Table 1 Clinical safety evaluation in perioperative period

Complications in perioperative period: any of following conditions is considered as complication of perioperative period.

1	**Postoperative arrhythmia**	postoperative atrial fibrillation, atrial flutter, supraventricular tachycardia, ventricular tachycardia, ventricular fibrillation, ventricular flutter, cardiac arrest, atrioventricular block of II° or above, frequent atrial premature beat and ventricular premature beat significantly affecting the stability of haemodynamics (indicated in ECG)□		
2	**Pneumonia**	body temperature above 38.5℃ (indicated in chest film);		
3	**Acute lung injury**	(1) acute onset, with pathogenic factors;		
		(2) oxygenation index (arterial partial pressure of oxygen/fraction of inspired oxygen, $PaO_2/Fi0_2$) <300 mmHg (1 mmHg=0.133 kPa) not referring to positive end-expiratory pressure (PEEP) level;		
		(3) frontal X-ray chest film revealed patchy shadows in both lungs;		
		(4) pulmonary artery incarceration pressure < 18 mmHg or no clinical evidence of increased pressure in left atrium;		
		(5) Acute paroxysmal respiratory failure.		
4	**Pulmonary atelectasis**	indicated in chest film;		
5	**Intraoperative and postoperative myocardial infarction**	manifestation of myocardial infarction symptoms or change of ECG ST segment, continuous increase of myocardial enzyme, especially cardiac troponin I (cTnI), accompanied with dynamic change of ST segment;		
6	**Postoperative cardiac insufficiency**	the postoperative cardiac output (CO) is lower than lower limit of normal value or there are symptoms and vital signs of heart failure;	(1) Left cardiac insufficiency	**Symptoms:** dyspnea; coughing, expectoration and hemoptysis; cyanosis, fatigue and weakness;
				Vital signs: expansion of border of cardiac dullness, left lower shifting of cardiac impulse with elevating sensation. Accelerated heart rate, diastolic gallop heard in apex, alternative pulse in severe case. Moist and dry rales are heard in the bottom of both lungs. Wheezing rale and dry rales may be accompanied with secondary bronchial spasm.
			(2) Right cardiac insufficiency	**Symptoms:** reduced urine volume, increased nocturnal enuresis, swelling pain in liver region or even occurrence of jaundice; inappetence, dyspepsia, nausea, vomiting and diarrhea.
				Vital signs: expansion of border of cardiac dullness, apex beating showing elevating sensation, diffuse beating range, accelerated heart rate;
				Distention of jugular vein, liver swelling with tenderness, hepatojugular reflux sign positive; pitting edema, right heart failure
				Typical vital signs of failure, mostly in the body drooping part.
				Coexistence of clinical

			(3) Whole cardiac insufficiency	manifestations of left and right cardiac insufficiency, but principally one of them
7	**Postoperative respiratory insufficiency**	breath in indoor air at static conditions, and intracardiac anatomical shunt and originated from the decrease of cardiac volume are excluded; arterial partial pressure of oxygen (PaO_2) is lower than 8 kPa (60 mmHg) or accompanied with partial pressure of carbon dioxide ($PaCO_2$) higher than 6.65kPa (50 mmHg).		
8	**Postoperative hemorrhage of digestive tract**	including ulcer bleeding or bloody gastric content caused by mucosal ischemia of gastrointestinal tract, haematemesis, tarry stool or hemafecia;		
9	**Postoperative hepatic insufficiency**	severe hepatocellular damage, causing significant metabolism, secretion, synthesis, biotransformation and immune function disorder, clinical syndrome of edema in the organism, jaundice, haemorrhage, infection, renal function disorder and hepatic encephalopathy, etc.		
	Postoperative renal insufficiency	rapid decrease of renal excretory function in short term, and daily mean increase of serum creatinine ≥44.2 umol/L and exacerbation of existing renal insufficiency.		
10	**Postoperative infection other than lung infection**	including hematogenous infection, infections of digestive tract, urinary system, wound, skin, and indwelled catheter.		
11	**Postoperative cerebral ischemia and hypoxic disease**	including cerebral infarction, cerebral thrombosis, cerebral hemorrhage, transient cerebral ischemic attack and diffuse cerebral ischemia and hypoxic disease.		
12	**Prolongation of postoperative hospital stay**	postoperative hospital stay exceeds 14 days.		
13	**Acute kidney injury**	(1) the increase of plasma creatinine within 48 hours ≥ 0.3 mg/dL (≥ 26.5 μmol/L);		
		(2)Plasma creatinine within 7 days ≥ 1.5 times of basic value;		
		(3) Urine volume within 6 hours lower than 0.5ml/kg/h.		
14	**Death in perioperative period: definition**	(1) Death within 30 days after surgery;		
		(2) Death in hospital stay after surgery;		
		(3) Death caused by surgical reasons after discharge.		

