A Randomised Controlled Trial of Intraoperative Cell Salvageduring Caesarean Section in Women at Risk of Haemorrhage in China

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Statement of Principal Investigator:

I hereby sign that I have read and understand this study, agreed the contents of the study, and will strictly follow all the provisions of this study, 'Good Clinical Practice of Drugs' and the implementation of relevant state laws and regulations. I will provide a copy of this document to all investigators who are responsible for this study, and discuss with them about the study and the related information to ensure that they are fully familiared with the drug we study, and how the study is conducted.

Name of Principal Investigator: Qingquan Lian

Signature of Principal Investigator: _____

Date: _____

Name of Principal Investigator: Fang Gao

Signature of Principal Investigator: _____

Date: _____

Statement of Statistical Analyst:

I hereby sign that I have read and understand this study, agreed the contents of the study, and will strictly follow all the provisions of this study, 'Good Clinical Practice of Drugs' and the implementation of relevant state laws and regulations. I am responsible for the data management and statistical analysis of this clinical research. I will conscientiously perform the duties of statisticians, follow the relevant guidelines for clinical statistics, and ensure that the statistical results are real, scientific and standardized.

Name of Statistical Analyst: Zuokai Xie

Signature of Statistical Analyst: _____

Date: _____

Statement of Major Investigator(s) of Test Institution(s):

I have carefully read the study protocol, and have agreed to follow this protocol to perform this clinical study.

1) I understand that the study protocol needs to be approved by the Ethics Committee, signed and approved before implementation. During the study, any change makes to this study protocol need to be approved by the Ethics Committee; any serious adverse event occurs in the study need to be promotly reported to the Ethics Committee.
2) I know that according to the Regulations on 'Good Clinical Practice of Drugs' and the Helsinki Declaration (2000), before each patient's enrollment in the study, it is my responsibility to present a written form to give him/her or his/her designated representative a complete and overall introduction of the purpose, procudures and possible risks in this study. Patients should be aware that they have the right to withdraw from the study at any time. Each patient or relative must be given a written version of informed consent before being enrolled. It is my responsibility to have every patient receive the informed consent before enrolling the study and the informed consent should be retained for future reference as a clinical research document.
3) I will enroll subjects who meet the criteria of this study. I will be responsible for making medical decisions related to clinical research, and promise that subjects will receive timely treatment once any adverse event occurs during the clinical trials. I know the requirement of correctly reporting serious adverse events, so I will be record and report these incidents based on these requirements.
4) I promise that the data loaded into the case report form is accurate, complete, prompt and legal. I verify that the signature on each completed case report form indicates that I have carefully reviewed each page and took full responsibility for the relevant content.
5) I accept the scrutiny or inspection of the CRA or CRAs sent or entrusted by the sponsor, and I accept the inspection of the drug regulatory authority to ensure the quality of the clinical trial.
6) I know that the information in this trial is confidential. I hereby certify that I will not disclose any information in this study without the consent of the sponsor, except as required by the State Food and Drug Administration or other regulatory agencies.
7) I will provide a resume before the the study starts, submit it to the Ethics Committee for reviewing, and may submit it to the drug regulatory authority.

Name of major investigator: _____

Signature of major investigator: _____

Date: _____

Table 1：Proposal

Title	A Randomised Controlled Trial of Intraoperative Cell Salvage during Caesarean section in women at risk of haemorrhage in China
Abbreviation	Cell Salvage during Caesarean Section- CSCS
Version	Version 2.1 dated 2016/2/12
Method	A randomized, controlled, single-blind and multi-centre clinical intervention trial with cost-effective analysis.
Trial Duration	2 years recruitment 6 months data analysis 6 months write dissertation (3 years in total)
Trial Center(s)	6 Centers of Obstetric who are eligible for clinical trials and are eligible for intraoperative cell salvage: 1. The Second Affiliated Hospital of Wenzhou Medical University 2. The First Affiliated Hospital of Wenzhou Medical University 3. Wenzhou People's Hospital 4. Taizhou Hospital of Zhejiang Province 5. Lishui City People's Hospital 6. The Central Hospital of Lishui City
Objectives	• To determine the difference of blood loss postoperation in whether use of intraoperative cell salvage during caesarean section, or current practice of allogous transfusion. • To look into and compare whether there is any adverse effects or reaction after IOCS or allogous transfusion. • To determine if the routine use of IOCS during CS, in women at risk of haemorrhage, is cost effective in comparison to current practice.
Estimated number of subjects	120 cases

Objects	Inclusion Criteria: • 18 years of age or elder • delivery by elective or emergency caesarean section with an identifiable increased risk of haemorrhage • ability to provide informed consent • needing for blood transfusion during CS Exclusion Criteria: • with blood transfusion history before the CS • Maternal preoperative Hemoglobin(Hb) < 70g/dL • Maternal Rh(Rhesus Macacus) negetive blood type • Sickle cell disease • contraindicated to intraoperative cell salvage e.g. abdominal cancer, abdominal infection, intrauterine infection with blood transfusion history before the CS • Maternal preoperative (Prothrombin Time)PT or (activated partial thromboplastin time) APTT is 1.5 times greater than normal and above; or preoperative platelet(Plt) < $50*10^9$L • Cultural or social beliefs contraindicating blood transfushion, e.g. Jehovah's witnesses18 years of age or elder • Significant antibodies making it difficult to find cross matched blood for transfusion • ability to provide informed consent • Joined other clinical trial within 3 months. • Investigator thinks maternal is not suitable to participate
Statistical Method	use safety dataset to analyze the security, and use Full Analysis Set(FAS) and Per Protocol Set(PPS) to analyze the efficacy accordingly. Use SAS9.13 for coding analysis

Contents

Introduction

1.1 Background

Haemorrhage(excessive blood loss) is one of the major causes of maternal death. It is more likely to occur during caesarean section, especially for those maternal who have placenta accreta or placenta previa.

