Improvement of Postoperative Immunosuppression in Patients Receiving Coronary Artery Bypass Grafting by Transcutaneous Electrical Acupoint Stimulation: Study Protocol for a Double-blind Randomized Controlled Trial

CURRENT STATUS: ACCEPTED

Wen-ting Chen
Anesthesiology department Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine Shanghai China

Jin-feng Wei
Guangdong Cardiovascular Institute & Guangdong General Hospital Guangdong Academy of Medical Sciences Guangzhou Guangdong Province China

Lan Wang
Anesthesiology department Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine Shanghai China

Deng-wen Zhang
Guangdong Cardiovascular Institute & Guangdong General Hospital Guangdong Academy of Medical Sciences Guangzhou Guangdong Province China

Wei Tang
Anesthesiology department Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine Shanghai China

Jian Wang
Anesthesiology Department Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine Shanghai China

Yue Yong
Anesthesiology Department Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine Shanghai China

Jing Wang
Anesthesiology Department Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine Shanghai China
Ya-lan Zhou  
Anesthesiology Department Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine Shanghai China

Lan Yuan  
Anesthesiology Department Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine Shanghai China

Guo-qiang Fu  
Anesthesiology Department Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine Shanghai China

Sheng Wang  
Guangdong Cardiovascular Institute & Guangdong General Hospital Guangdong Academy of Medical Sciences Guangzhou Guangdong Province China  
Email: shengwang_gz@163.com  
Corresponding Author  
ORCiD: https://orcid.org/0000-0003-0065-0102

Jian-gang Song  
Anesthesiology Department, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, China; Acupuncture and Anesthesia Research Institute, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medi  
Email: songjg1993@126.com  
Corresponding Author  
ORCiD: https://orcid.org/0000-0002-9485-0908

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Abstract

**Background:** The coronary artery bypass grafting (CABG) is a surgery to relieve angina and reduce mortality of coronary heart disease. However, the patient is more prone to have immunosuppression after the surgery, causing secondary infection, and it is closely correlated to the occurrence and progress of tumor after surgery. More and more studies revealed that needle puncture was able to effectively regulate the function of immune system. However, its perioperative application is unclear. Therefore, this clinical study is aimed to evaluate whether the effect of Transcutaneous Electrical Acupoint Stimulation (TEAS) on improvement of immunosuppression of patients after receiving CABG.

**Methods:** This clinical study was a single-center clinical trial. The 88 patients scheduled to receive CABG under extracorporeal circulation were randomized into 2 groups: the group of TEAS combined with general anesthesia (TEAS+GA), and the group of transcutaneous acupoint pseudo-electric stimulation combined with general anesthesia (Sham TEAS+GA). Human leukocyte antigen of monocyte (mHLA-DR) was the main endpoint to evaluate on the improvement of immunosuppression of patient after surgery. In addition, the monitoring indicators, such as interleukin-6 (IL-6), reactive protein C (CRP), high mobility group protein 1 (HMGB1), regulatory T cell (Treg) and secondary infection, etc., were recorded, and further statistical analysis was conducted.

**Discussion:** This study performs TEAS intervention on patients scheduled to receive CABG under extracorporeal circulation in perioperative period, to improve their postoperative immunosuppression, reduce postoperative complications, reduce secondary infection rate, shorten hospital stay and improve the patient’s outcome. This study also aims to provide clinical evidence for application of this technique. However, TEAS related parameters in this study (stimulus intensity and waveform, intervention timing and duration, etc.) should be further optimized.

**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
Coronary artery bypass grafting (CABG) is a surgery to use the artery or vein of the patient himself/herself to bridge the blocked coronary artery, which is able to relieve angina and reduce the mortality of coronary disease. It can be operated at the support of extracorporeal circulation (on pump CABG) or directly on beating heart (off-pump CABG).