Table 2 TEAS operation standard and procedure

1	Determination of position	the patient takes a supine position;
2	Inspection of equipment	confirm normal operation of electric acupuncture apparatus;
3	Area and acupoint locating	the Zusanli acupoint and Sanyinjiao acupoint are determined by feeling and pressing the point for acupuncture;
4	Local skin preparation	prepare the skin at the acupuncture point, disinfect from the center with 75% ethanol cotton ball in circle to wipe off the sebum;
5	Selection of electrode slices	select electrode slices specially used for TEAS;
6	Acupoint patching	attach the electrode slices specially used for TEAS on the acupoints, press to confirm securely attached;
7	Connection of electrode slices to equipment	connect Zusanli acupoint and Sanyinjiao acupoint on one side to 2 electrodes of the same wire, and those of the other side to another 2 electrodes of the same wire; both wires are connected to the same electric acupuncture apparatus;
8	Acupoint electric stimulation	confirm the electric acupuncture apparatus is in power-up state, turn on the electric acupuncture apparatus, select corresponding parameters, and initiate TEAS therapy according to the patient's tolerance to electric stimulation;
9	Maintenance treatment	maintain electric stimulation for 30 min, instruct the patient to protect the surgery area in acupuncture pin setting process, and closely examine the patient for adverse reactions of fainting, vomiting and pain during acupuncture treatment, and symptomatic treatment is provided if any;
10	End of treatment	turn off the electric acupuncture apparatus, remove the electrode slices, and clear away connection wires. Check redness and swelling, injury on the skin for electrode slice attachment, and provide symptomatic treatment in case of occurrence above symptoms.