Blood is a scarce and expensive resource. Hospitals in China at all levels have varying degrees of tension with blood. Due to the scarcity of blood and maternal's risk of blood transfusions, it is common that mothers lose their blood transfusions in time, and result in postpartum anemia (low hemoglobin levels) in their mothers. A seriesof reports confirmed: anemia is a direct cause of maternal deaths[1]. Life-threatening blood loss is an indication of emergency hysterectomy[2]. A retrospective analysis involving 24 hospitals shows that at present, the total domestic cesarean section rate is 57.84% (2005 ~ 2006) [3]. Postoperative anemia will further prolong hospitalization, increase postoperative infection rates and delay the time of first mobilisation. Intraoperative cell salvage collects patients' own lost blood, wash and filter into the patient's own blood circulation. It may help to reduce the incidence of postoperative anemia for patients and avoid prolonged hospitalization. Although it has been confirmed that using cell salvage technology in some operations can reduce allogeneic blood transfusion, since clinical concerns about the amniotic fluid contamination of the blood transfused by the mother, intraoperative cell salvage during caesarean section has not yet been fully validated. At present, the National Institute of Health and Clinical Excellence has developed guidelines which recommend to use intraoperative cell salvage in the event of excessive haemorrhage in emergency cesarean section. In addition, intraoperative cell salvage is also recommended by the Royal College of Obstetricians and Gynecologists and the American Society of Anesthesiologists. At present, only a few clinical cases in China have been reported for washing autologous blood transfusion for maternal hemorrhage. No relevant randomized controlled studies were found in the literature. The risks of allogeneic blood transfusion include: death from transfusion errors, acute transfusion reactions, fatal lung injury and infections[4]. Therefore, applying allogeneic blood transfusion to the healthy maternal need to be very cautious, and intraoperative cell salvage may possibly avoid these risks. Intraoperative cell salvage is a technique that can not only simultaneously reduce the need for allogeneic transfusions and prevent anemia, but also avoid bleeding-related serious complications. It has been shown to be well documented in non-cesarean sections, but current information is not able to demonstrate the safety and efficacy of autologous blood collection for bleeding during cesarean section, and have reported several cases of coagulation dysfunction associated with autologous blood collection.[5]. Therefore, it is necessary to carry out the monitoring of coagulation function and other checking of related

indexes for obstetric autologous blood transfusion patients[6, 7]. In addition, scientific rigorous test is necessarily to be carried out to demonstrate the safety and effectiveness of intraoperative cell salvage in cesarean section. We plan to construct a randomized controlled multicenter study to evaluate the safety and efficacy of intraoperative cell salvage in caesarean section, and provide some statistics for application of autologous blood transfusion in obstetrics.

1.2 CSCS

Intraoperative cell salvage collects patient's blood loss during surgery, and after separation purification and filtration, and then returns to the patient's own. This technique has been optimized in recent years, and it has been routinely used in departments which is at risk of heavy bleeding, such as cardiac surgery, orthopedics, hepatobiliary surgery and vascular surgery. British National Code of Practice is now only recommended to use of intraoperative cell salvage in the case of emergency cesarean section with heavy bleeding. However, in China, there's no guidelines recommending to use of intraoperative cell salvage in the case of cesarean section, but some hospitals in China have now tried to perform intraoperative cell salvage when lack of suitable allogenic blood. However, there's still a lack of evidence demonstrating the medical and economic benefits of intraoperative cell salvage for cesarean section. Regardless of amniotic fluid embolism, people generally think that intraoperative cell salvage technology may be superior to allogeneic blood. This protocol focuses on evaluating the difference in the efficacy of the two transfusion methods. In addition, this study will also compare the adverse reactions and economic benefits of two transfusion methods. Although currently the cost of intraoperative cell salvage is not low, compared with the cost of allogeneic blood transfusions and the extra cost of longer hospital stay and additional treatment, intraoperative cell salvage may reduce overall maternal medical costs.

1.3 Risk and Benefit of CSCS

1.3.1 Risk

As mentioned earlier, cesarean section has been considered a contraindication to the use of intraoperative cell salvage, due to the theoretical risk of amniotic fluid embolism(an extremely serious, but very low (about one in 200) incidence of pregnancy and maternal complications. However, with the update of technology (new filter equipment), this risk is theoretically even lower. After testing the mothers who has been used cell salvage in cesarean section, we found that after the modernization of equipments, those salvaged, purified and returned blood in cesarean section is the same

as the normal maternal blood[8, 9].

In addition, during autologous blood transfusion, in theory, the contamination of fetal red blood cell may cause fetal hemolysis in another pregnancy, but there is no evidence that autologous blood transfusion can increase future fetal hemolytic disease.

About autologous blood transfusion and coagulation disorders, the general view is that autologous blood transfusion can wash out heparin and other anticoagulant substances, but there are also typical cases report that after autologous blood transfusion, abnormalities in coagulation index may happen. Since coagulation function and a variety of factors are related, in this trial, we will monitor some of the indicators.

The risks of allogeneic blood transfusions include death from transfusion errors, acute transfusion reactions, fatal lung injury, and transfusion infections.

Compared with the allogeneic transfusion group, there exsits potential or undetected adverse events to subjects themselves.

1.3.2 Benefit

Intraoperative cell salvage may reduce the demand for allogeneic transfusions, and therefore less transfusion reaction and infection would happen than allogeneic transfusions. Postoperative anemia is related to the prolonged hospitalization, the increased wound infection and the delayed recovery of the patient's activity. Intraoperative cell salvage allow those original blood that may be discarded goes back to the patient, which greatly reduces the incidence of postoperative anemia.

1.4 Set-up

Prior to the protocol development, a systematic review of the literature has been conducted. Prior to the pretest, a series of surveys of maternal and obstetricians and anesthesiologists has been conducted as well to understand their acceptance of intraoperative cell salvage.

At the Second Affiliated Hospital of Wenzhou Medical University, we randomly invited 30 maternal to conduct a questionnaire survey. There are 29 successfully returned copies, and the response rate reached 96.7%. Data analysis shows that 75.86% of the maternal have some concerns on allogeneic blood transfusion, and 82.76% of the maternal have some concerns on intraoperative cell salvage. However, when maternals were asked, “if blood transfusion is needed during cesarean section, which method do you prefer?”, 13.79% of mothers are willing to receive intraoperative cell salvage, 55.17% of mothers are willing to follow doctors’ decision, and only 31.03% of mothers are willing to accept allogeneic blood transfusion only. And 24.14% of the maternals clearly declare that they are willing to participate in our trials. Therefore, during the trial, there was some difficulty in obtaining subjects' informed consent and joining the trial, but there was still a certain acceptance rate.

At the Second Affiliated Hospital of Wenzhou Medical University, we randomly invited 30 obstetricians to conduct a questionnaire survey. 29 of the surveys were successfully returned with a response rate of 96.7%. Data analysis shows that 89.66% of obstetricians were affected during their work due to blood difficulties. 89.66% of obstetricians have heard of intraoperative cell salvage, and 41.38% of obstetricians have used this technique in their work. 72.41% of obstetricians think autologous cell salvage is more beneficial than allogeneic blood transfusion, 27.59% of doctors are not sure which method is more favorable, but none of the doctors think that allogeneic cell salvage is more favorable than autologous blood recovery. Meanwhile, 100% of the doctors think it is necessary to have some research on the safety and efficacy of the two transfusion methods. When asking “if the mother needs blood transfusion during the cesarean section, are you willing to let the mother participate in this study”, 48.28% of doctors are willing to, and 48.28% of doctors will be based on the condition. When asking the influence of postoperative bleeding according to the two transfusion methods, 68.97% of doctors are not sure about it. The above data not only show the good acceptance of autologous cell salvage from obstetricians, but also show the value of this trial.

