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A randomised sham-controlled double-blind trial evaluating remote ischemic preconditioning in solid organ transplantation – A study protocol for the RIPTRANS trial

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Abstract

Introduction

Remote ischemic preconditioning (RIPC) using a non-invasive pneumatic tourniquet is a potential method for reducing ischemia-reperfusion injury. RIPC has been extensively studied in animal models and cardiac surgery, but scarcely in solid organ transplantation. RIPC could be an inexpensive and simple method to improve function of transplanted organs. Accordingly, we aim to study whether RIPC performed in brain-dead organ donors improves function and longevity of transplanted organs.

Methods and analyses

RIPTRANS is a multi-center, sham-controlled, parallel group, randomised superiority trial comparing RIPC intervention versus sham-intervention in brain-dead organ donors scheduled to donate at least one kidney. Recipients of the organs (kidney, liver, pancreas, heart, lungs) from a randomised donor will be included provided that they give written informed consent. The RIPC intervention is performed by inflating a thigh tourniquet to 300 mmHg 4 times for 5 minutes. The intervention is done twice: firstly right after the declaration of brain death and secondly immediately before transferring the donor to the operating theatre. The sham group receives the tourniquet, but it is not inflated. The primary endpoint is delayed graft function (DGF) in kidney allografts. Secondary endpoints include short-term functional outcomes of transplanted organs, rejections, and graft survival in various time points up to 20 years. We aim to show that RIPC reduces the incidence of DGF from 25 % to 15%. According to this, the sample size is set to 500 kidney transplant recipients.

Ethics and dissemination

This study has been approved by Helsinki University Hospital Ethics Committee and Helsinki University Hospital's Institutional Review Board. The study protocol was be presented at the European Society of Organ Transplantation congress in Copenhagen 14-15th September 2019. The study results will be submitted to an international peer-reviewed scientific journal for publication.

Trial registration number

NCT03855722 (ClinicalTrials.gov)

Strengths and limitations of this study

- The study method, a multi-center, double-blinded, sham-controlled, randomised superiority trial, is the best available method to investigate the effects of remote ischemic preconditioning (RIPC) performed in the donor on the function and longevity of transplanted organs in the recipient

-Remote ischemic preconditioning is an extremely simple, reproducible, and inexpensive method

-The sample size, 500 kidney transplant recipients, is large enough to provide confidence in the estimates of outcomes.

-Primary outcome, delayed graft function of kidney allograft, is clinically highly relevant, easy to measure, and objective.

-As the sample size is calculated for kidney transplantation, outcomes of other organ recipients might be underpowered.

Introduction

Solid organ transplantation is an established standard of care for end-stage dysfunction of different organs, but the availability of the treatment is greatly limited globally by the shortage of organ donors. On the other hand, the lifetime of a transplanted organ is often limited and there is a number of patients waiting for a second or subsequent transplant (1-6). A transplanted organ is exposed to ischemia-reperfusion injury during the transplantation process (7). Alleviating this injury could improve the function and lifetime of transplanted organs.

Remote ischemic preconditioning (RIPC) is an old concept where remotely produced ischemia induces protective changes in distant organs or tissues and renders them less susceptible for future ischemia via hormonal, metabolic, and neuronal mechanisms (8). As an intervention, RIPC is easy and cheap to perform – an inflatable tourniquet is used to occlude thigh 4 times for 5 minutes. RIPC has been extensively studied in animal models (9-11), and in human clinical trials of cardiac surgery. The largest of these clinical trials (12-13) have not been successful in terms of benefit from RIPC, but this might be due to the fact that the patients suffering from chronic myocardial ischemia already have maximal compensatory mechanisms in use. Organ transplantation is a lucrative field to study RIPC, as the donor organs are healthy, and do not suffer from chronic ischemia, but face invariable acute ischemia of various durations.

RIPC has been studied little in clinical transplantation and results have been controversial. In a multi-center randomised controlled trial (RCT), a RIPC performed in both donor and recipient immediately before a living-donor kidney surgery improved the estimated glomerular filtration for the whole follow-up period of 5 years (14-15). The kidney allografts from living donors are subjected to very short ischemia (in Finland this is typically less than two hours) and even greater benefits could be obtained if RIPC is performed in deceased donors, where ischemia times are much longer (median 15 hours for kidney allografts in Finland, even longer in other countries). RIPC intervention performed to the recipients of deceased donor kidneys during the transplantation surgery did not improve kidney function in another RCT (16). This study can be criticized for performing RIPC in the recipients instead of donors, because the ischemic injury has already taken place before RIPC.

The aim of this study is to show that RIPC performed in brain-dead donors (DBD) can be used to improve function and longevity of transplanted organs.