The studies in recent years revealed that the patients who received CABG at the support of extracorporeal circulation were more prone to have immunosuppression in perioperative period, which not only easily led to complications of secondary infection, but also closely correlated to the occurrence and progress of postoperative tumor. The study by Justus et al, revealed that the state of immunosuppression induced by heart surgery and cardiopulmonary bypass (CPB) were more easily subjected to secondary infection, which might be correlated to significant decrease of postoperative reaction of monocyte against antigen stimulation of the patient. Another study comparing the effects of percutaneous coronary intervention (PCI) therapy and CABG on patient’s prognosis revealed that in the 6-year post-treatment observation period, there was no significant difference in the total mortality between the patients of 2 groups; In comparison with PCI therapy, the mortality of patients receiving CABG caused by cardiac reasons significantly decreased (47.1% vs 76.8%), but that caused by tumor reasons significantly increased (20.6% vs 7.2%). In addition, for the patients with malignant tumor before the surgery, the interval between the diagnosis of tumor and time for cardiac surgery had significant effect on tumor growth and survival time of the patients: Being compared with the patients with an interval of 2-5 years, the mortality rate due to recurrence of prior tumor of patients with an interval of less than 2 years significantly increased (58.8% vs 25%); Being compared with the patients with no tumor, the survival rate of patients with a malignant tumor in the history was also significantly reduced, the 5-year survival rate (72 ± 3% vs 91 ± 2%) and 10-year survival rate (40 ± 5% vs 73 ± 4%).

Large surgical trauma, extracorporeal circulation and myocardial ischemia reperfusion injury may be the important causes leading to immunosuppression. Therefore, it is
necessary to intervene with the patient’s immune functions in perioperative period, improve his/her state of immunosuppression and increase survival rate.

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.). Since 1980s, the application of electroacupuncture in heart surgery was able to increase the stability of circulatory function during surgery period, reduce myocardial ischemia reperfusion injury following extracorporeal circulation, reduce the time of mechanical ventilation, shorten postoperative recovery time and reduce the mortality. Our recent experimental study also revealed that the electroacupuncture was able to regulate the over reaction of innate immune system against endotoxin, and over inflammatory reaction caused by myocardial ischemia reperfusion. However, whether the application of TEAS in perioperative period is able to improve postoperative immunologic function of the patient after receiving heart surgery is still unknown.

At present, monocyte human leukocyte antigen-DR (mHLA-DR) is believed to be a representative marker reflecting human immunosuppression in blood; More importantly, decreased mHLA-DR expression is correlated to the increase of infection and death risk in hospital. Under normal circumstances, high mHLA-DR expression is shown in peripheral blood, but it will significantly decrease after major abdominal and heart surgery, which is closely correlated to secondary infection and patient prognosis. The study by Volk et al proved that < 30% expression rate of CD14^+mHLA-DR could be regarded as a threshold and reliably screened out the patient with sepsis complicated with immunosuppression, and the immunostimulation therapy on such patient was able to effectively reverse the immunosuppression and improve the prognosis.

Clinical and experimental studies both revealed that TEAS and electroacupuncture could increase adaptive immune function, and improve immunosuppression. In addition, the up-regulation of the quantity and function of HMGB1 mediated regulatory T-cell (Treg) was one of key causes leading to
immunosuppression.\textsuperscript{19-21} Postoperative blood Treg quantity and HMGB1 of patients receiving heart surgery both significantly increased, and such findings revealed that HMGB1 and Treg might be involved in the occurrence of postoperative immunosuppression in patients receiving CABG.\textsuperscript{22-23} The early-stage pilot study of this clinical trial showed that the electroacupuncture was able to inhibit the increase of HMGB1 caused by myocardial ischemia reperfusion, and reduce the incidence of myocardial injury.\textsuperscript{23} Based on above studies, we hypothesized that TEAS could improve the immunosuppression of the patients receiving heart surgery, reduce postoperative expression level of mHLA-DR, as well as reduce Treg quantity and function by inhibiting the release of HMGB1, and thus increase the function of CD4\textsuperscript{+}T-cell.

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 extracorporeal circulation as observation subjects and randomized them into 2 groups: the group of TEAS combined with general anesthesia (TEAS+GA), and the group of transcutaneous acupoint pseudo-electric stimulation combined with general anesthesia (Sham TEAS+GA). mHLA-DR was primary endpoint for measuring improvement of postoperative immunosuppression, and the patients of both groups were observed for the change of mHLA-DR 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) to evaluate TEAS effectiveness, which would possibly provide a clinically technical method to improve postoperative immunosuppression of patients receiving heart surgery and also provide evidence for clinical application of this technique.