At the Second Affiliated Hospital of Wenzhou Medical University, we randomly invited 30 anesthesiologists to conduct a questionnaire survey. All 30 of the surveys were successfully returned with a response rate of 100%. Data analysis shows that 76.67% of anesthesiologists were affected during their work due to blood difficulties. 26.67 % of anesthesiologists think autologous cell salvage is more beneficial than allogeneic blood transfusion, 66.67% of anesthesiologists are not sure which method is more favorable. The "uncertainty" is higher than obstetricians. Perhaps it is because anesthesiologists know more about intraoperative blood collection. When asking “if the mother needs blood transfusion during the cesarean section, are you willing to let the mother participate in this study”, compared to obstetricians, there are also 48.28% of anesthesiologists would like to, and 48.28% of anesthesiologists will be based on the condition. When asking the influence of postoperative bleeding according to the two transfusion methods, compared to obstetricians, 68.97% of anesthesiologists are not sure about it.

1.5 Trial Feasibility

After obtaining the approval of ethics committee, a randomized controlled trial of cell salvage in cesarean section will be conducted at the Second Affiliated Hospital of Wenzhou Medical University. This pretest will help to adjust the protocol and operation, test the effectiveness of the data collection method, and the acceptability of the test design.

2 Objectives and Design

A randomized, controlled, single-blind and multi-centre clinical trial

2.1 Study Objects

2.1.1 Primary Objective

- Blood loss of the subjects in 5 days post operation

2.1.2 Seconday Objective

- PT/APTT, Hct and Hb in first and fifth day after surgery
- the number of units of donor blood transfused
- transfusion-related adverse reactions
- postoperative complications: amniotic fluid embolism, sepsis, acute respiratory distress syndrome, disseminated intravascular coagulation, wound infection, acute hemolysis, etc.
- mortality during hospital stay
- time to first mobilisation,
- duration of hospital stay
- intraoperative salvaged cell; before and after cesarean section, maternal‘s squamous epithelial cells, fetal red blood cells, serum potassium, free hemoglobin.
- Before and after blood transfusion, maternal’s electrolytes, PH value, fetal red blood cells, serum potassium, free hemoglobin
- Total cost in hospital

Second Stage: Follow-up when pregnancy again:

- Neonatal hemolytic disease（Appendix 2）
  Determine blood type, antibody, and (Immunoglobulin G)IgG titer

2.2 Study Design

A randomized, controlled, single-blind and multi-centre clinical trial with cost-effective analysis.

3 Subjects Screening

Subjects: Woman who are admitted to a participating labor ward who fulfil all the following criteria will be eligible to be randomized.

3.1 Eligible Criteria

3.1.1 Inclusion Criteria

- 18 years of age or elder
- admitted to a participating labor ward
- delivery by elective or emergency caesarean section with an identifiable increased risk of haemorrhage
- ability to provide informed consent
- needing for blood transfusion during CS (according to blood transfusion guidelines [Appendix 1], obstetrician or anesthesiologist's advice)

3.1.2 Exclusion Criteria

- with blood transfusion history before the CS
- Maternal preoperative Hemoglobin(Hb) < 70g/dL
- Maternal Rh negetive blood type
- Sickle cell disease
- previous Caesarean section or breech history
- contraindicated to intraoperative cell salvage e.g. abdominal cancer, abdominal infection, intrauterine infection with blood transfusion history before the CS
- Cultural or social beliefs contraindicating blood transfushion, e.g. Jehovah's witnesses18 years of age or elder
- Significant antibodies making it difficult to find cross matched blood for transfusion
- admitted to a participating labor ward
- delivery by elective or emergency caesarean section with an identifiable increased risk of haemorrhage
- ability to provide informed consent
- Maternal preoperative PT or APTT is 1.5 times greater than normal and above; or preoperative platelet(Plt) < $50*10^9$L
- Joined other clinical trial within 3 months.

- Investigator thinks maternal is not suitable to participate

3.2 Midway-Withdraw of Subjects

After completing randomization, the subjects will be considered as midway-withdraw from the study if:

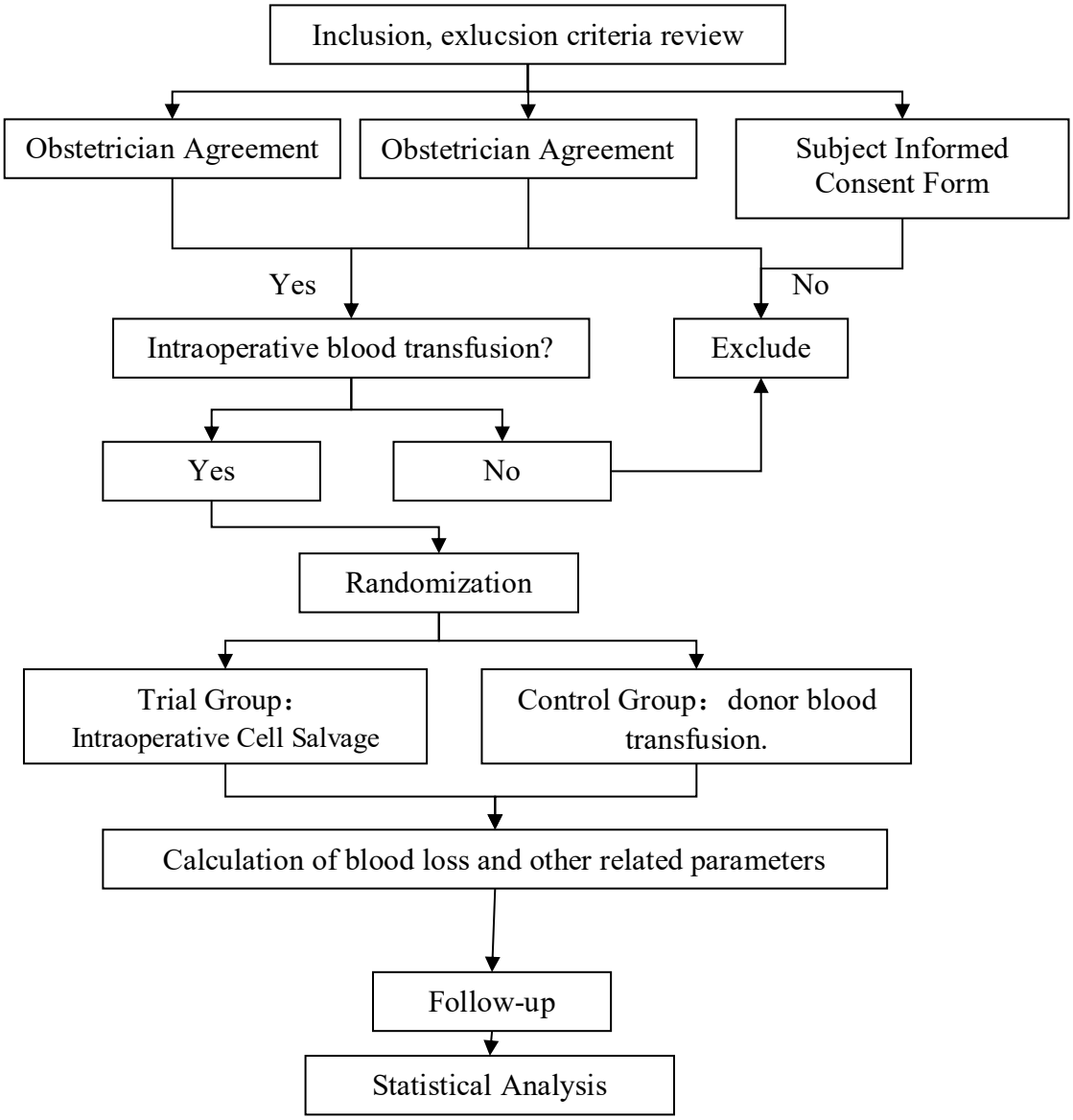
- does not meet the inclusion criteria;
- for safety issue, one of the transfusion methods must be applied;
- subjects is no longer able to agree to participate the trial;
- subjects withdraw their consent form during the trial or during data collection;
- subjects no longer need cesarean section.