Figures

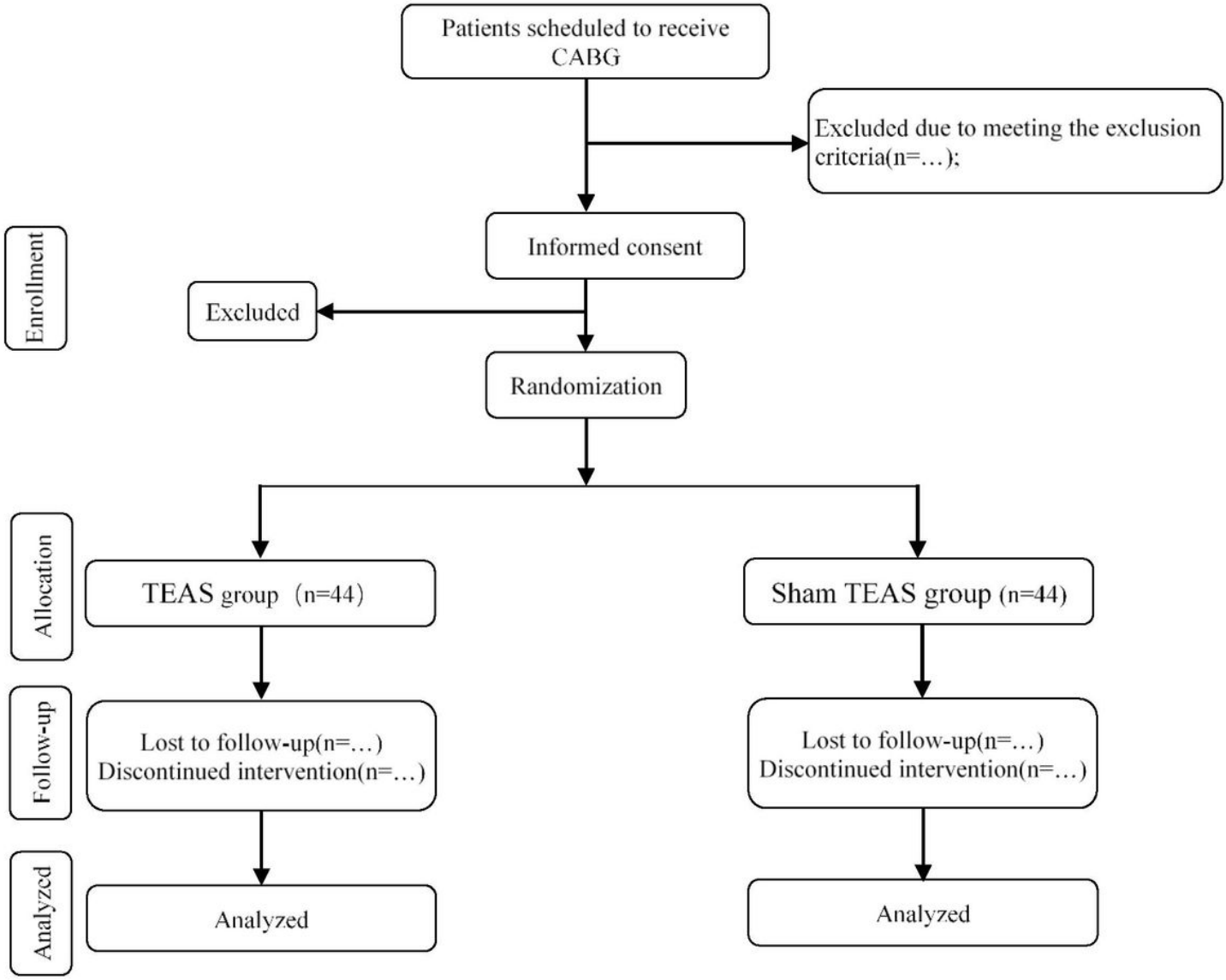

Figure 1

Study design and participant flow chart. CABG under extracorporeal circulation

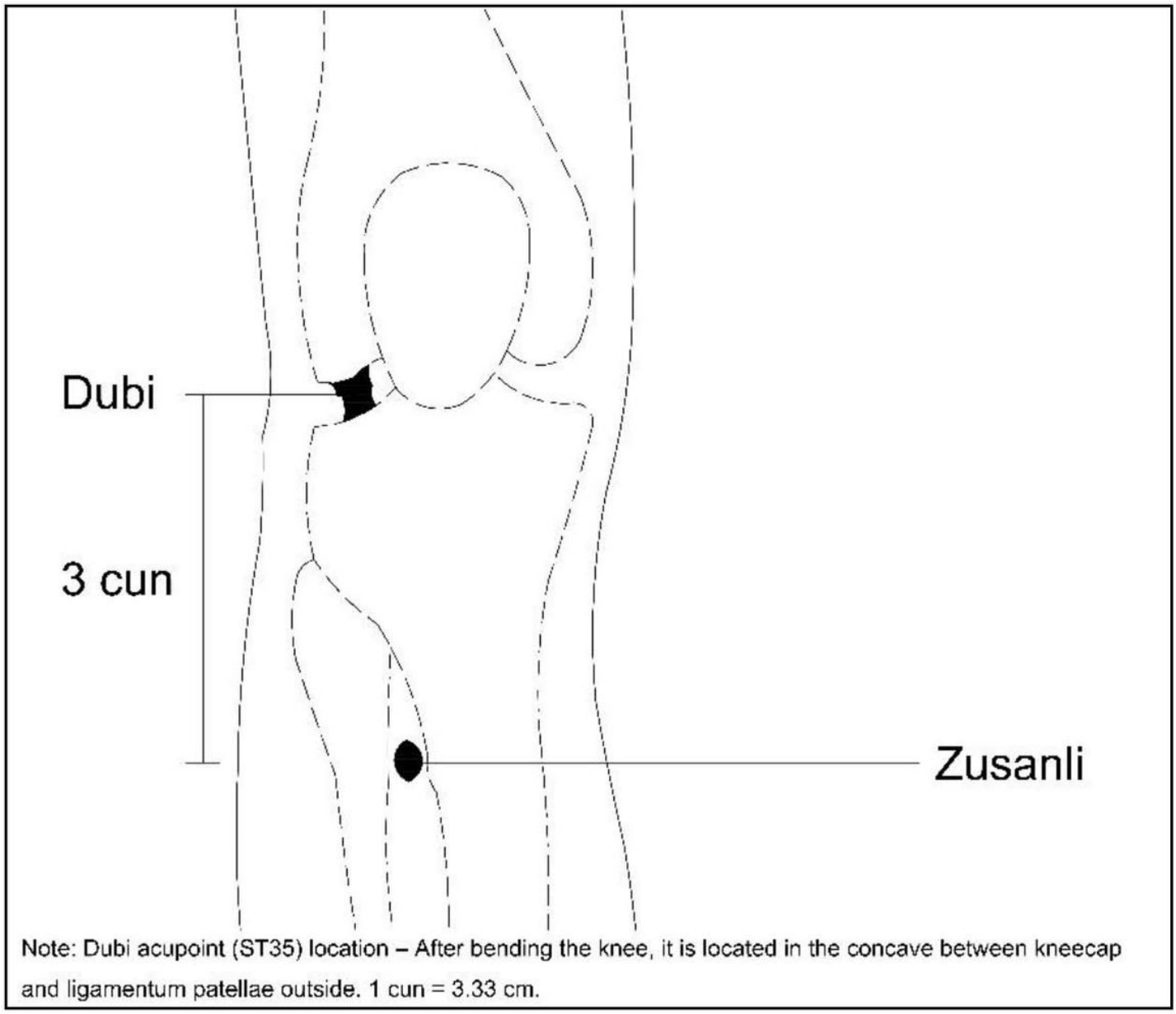