Methods

Patient and public involvement

The research question and outcome measures were developed from a desire to evaluate whether the effect of TEAS on improvement of immunosuppression of patients after receiving CABG. We seek to
promote this clinical technique and provide theoretical basis for further improvement of this
technology. Patients, however, were not directly involved in the design or conduct 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), reactive C (CRP), secondary
infection (incision infection, lung infection, hematogenous infection and indwelled catheter infection).
The indicators of related mechanism study include high mobility group proteins-1 (HMGB1),
regulatory T-cell (Treg) and CD4+ T-cell.

**Study location**

A prospective, single-centre, randomised, 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 under extracorporeal circulation.

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: $18.5\text{kg/m}^2 < \text{BMI} \leq 30\text{kg/m}^2$
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 (ST36) / 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.

Endpoints

Primary endpoint

With mHLA-DR as primary endpoint 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). Flow cytometry is used for determination. (Becton-Dickinson, New Jersey, U.S.)

Secondary endpoints

1. Related indicators of immunosuppression include interleukin-6 (IL-6), reactive C (CRP), secondary infection (incision infection, lung infection, hematogenous infection and indwelled catheter infection). The examination methods and time points are as follows:
   - IL-6: ELASA kit (ABCAM, Shanghai, China), One day before surgery, Day 1, 3 and 5 after surgery;
   - CRP: automatic biochemical analyzer (Beckman Coulter, Georgia, U.S.), One day before surgery, Day 1, 3 and 5 after surgery;
   - Secondary infection: observe the incidence of incision infection, lung infection, hematogenous infection and indwelled catheter infection after the 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:

**HMGB1:** ELASA kit (ABCAM, Shanghai, China), One day before surgery, Day 1, 3 and 5 after surgery;

**Treg (CD4+/CD25⁺ T-cell):** flow cytometry (Becton-Dickinson, New Jersey, U.S.) One day before surgery, Day 1, 3 and 5 after surgery;

**CD4⁺ T-cell:** flow cytometry (Becton-Dickinson, New Jersey, U.S.) One day before surgery, Day 1, 3 and 5 after surgery;

**Randomization and blinding**

Stratified randomization will be used to assign the candidate subjects to 2 group according to age (elderly patients ≥65 and adult patients <65). 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**

Human leukocyte antigen of monocyte (mHLA-DR) is the primary endpoint to evaluate on the improvement of immunosuppression of patient after surgery. 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%.

**Study design**

This study is a prospective, single-center, double-blind, randomized and controlled clinical trial to explore the effects of TEAS therapy on improvement of postoperative immunosuppression of patients receiving CABG (Fig. 1). The trial was 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 combined with general anesthesia (TEAS+GA), and the group of transcutaneous acupoint pseudo - electric stimulation combined with general anesthesia (Sham TEAS+GA). In each group, there will be 44 patients receiving CABG under extracorporeal circulation. The patients of TEAS+GA group will receive TEAS therapy in perioperative period, and the Sham TEAS+GA will receive none TEAS in perioperative period.

**Study time**

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

**Interventions**

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

   Zusanli location: located at outside of the shank, 3 cun (10 cm) below Dubi acupoint (ST36) and a finger’s width (middle finger) to tibial front edge;
   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 (Figure 4)

   30 mins before anesthesia: one stimulation for 30 mins;
   Intraoperative: stimulating in the whole course;
   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**

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

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

TEAS+GA group: the acupoints including Zusanli and Shenshu, were identified before electrical stimulation with surface electrodes (Figure 6). Selection of these acupoints was based on a consensus between the acupuncturists of the study.
Sham TEAS+GA 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

Medication before the anesthesia
Morphine: 0.1mg/kg
Anesthesia induction
Sufentanil 0.3−5ug/kg
Propofol TCI: 2.0−5ug/ml
Dextromidine 0.5−10ug/kg/hr or midazolam 0.05−0.1mg/kg
Lidocaine 1mg/kg (Maximum dose not higher than 50 mg)
Rocuronium bromide 0.9−2mg/kg