3.3 Data Collection and Follow-up for Midway-Withdraw Subjects

If the investigator or the clinician or medical team providing the medical service believes that the subject needs to withdraw from the trial, it must be accepted from the medical point of view. It is the participants' freedom to determine whether continue follow-up after surgery or not. However, it is unavoidable that those midway-withdraw subjects will lead to biased outcome and the efficacy of the clinical trials will be worsen. Investigators need to collect and record the reasons and adverse events to any violation of the protocol as far as possible. The follow-up of maternal is also needed for safety issues. Maternal is still free to quit the program after completing the randomization. We will have to keep a record for the quit. Meanwhile, whether maternal agrees to be followed is also necessary to be recorded, if the participant decides to withdraw the study.If the subject explicitly requests to return all the data records, their decisions should be respected and be recored to the final study form. When maternal is unable to obtain the informed consent during the trial, it turns to her legal representative's decision of whether continue or not.

4 Study Procedure

4.1 Flow Chart of Study



4.2 Informed Consent Acquisition Process

4.2.1 Elective Cesarean Section

Selected maternal in cesarean section will receive detailed information. They will

also have the opportunity to ask questions after the appointment of surgery. The paper version informed consent will also be obtained on the day of admission to the ward or the day of operation. The informed consent forms need to make it clear that they can withdraw from the trial at any time for any reason, without discrimination and without any justification for their withdrawal from the trial. Randomizaion will be carried out on the day of operation.

4.2.2 Emergency Cesarean Section

For independent randomized controlled trials of intraoperative cell salvage, it is a huge challenge to obtain informed consent during emergency cesarean section. Subjects in this group will be a special category among all participants. They will obtain particular benefits form this technology, therefore their participation is very important.

Emergency cesarean section also requires a paper informed consent form. The informed consent forms also need to make it clear that they can withdraw from the trial at any time for any reason, without discrimination and without any justification for their withdrawal from the trial. Randomizaion will be carried out on the day of operation.

Mostly, when there's no feral distress during the emergency cesarean section, anticipate adequate time will be required for routine anesthesia. Therefore, after patients' decision of having a cesarean section, there is still some time to obtain informed consent of the paper.

For safety, in this trial, we do not accept the oral informed consent and the case that re-sign the informed consent after the operation.

4.3 Patient Screening Process

Researchers need to understand the situation of new hospitalized maternal, such as the presence of potential subjects. Researchers also need to read their medical case reports in detail, and then confirm the inclusion criteria and exclusion criteria. When all the criteria meet, we need to get informed consent from potential subjects, and informed consent needs to be obtained prior to surgery. After receiving the informed consent, randomization will be performed directly in the delivery room at the time of preparation (in order to provide enough consideration to maternal time) and subjects will be assigned to the respective groups based on randomization results. The screening process needs to be recorded in the patient enrollment form.

4.4 Randomization Process

Once subjects are confirmed through all the criteria and have received theinformed

consent, they can be randomized to either the treatment group(Caesarean section with IOCS) or control group(Caesarean section without IOCS).The randomization will be proceed in operation if the patient needs blood tranfusion.

Randomization (ratio:1:1) is used through a network-based randomization system, which follows SOP. We use central dynamic randomization system.

The randomization will be stratified by 5 conditions:

1. centers
2. type of cesarean section（Emergency/Elective）
3. whether there exist abnormality in placental
4. number of fetus (twins or more)
5. whether the ASA level is in I – II?

The randomization system will be tested and validated before the trial starts and will be supervised by the leader center's CRA and statistical expert throughout the trial.Once the subjects have been successfully grouped, they will be recorded in theenrollment form.

Each test center has their independent account and password for randonmization system. The account number and password are authorized to the relevant staff by the major researchers in each center. After the staff has filled in the subject's ID and details into the randomization system, the system will display the grouping result.

4.5 Methods of Minimizing Risk and Bias

Using a third party to hide the grouping will help reduce the errors caused by selection. The randomization will be proceed in operation, and the staff should set blinding to the subjects.

Operational errors could result in change in blood transfusion rate.To minimize this risk, we need to make sure that all operating rooms and recovery wards in each center use an unified blood transfusion plan. Each medical team within each center should have same start-up criterion for setting up blood transfusion. Before participating in the trial, each center should provide a blood transfusion plan to the leader center for record. Some centers would use a prespecified value of Hb to determine whether blood transfusion is needed and this value should be unified among each center's medical team. If any of the following sympotoms presents, such as inconscious, dyspnoea, exhaustion, which could clearly indicate level of red cell, blood transfusion is needed. Maternal mobility and neonatal care after cesarean section are different from the postoperation rehabilitation activity of most bedridden patients. According to the practical issues, since the clinicians may have different point of view, it is allowed to have little variation between each center. It represents the difference in reality cross the clinic, so it will not increase the bias.

Postpartum ward medical staff should use an unified blood transfusion plan as far as possible, according to the postoperative Hb levels and maternal symptoms. Do not deliberately observe the grouping of subjects.

4.6 Preparation and Support for Medical Staff in the Trial

For obstetric, operating room nurses, obstetricians and anesthesiologists , who involve in the study, the application of intraoperative cell salvage during caesarean section surgery would be an extra workload. Especially in emergency caesarean section surgery, it would be more challenging to test how skilled clinicians are. Adequate training of all medical staff and the use of intraoperative cell salvage machine are very important to the success of trials. Intraoperative cell salvage needs to have been on-going at all participating centers, and many operating room staff should be highly-skilled in use of technology. Prior to the start of the trial, each center will need to provide a list of staff, who would involve in intraoperative cell salvage operations and have been assessed by the main investigators of each center. During the inspection, the CRA will assess the standardization of operations (SOP) of relevant staff.