Figure 2

Zusanli (ST36) illustration

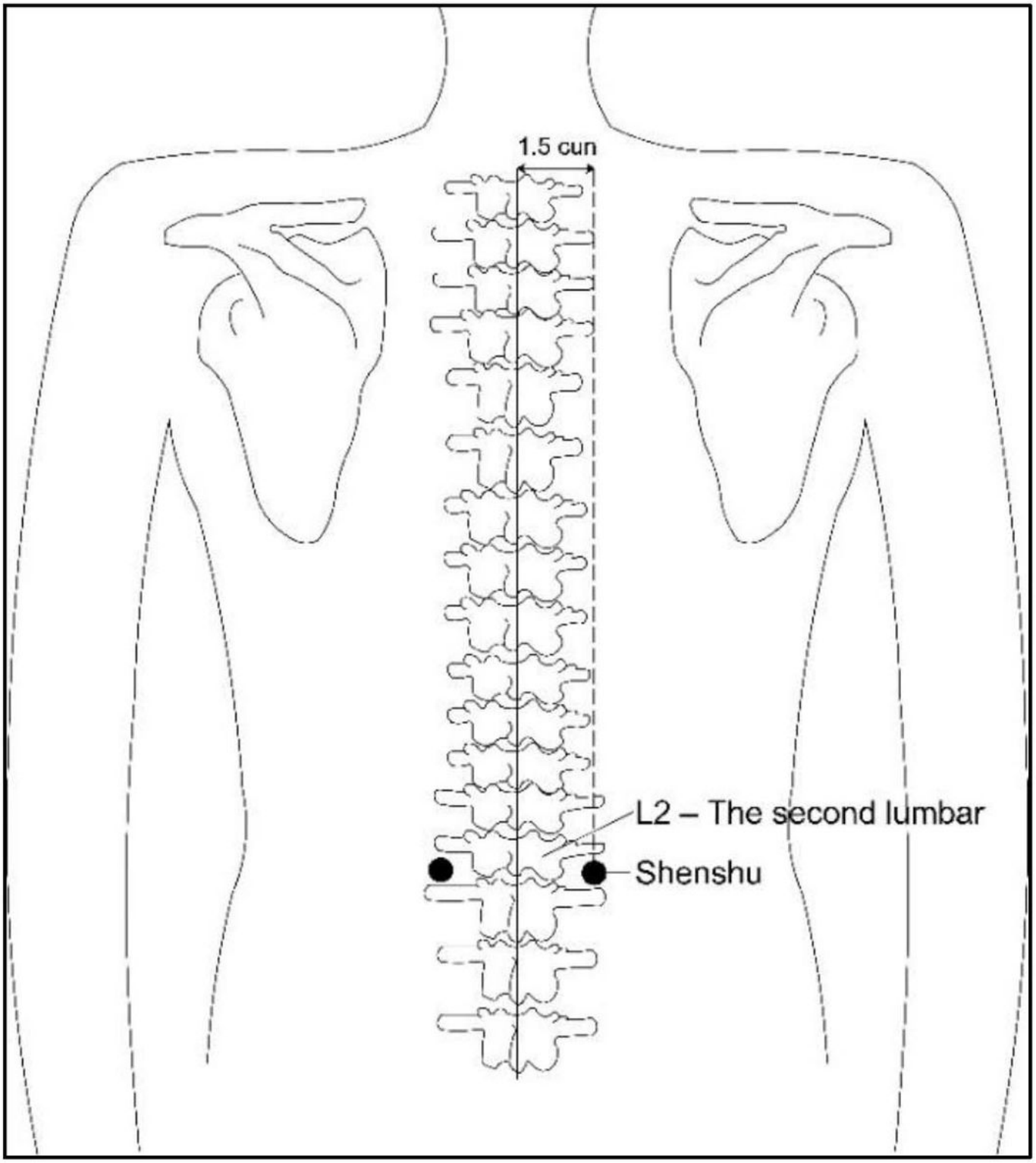

Figure 3

Shenshu (BL23) illustration

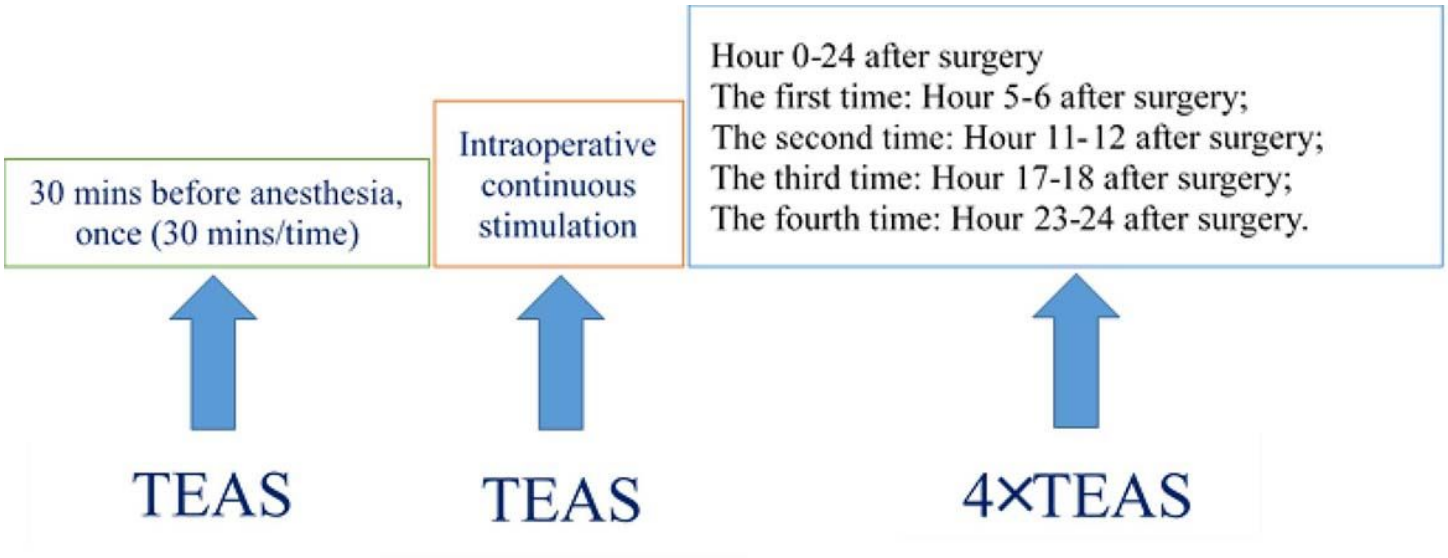

Figure 4

Illustration of the timing for TEAS intervention in perioperative period

Figure 5

Low-frequency electronic pulse therapeutic device G6805-2 (Huayi, Shanghai, China)