Maintenance of anesthesia
Narcotic analgesics: common sufentanil by 0.2−5ug/kg by times (i.v) or remifentanil by 0.05−5ug/kg/min continuous intravenous pump injection, addition of sufentanil by 10−20ug before skin incision and sternum splitting.
Inhaled general anesthetics: sevoflurane and isoflurane can be inhaled discontinuously as requested with MAC 0.7−0.0.
Muscle relaxant: common vecuronium bromide and rocuronium bromide, etc.
After the completion of tracheal intubation, the anaesthesia machine is connected immediately, and ETCO₂ is examined, and the breathing sound of both lungs is auscultated to determine the position of endotracheal tube.
Common parameters of mechanical ventilation: VT: 7−8 ml/kg, RR: 10−12 bpm; PaO₂: 200 mmHg, PaCO₂: 35−45 mmHg, FiO₂: 80%.

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).

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).

**Data collection and management**

The data will be collected as primary and secondary endpoints, 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.

**Discussion**

The patients who have received CABG under extracorporeal circulation are more prone to have immunosuppression, causing secondary infection and occurrence of malignant tumor, and severely affecting patient’s outcome. Previous studies proved that the acupuncture was able to improve the patient’s immune function, and therapy of TEAS had the same efficacy as acupuncture, and TEAS was also characterized by easy operation, non-invasiveness and easy acceptability. This study performs TEAS intervention on patients scheduled to receive CABG under extracorporeal circulation in perioperative period, to improve their postoperative immunosuppression, reduce postoperative complications, reduce secondary infection rate, shorten hospital stay and improve the patient’s outcome. This study also aims to provide clinical evidence for application of this technique.

In addition, this clinical study has certain limitation. This is a single-center study, and the experimental results may be biased. Therefore, we need larger sample size study in future to increase the reliability of research results. TEAS related parameters in this study (stimulus intensity and waveform, intervention timing and duration, etc.) should be further optimized, which needs us to carry out further comparison in groups in the following clinical trials and to discuss the superiority of stimulus parameters one by one.

**Trial status**

This is the second protocol version, updated on Nov. 8\(^{th}\), 2018. The recruitment began on Dec. 1, 2017 and would be completed by Dec. 31, 2019. At the time of manuscript submission, the study was
in recruitment phase.

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.

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**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.

Abbreviations

TEAS: Transcutaneous electrical acupoint stimulation
GA: General anesthesia
CAGB: 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 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\(_2\)

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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.

|   | Postoperative arrhythmia | Pneumonia | Acute lung injury | Pulmonary atelectasis | Intraoperative and postoperative myocardial infarction |
|---|--------------------------|-----------|-------------------|-----------------------|------------------------------------------------------|
| 1 | 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) | Body temperature above 38.5°C (indicated in chest film); | (1) acute onset, with pathogenic factors; | Indicated in chest film; | 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; |
| 2 | | | (2) oxygenation index (arterial partial pressure of oxygen/fraction of inspired oxygen, PaO_2/FiO_2) <300 mmHg (1 mmHg=0.133 kPa) not referring to positive end-expiratory pressure (PEEP) level; | | |
| 3 | | | (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. | | |
| 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. |
| 3 | Whole cardiac insufficiency | Coexistence of clinical 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 (PaO₂) is lower than 8 kPa (60 mmHg) or accompanied with partial pressure of carbon dioxide (PaCO₂) higher than 6.65kPa (50 mmHg). |
| No | Condition                                      | Description                                                                 |
|----|------------------------------------------------|-----------------------------------------------------------------------------|
| 8  | Postoperative hemorrhage of digestive tract   | including ulcer bleeding or bloody gastric content caused by mucosal ischemia of gastrointestinal tract, haematemesis, tarry stool or hemaefecia; |
| 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 μmol/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

Note: Dubi acupoint (ST35) location – After bending the knee, it is located in the concave between kneecap and ligamentum patellae outside. 1 cun = 3.33 cm.
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)
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
This is a list of supplementary files associated with this preprint. Click to download.
Research Checklist.pdf