4.7 Treatment Plan

According to local transfusion guidelines and medical staff's opinion (participant's Hb is lower than 70g/L before transfusion, or Hb is between 70~100g/L, depending on transfusion plan and medical staff.), women will be randomly allocated to either:

1. Caesarean section with IOCS(treatment group）
2. Caesarean section without IOCS. eg. donor blood transfusion(control group）

Theoretically, intraoperative blood transfusion need to be based on the following formula:

the volumn of blood transfusion needed(ml) * Hb of transfusion samples = (Hb_2-Hb_1) * blood volumn,

where Hb_1:actual Hb; Hb_2: target Hb(100g/L）

blood volumn = 100ml/kg[10]*weight

Either in treatment group or control group,the difference between the actual volumn of blood transfusion and the theoretical volumn should be controlled within ±15% (in terms of mass of Hb).

a. IOCS group

Eg.: Maternal actual Hb = 60g/L, weight = 60kg, Hb of blood sample = 200g/L

Volumn of blood transfusion needed = 1200ml, range of blood transfusions = 1200*(1±15%), 1020-1380ml; If there's insufficient volumn of self blood for five-minutes' infusion, then we use formula to calulate the volumn of blood transfusion needed. After five-minutes' infusion by self blood, if participant's Hb is still lower than 70g/L before transfusion, or Hb is between 70~100g/L, according to transfusion guidelines and medical staff's opinion, we continue to use formula to calulate the volumn of blood transfusion needed.

b. Donor blood transfusion group

Eg.: Maternal actual Hb = 60g/L, weight = 60kg, assumed Hb of allogeneic red cell is 240g/L[11]，per U red cell is around 200ml.

Volumn of blood transfusion needed = 1000ml，range of blood transfusions = 1000*(1±15%), 850-1150ml，around 4.25-5.75U; 4.5U red blood cell allogeneic blood can be requested from the blood bank. Following the concept of using of blood economically, we should take as less suitable blood as possible. If needed, we can communicate with the transfusion department. After five-minutes' infusion, we measure maternal's Hb again. If participant's Hb is still lower than 70g/L before transfusion, or Hb is between 70~100g/L, according to transfusion guidelines and medical staff's opinion, we continue to use formula to calulate the volumn of blood transfusion needed.

When emergency life-threatening bleeding events happen in donor blood transfusion group (control group), based on the actual situation, clinical staff there can take the most reasonable step for mothers, including the intraoperative cell salvage. We expect the probability of this situation to be extremely low.

4.8 Procedure Table

	Preoperative	**Intraoperative**	**Postoperative**	**Hospital Discharge**	**Long-term Follow-up**
Eligible criterion and Grouping					
Inclusion, exlucsion criteria review	X				
Informed Consent Acquisition	X				
Record demographic information, contact info, diagnosis, type of surgery, cesarean section indication, birth history, preoperative blood loss, comorbidities (past history), blood group ABO, and other abnormalities	X				
Randomization （Dynamic Randomized）		X			
Blood Transfusion					
Intraoperative blood transfusion and other blood products		X	X		
Intraoperative autologous blood transfusion, and related data		X			
Maternal					
Intraoperative anesthesia operation time, special circumstances (such as hypotension), urine output		X			

Intraoperative and postoperative daily bleeding and transfusion, infusion volume (crystal, gel), blood-related drugs		X	X		
The highest body temperature, Hb / Hct / Plt, PT / APTT, ALT / AST / total G / D and renal function (creatinine and urea)	X	X	X		
Postoperative bleeding (drainage, bleeding, lochia, etc.)			X		
Specific causes of postoperative bleeding (uterine inertia, placental conditions, soft birth canal laceration situation)			X		
Test of blood component in recover blood and maternal blood fetal		X（Specimen only）	X（Specimen only）		
Transfusion reaction		X	X		
Other hospitalization					
Time to first mobilisation			X		
Duration of hospital stay				X	
Intraoperative and postoperative adverse events		X	X		
Reoperation caused by bleeding			X		
Inpatient mortality				X	
Overall medical expenses				X	
Tracking for hemolytic disease in future birth					X

4.9 Relevant Operating Plan

4.9.1 Assessment Method for Intraoperative and Postoperative haemorrhage in Obstetrics

- Assessment Method for Intraoperative blood loss in Cesarean Section
  - ✧ Weighing Method: blood loss (ml) ≈ (weight of gauze with blood – weight of dry gauze) /1.05
  - ✧ Measuring Cup Method: used to measure amount of blood in a curved plate; blood loss ml=Volume of measuring cup (blood clots crush as much as possible).
  - ■ Intraoperative blood loss = Recycling machine blood volume (recovery machine capacity - flushing, washing capacity) + Placental bleeding (using measuring cup to measure the amount of blood flow into the curved plate by

squeezing the placenta) + blood volumn of cleaning the vagina (bleeding with weighing method, blood cluts with measuring cup method) + blood volumn of gauze (bleeding with weighing method, blood cluts with measuring cup method)

- Postoperative blood loss = blood volume of postpartum sterile mattress + blood volumn of gauze (bleeding with weighing method, blood cluts with measuring cup method) + volumn of shed blood（using measuring cup）

4.9.2 Operation Precautions of Obstetric Cesarean Section

In cesarean section, the absorption of amniotic fluid should be sufficient, so that the residual of intrauterine amniotic fluid can be minimized. When cut the umbilical cord, cord blood should be guaranteed not to enter the uterine cavity. Any action that makes fetal bleeding should be avoid.

Prepare two sets of suction equipment, one for amniotic fluid and other.

4.9.3 Anesthesia Cell Salvage Operation（See Appendix 3）

4.10 About Early Termination of Trial

If the blood collection machine manufacturer issues an important safety notice, then we will temporarily stop the trial. If the ethics committee, the national clinical trial management agency, or the funders need to suspend the trial due to the consideration for subjects'maximum benefit or the trial itself, then the principal investigator will receive a written notice, and stop the trial. Usually the concerns will involve safety, efficacy of the trial, violations of relevant provisions and protocol, etc.

4.11 About the Ending of Trial

The ending time of Phase I in this trial is defined as the date that the last subject discharges from hospital. The ending time of Phase II in this trial is defined as when the last subject exceeded the childbearing age and has been followed for 10 years. At the end of trial, we will notify the Ethics Committee. The final study report will be completed within six months after the end of trial.

5 Cell Salvage Equipment

5.1 Blood Collection Machine （National certified washing cell salvage machine）

5.2 Leucocyte Filter

6 Laboratory(Biochemical Test)

6.1 Centers/Local Laboratory

The test is basically conducted in the local laboratory. Only part of the blood samples will be sent to the Second Affiliated Hospital of Wenzhou Medical University (Central Laboratory) for testing (eg, fetal components from maternal and cell salvage). It can be raised individually if anything else needs to be submited to the Central Laboratory.

6.2 Sample Collection/Marking/Registration

Sample:
a. In operating roon, take the peripheral blood of maternal before anesthesia and after transfusion, 8ml respectively;
b. self-salvaged blood, 8ml.
Using EDTA or other anticoagulant whole blood, samples from other hospitals can be placed in a refrigerator at 4 ℃, prepare within 48h.
Symboling and registering the samples with the unique study number.

6.3 Sample Reception/Management/Responsibility

Before sample processing, all samples need to be checked in by laboratory personnel. Once any error is found, it will be returned to the sample collector or research team.