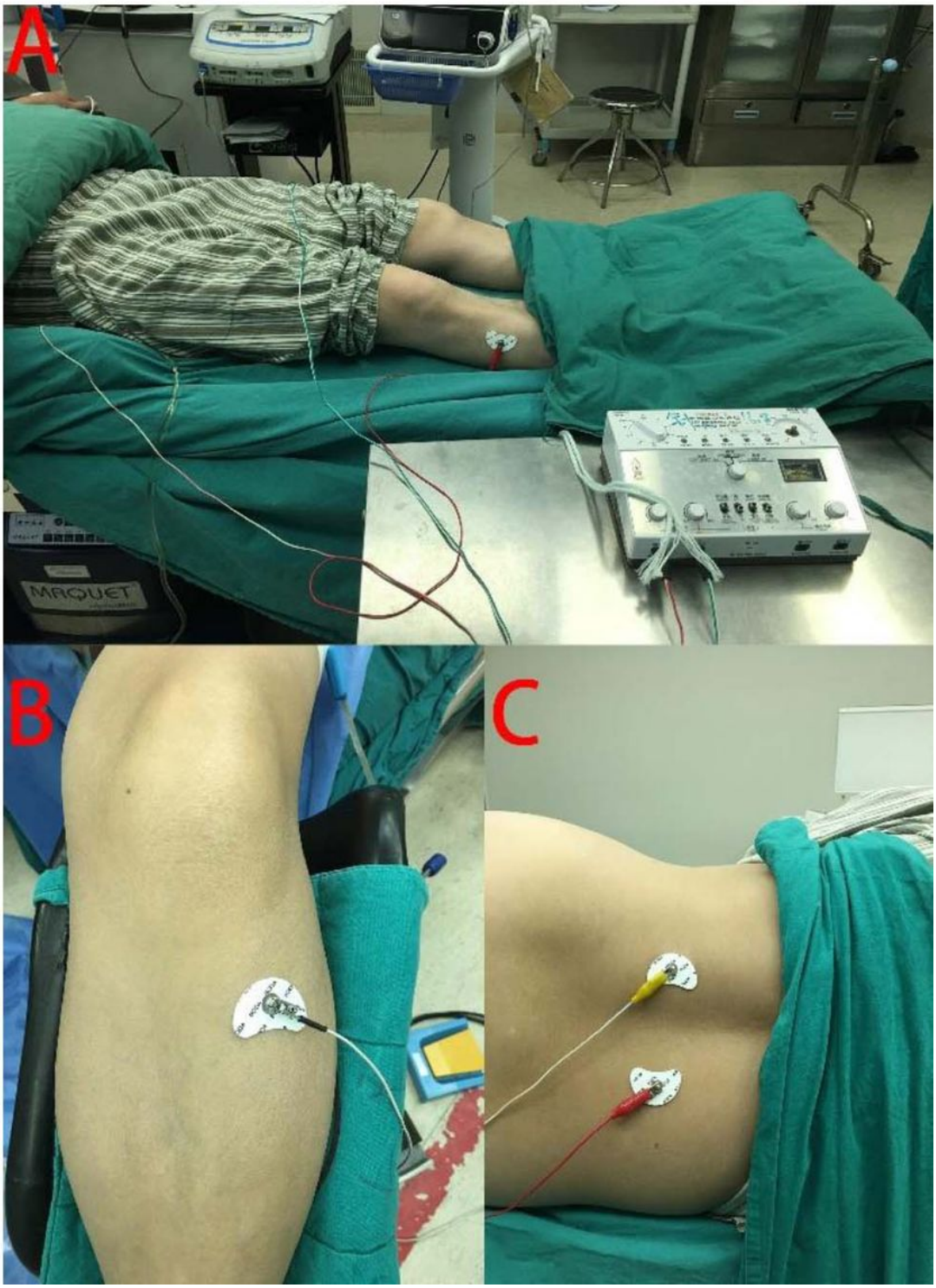

Figure 6

Acupoints selected in this trial. A: Intraoperative Zusanli acupoint (ST36) TEAS therapy; B: Intraoperative Shenshu acupoint (BL23) TEAS therapy; C: Intraoperative bilateral Zusanli acupoint (ST36) + Shenshu acupoint (BL23) TEAS therapy.

Supplementary Files

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