6.4 Sample Analysis Process

6.4.1. Set 8ml blood sample with NH4Cl lysis solution for 30min (Due to the large amount required and several bottles of commercial hemolysin may be required, it is preferred to formulate NH3CL by self.). The sample is centrifuged at 2000 rpm for 5 minutes at room temperature and the supernatant is discarded;

6.4.2. Use perfix-nc kit, punch and fix. (The usage reference of perfix-nc kit is specified in kit instructions);

6.4.3. Antibody, CD45PECY7 / CD71-PE / HBF-FITC / 7-AAD (30ul / antibody) is added and incubated for 20 minutes;

6.4.4. Use up-flow cytometer to circle non-cellular impurity groups, calculate the value of fluorescence;

6.4.5. Use upstream cytometer to sort and select cells that expressing CD45- / CD71 + / HBF + / 7-AAD + at a rate of 30,000 cells per second or above (Due to large background mass of the sample cells, the time required to sort each sample at an effective sorting rate of 25-30 thousands cells per second is between 1 and 1.5 hours, so we need to ensure that cells are not lost and are complete, and try to improve the effective sorting speed);

6.4.6. Receive and accept the sorted fetal with nucleated red blood cells in the 100 ulPCR centrifuge tube;

6.4.7. Fetal has nucleated red blood cell count.

6.5 Sample Preservation Process(if needed)

Keep autologous blood samples until 3 years after the end of the study

6.6 Results Report and Registration Time

All sample results need to be truthfully recorded in the original record sheet and ECRF. If needed, the test results can be printed to the original record sheet.

7 Safety of Trial（Adverse Events）

7.1 Definitions

7.1.1 Adverse Events（AE）

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavourable and unintended sign, an abnormal laboratory finding, symptom, or disease temporally associated with the use of a medicinal (investigational) product.

7.1.2 Severe Adverse Events（SAE）

In trials, if any untoward medical occurrence, it is defined as severe adverse event.

- death of maternal or fetus (note: death is a termination indicator, not an event)
- Is life-threatening
- after caesarean section，causes prolongation of existing hospitalization for more than 7 days by adverse event
- results in persistent or significant disability/incapacity
- fetus has congenital abnormalities or defects
- investigators think it is a severe medical event

7.2 Investigator's Assessment of Adverse Events

7.2.1 Seriousness of Assessment

The principal investigators at each center are responsible for participants' medical treatment. If the principal investigator is unable to observe the abnormality, the authorized physician in this test team is responsible for assessing the severity of the adverse events as described in 7.2.4.

7.2.2 Causal Analysis

Investigators must assess the causal relationship between all adverse events and the trial.

7.2.3 Urgency of Report

Investigators must assess the prognosis of all severe adverse events. If the cause of this serious adverse event is unpredictable related to the trial, it must be reported immediately.

7.2.4 Severity of the Adverse Events

According to the criteria described below, investigators need to assess the severity of the adverse events. The severity grades of the event can not be confused with the word ‘serious’ in a serious adverse event(SAE). ‘Severity’ is defined based on the patient / event outcome criteria.

- General: observed discomfort but does not affect subject's daily life
- Moderate: the level of discomfort is sufficient to affect or reduce the level of activity of a normal person
- Serious: completely unable to carry out daily activities, and seriously affected the normal life.

7.3 Record and Report of Adverse Event

If the adverse event is not rated as a serious adverse event, then the adverse event needs to be recorded in the appropriate place or adverse event testing registration form for participant’s record. The research team also needs to track the progress of the participants.

7.4 Record and Report of Severe Adverse Event

All serious adverse events found in the trial or reported by the participants themselves, whether related to the trial or not, need to be recorded in the participants' case report forms and adverse event registration forms. All serious adverse events need to be followed until a new solution appears or the event is stable. Investigators need to provide follow-up information. All serious adverse events that are related to the withdrawal of the participants or happen at the end of the trial, need to be tracked until a satisfactory solution has been found.

After cesarean section, due to adverse events resulting in the longest hospital stay than 7 days, we need to be considered as prolonged hospitalization, and recorded in the

serious adverse events record form or adverse events record form.

According to local regulations, in each center, it is the major investigators' responsibility to report serious adverse events to the principal investigators and their local authorities. All serious adverse events need to be reported to the principal investigator within 24 hours of occurrence. Within 24 hours, PI needs to give feedback to quality control managers and sponsors of the Practical Clinical Trial Unit (PCTU) about all relevant or unanticipated serious adverse events. Within 15 days, or within the time as requested by the sponsor or PCTU, PI is required to report a serious adverse event to the General Ethical Comittee.

7.4.1 Expected Adverse Events

Although no serious adverse events are expected, it is still possible.

Risks of trial operation include:

- Maternal exposed to baby's blood
- Amniotic embolism
- Severe hypotension
- Transfusion reaction

For participants, the frequency of these risks is unlikely to increase substantially. Based on the criteria for a serious adverse event, if these risks meet the criteria for a serious adverse event, they are also reported as serious and unexpected adverse events.

7.5 Annual Safety Report

Principal investigators need to send an annual safety report to the Ethics Committee (MREC). The principal investigator needs to report annually to the Ethics Committee about all relevant and unexpected serious adverse events in tabular form with time order. Meanwhile, this form is reported to Data monitoring & Safety monitoring committee and Trial Steering Committee every 6-12 months.

8 Statistical Analysis

8.1 Estimation of Sample Size

8.1.1 Sample Size Calculation

In this study, we use data from control group (donor blood transfusion) to estimate the sample size, and then use 1: 1 ratio to calculate the sample size of IOCS sample.

The calculation is shown below:

A pilot study has commenced at the The 2nd Affiliated Hospital of Wenzhou Medical University, Zhejiang Province, China, involving women having Caesarean sections and the need for red cell transfusion. We collected a samle of 50 from the study and use the following foemula to estimate the blood loss after te caesarean section:

Blood loss = BV * ($Hct_{\text{first day post operation}} - Hct_{\text{xth day post operation}}$) + Vt

where BV is the blood volume, Vt is the volumn of red cell (unit: ml), and Hct has a unit with decimal places.

1. Sample Statistics for Donor Blood Transfution

N	effective	48
	missing	0
mean		444
median		274
SD		709
minimum		0
maximum		4347
percentiles	25	0
	50	274
	75	700

2. Sample Size Estimation Based On Population Mean

$$n = \left(\frac{t_{\alpha} s}{\delta}\right)^2$$

If $t_{0.05}$=1.96, S=709, δ=200, the estimated minimum sample size is 50.

3. Thus, according to ratio 1:1, the sample size of IOCS group is also 50.

4. Taking into account the unpredictable factors in the experiment that cause the loss of sample size, we recommend to use 1.2 times the minimum sample size as the sample minimum sample size. Therefore, according to ratio 1:1 between treatment group and control group, the total sample size would be 120 (where 60 for treatment group, and 60 for control group).

8.2 Outcomes

8.2.1 Primary Outcomes

- Blood loss post operation

  Hb and Hct in first and fifth day after surgery.

  The direct measurement of is the accurate volume of blood loss in major haemorrhage is difficult. Alternatives such as haemoglobin (Hb) or hematocrit (Hct)

are more frequently used and blood loss can be calculated using these parametres. Since Hct or Hb will be recorded as per standard practice during and postoperative in the hospital.

In this proposed trial, we are planning that Hb and Hct in the first and fifth day after the surgery as the primary outcome to reflect the blood loss.

8.2.2 Secondary Outcomes

- PT/APTT, Hct and Hb in first and fifth day after surgery
- the number of units of donor blood transfused
- mean fall in serum haemoglobin level
- maternal morbidity resulting from postoperative anaemia
- time to first mobilisation,
- duration of hospital stay
- immediate multidimensional fatigue inventory.
- Total cost in hospital

Second Stage: Follow-up when pregnancy again:

Neonatal hemolytic disease（Appendix 2）

Determine blood type, antibody, and IgG titer）

8.3 Statistical Methods

8.3.1 General Principle

Using SAS 9.13 for statistical analysis. All statistical tests should be two-sided. If p-value is less or equal to 0.05, then it is considered to be statistically significant.

Variables data are described by mean±standard deviation, median, maximum and minimum. Use paired t-test to compare intra-group variations with respect to baseline and use ANOVA or Wilcoxon rank-sum test to compare variations between groups.

Attributes data are descreibed as frequency (percentage). Between group comparison of percentages is tested by Fisher's exact test

8.3.2 Significance Level

Use superiority test to compare the primary objective between groups. The significance level $\alpha = 0.05$ (two-sided).

8.3.3 Hypothesis Test

Primary objective: The amount of blood lose post operation:

$$H_0: HR = \frac{\lambda_T}{\lambda_C} < 1, \quad H_1: HR = \frac{\lambda_T}{\lambda_C} \geq 1$$

α= 0.05 (two-sided).

By Log-Rank test, if p-value is less or equal to 0.05, and the median amount of blood lose in treatment group is less than in control group, then the superiority of the treatment group relative to the control group holds.

Based on the Cox Proportional hazards model, hazard ratios and the 95% confidence interval (CI), if the lower bound of the CI is greater than 1, then the efficacy of the treatment group is better than that of the control group.

8.3.4 Trial Population

8.3.4.1 Distribution of subjects

- Situation of subjects enrolling in the group and the completion of trail: frequency, percentage;
- Description of the proportion of diagnosed cases in each group.

8.3.4.2 Demographic characteristics, disease characteristics and medical history

Statistically describe indicators, compare between groups to assess the equivalence between the groups

- age;
- height, weight;
- related medical history.

9 Data and Record Keeping

9. 1 Confidentiality Plan

Any research-related documents must be filed with the research center and kept by the investigator. Patient-related documents are filed in accordance with the relevant laws of China. All research related documents must be kept for at least 15 years after the study ends. After this time limit expired, investigators must obtain the consent of the Second Affiliated Hospital of Wenzhou Medical University before discarding any documents. All patient-related data, including identification information and all personal medical information, should be treated as confidential documents. Data verification procedures should be based on the most stringent methods of confidentiality. Prior to obtaining the informed

consent, each patient should be provided with the confidentiality of the personal data, the original data verification, inspection, electronic data processing and file transfer procedures. During the trial, Study team members should keep any relevant information and results confidential. It is strictly forbidden to disclose any of these information to anyone not participating in this study. After the completion of the trial, all case report forms shall be filled in, reviewed and signed by the investigators and ombudsmen in accordance with the data management requirements of this study and kept together with the 'informed consent'.

9.2 Trial Required Documents

Serial No.	Name of Document	Format of document
D1	Trial Preparation Stage	
D1.1	Proposal	
D1.2	Standardization of Operations （SOP）	
D1.3	Original Record Form	
D1.4	CRF and its Manual	
D1.5	Randomization System and its Manual	
D1.6	Informed Consent	
D1.7	Subjects' Recruitment Ads	
D1.8	Project Contract	
D1.9	Table of Ethics Committee Members	
D1.10	Ethical Approval	
D1.11	Survey Questionnaire of Test Unit	
D1.12	Multi-center Cooperation Agreement	
D1.13	Resumes of Investigators	
D1.14	Investigators' Work Division and Signature Proofs	
D1.15	Copy of GCP Certificate and Registration Form	
D1.16	Participants' Screening Registration Form	
D1.17	Participants' Follow-up Registration Form	
D1.18	Research Tools (scales, etc.)	
D1.19	Follow-up Letter to Subjects	
D2	Trial Stage	

D2.1	Revision and Record of Documents	
D2.2	Meeting Munites	
D2.3	Signed Informed Consent	
D2.4	Filled Original Record Form	
D2.5	Filled CRF	
D2.6	Filled Screening Form of Subjects	
D2.7	Filled Follow-up Form of Subjects	
D2.8	Report Record of Adverse Event	
D2.9	Midterm Report	
D2.10	Audit Report	

9.3 Data Collection Process and its Regulatory Plan

Data is collected by related personnels. The data will be entered by researchers twice (stage I: hospitalization, stage II: follow-up) and the CRAs will check the authenticity and traceability of the data. If you have questions to fill in, send a questionnaire to the researchers, and answer it in writing. The whole process of the study was supervised by the supervisory staff: the participants' informed consent, screening and inclusion, correct and single-blind randomization, correct entry of case report forms and confirmation of all adverse events were recorded.

After the database is established, and information, data is reviewed and confirmed correct by CRA, then the data should be locked, and the locked data files are no longer allowed to make change. The data will be analyzed according to the statistical methods.

9.4 Quality Control and Assurance

The responsibilities and division of labor among relevant personnel should be clearly defined and it should be ensured that the data is correctly collected, randomized and single-blinded properly. We should obtain the subjects’ full informed consent and understanding so that we could improve the subject's compliance, and ensure the participants' perioperative information and postoperative follow-up information is authentic and complete.

Regular audits will be conducted, including non-scheduled on-site inspections and telephone inspections before and during the study. Data will be entered twice to reduce errors in data entry. An independent data security committee will be established to audit data analysis. The clinical trial will be registered to make it transparent.

10 Trial Committees

This Study consists of main investigators, CRC and other researchers from each research center.

10.1 Exective Committee

Chair: Fang Gao, Qingquan Lian
Members: Ting Li, Zuokai Xie, Joyce Yeung, Teresa Melody and one member from each of other related centers

10.2 Steering Committee

Chair: Mingping Hu, Yuhuan Wang, Wangning Shangguan, Jun Li, Ting Li, Fang Gao, Zuokai Xie, Joyce Yeung, Teresa Melody

10.3 Operations Committee

Chair: Mingping Hu, Ting Li
Members: Zuokai Xie, Chenchen Jiang, Yan Zhang, Yi Wang, members from other centers

10.4 Data Monitoring & Safety Monitoring Committee

Chair: Jingwei Zheng
Members: Han Lin, Weihe Zhou

11 Publication Policy

Intellectual Property states that this statement is formulated in accordance with the ‘Copyright Law of the People's Republic of China’. The copyrights of this protocol and intellectual property are retained by the authors, with first publication

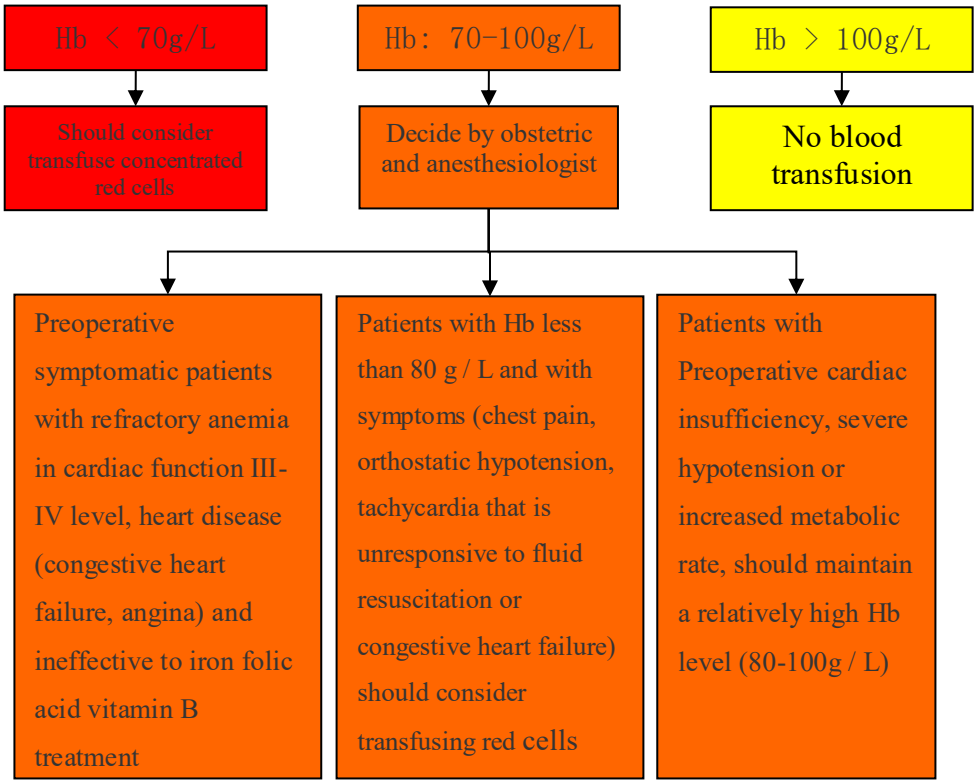
rights granted to the Second Affiliated Hospital of Wenzhou Medical University. During the study, investigators in each center are required to report the research data to the Second Affiliated Hospital of Wenzhou Medical Universit. They shall not privately disclose and publish the data in any forms during this trial. This trial respects all the contribution of collaborators. After the research center announces that the study ends, through consultation, the names and order of participating units will be determined, generally according to the number of selected patients, amount of work through the study and so on. Anyone who violates the above declaration will be held liable according to law.

Appendix

11.1 Appendix 1

Intraoperative and Postoperative transfusion Plan in Caesarean sections

1. Flowchart of Red Cell Transfusion Plan



2. Platelet (PLT)

1). PLT > $100*10^9$/L, could have no blood transfusion;

2). PLT < $50*10^9$/L， should consider to hanve blood transfusion (Considering the physiology of maternal hypercoagulability, the obstetrician decides whether or not to transfuse according to the condition or the bleeding condition of the wound);

3). PLT is between 50-$100*10^9$/L, and it will be decided based on whether there is spontaneous bleeding or wound bleeding;

4). If there's uncontrolled bleeding occurs during operation and PLT function is determined to be impaired, then transfusions of PLT has no ceiling restriction.

3. Fresh Frozen Plasma

For patients who lack of clotting factor

1)． PT or APTT greater than normal 1.5 times or INR > 2.0, diffuse wound bleeding;

2)．Patients with acute bleeding, transfuse a large amount of whole blood or red blood cells (amount of bleeding, or amount of transfusion is equivalent to the patient's own blood volumn).

3） There's advanced or acquired coagulation disorders in history or clinical performance.

4） Acute anti-coagulation effects for warfarin

Each unit is equivalent to the amount of plasma in 200ml of fresh whole blood, and it can increase about 2% -3% of clotting factor for adults. It needs to be based on clinical symptoms and monitoring results to adjust the dose when apply

4. Cryoprecipitate

Cryoprecipitate is generally used when bleeding and fibrinogen < 800 mg/L, and other treatments are performed according to the experience of obstetrician physicians.

11.2 Appendix 2

Diagnostic Criteria

If the following conditions meet, it can be diagnosed as neonatal ABO hemolytic disease:

① ABO blood group incompatibility between mother and child;

② Direct anti-human globulin test and/or Antibody release test result(s) in positive;

③ Early pathological jaundice and / or Anemia (Hb≤170g / L) and / or Hemolysis (Percentage of Reticulocyte: Full-term children ≥ 6%; Preterm children ≥ 10%).

11.3 Appendix 3

Procedures of Intraoperative Cell Salvage

Preparation of Equipment:

a) Cell salvage machines and consumables.
b) Heparin Anticoagulant: 500ml saline +3 heparin sodium injection for preparation of heparin saline.
c) Prepare two sets of suction device, one for intraoperative cell salvage suction, one for amniotic fluid, irrigation fluid, etc.
d) Large amount of saline for washing autologous blood.

2. Prepare two sets of suction device before operation; one for attracting amniotic fluid, irrigation fluid during operationand one for intraoperative cell salvage

suction, only for bleeding during operation.

3. Install the suction tube and blood reservoir first, and connect heparin anticoagulant fluid; Adjust the attraction pressure to the lowest available level (generally about 20.0kPa)
4. Pre-fill 200ml heparin saline into the blood storage tank, control the drop rate of heparin saline to 1-2 drops / second, and during the operation, adjust the drop rate of heparin saline according to the bleeding rate. When absorb blood, we should try to put the head of suction head below the surface. Whether or not recycle the blood on gauze during the operation is determined by each research center.
5. When the blood transfusion indicator is reached, or if the doctor thinks it is necessary to transfuse, install a centrifuge cup (Thus, generally use manual mode). Once start to centrifuges the blood, the centrifuge cup speed should set to 300ml / min, and adjust to 500ml / min in emergency. After the centrifugal pump is full, begin to wash. Use 0.9% saline for washing only. Do not use any other types of liquids for washing, such as Ringer's solution. The amount of liquid for washing should be about 8-10 times the total amount of centrifuge cup. The amount of washing liquid can increase according to real situation, but can not decrease. When finishing washing, the blood should packed into the blood storage bag.
6. When using the WBC filter, please do not squeeze blood bags, let it filter by gravity.

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