Methods and analysis

Study design

The RIPTRANS trial is a multi-center, double-blinded, parallel group, individual donor randomised superiority trial comparing RIPC with a sham-procedure performed in brain-dead donors. There is only one transplantation centre (Helsinki University Hospital) in Finland that covers the whole country and procurement team travels to all donor hospitals in Finland. This protocol was drafted in accordance with the SPIRIT (Standard Protocol Items: recommendations for Interventional Trials) statement (17). This trial is registered in ClinicalTrials.gov (NCT03855722), the first registration date was February 27th, 2019.

Participants

RIPC or sham procedure will be performed on a brain-dead donor fulfilling inclusion and exclusion criteria. All brain-dead donors in participating hospitals scheduled for at least one kidney procurement will be included. Donors with significant hemodynamic instability (assessed by the intensive care physician responsible for the treatment of the donor) and under the age of 18 years will be excluded. Donors (or potential recipients of organ from this donor), who are participating in a trial with conflicting interventions or outcomes, will also be excluded. Although the donors are randomised and the intervention is carried out in donors, the recipients are the actual participants of this trial. All patients receiving a kidney, liver, pancreas, heart, or lungs from a donor randomised in the trial will be included in the trial provided that they give a written informed consent (Supplement 3, in Finnish) to participate in the trial and are at least 18 years old. The informed consent will be presented to the patient by a study nurse or physician. As based on the previous studies, RIPC is supposedly not harmful for the donor, and the lack of consent from any of the transplant recipients does not exclude the donor from the study, nor the possible inclusion of the other recipients. There are no other exclusion criteria for recipients who receive abovementioned organs from a randomised donor.

Randomisation and masking

Eligible donors will be randomly allocated in a 1:1 ratio to either RIPC or sham-procedure group. The randomisation sequence was generated using a web-based commercial service (Sealed Envelope) with randomly variable block size (4, 6, or 8) and stratified according to donor age (under / over 60 years of age), planned organ to be procured (kidneys only / abdominal organs only / both thoracic and abdominal organs), and donor cardiopulmonary resuscitation (yes / no). The randomisation and allocation to either RIPC or sham-intervention is done by a transplant coordinator, who is not blinded to the allocated treatment, using the same web-based service. Once the donor is allocated, the transplant coordinator sends electronically or via fax written instructions on how to perform the allocated treatment to the intensive care team responsible for the treatment of the donor, who also are not blinded to the treatment. This intensive care team will collect data regarding the actual timing of the allocated procedure and whether this caused any noticeable changes in the donor hemodynamics. All researchers and all other treating personnel are blinded, such as procuring surgeons, transplant surgeons, treating physicians, data collectors, and data analysts as well as recipients. After the trial recruitment has been closed and data collected, the allocated group will be named as A and B before the data is analysed. Once the data analyses for primary and secondary outcomes are completed, the full blinding will be removed. No emergency unblinding is planned, but incidents of possible breaches in blinding will be recorded.

Procedures

RIPC will be performed as follows: Donor's thigh will be occluded 4 times for 5 minutes using tourniquet inflated to 300 mmHg each followed by 5 minutes of deflation. The intervention will be performed twice (once in both thighs). Once as soon as possible after brain death is determined, and once right before transferring the donor to the operation room for procurement. Sham-intervention will be performed by putting the inflatable tourniquet in place similarly, but not inflating it. Apart from the RIPC or sham-intervention,

the treatment of donors will be according to normal routine. Study blood samples will be acquired from donors before (selected centers) and after the intervention.

Outcomes

The primary outcome measure is delayed graft function (DGF) of kidney allografts, which is defined as the need for dialysis within the first week after transplantation. Secondary outcome measures are different for different organs (Table 1). Outcomes are assessed during the primary hospital stay, and thereafter at the routine follow-up visits. Helsinki University Hospital has a legal requirement to maintain a registry of all patients receiving a transplant in Finland, and data regarding visits in other hospitals are submitted to Helsinki University Hospital for registry purposes. Secondary outcomes are assessed directly from the registry, from the data provided by other hospitals, or at routine follow-up visits at Helsinki University Hospital. Survival status is automatically updated to the registry from the National Population Centre, which is an exact, complete, and up-to-date source for causes of death in Finland. Prespecified subgroup analyses are planned for characteristics that may potentially affect the results (Table 2). Further exploratory outcome measures will be done according to Supplement 1. In the informed consent, the patients are also asked to give their permission for using the excess study blood, urine and tissue samples in possible ancillary analysis.

Kidney allografts
Estimated glomerular filtration rate (eGFR) at 3 months, 1 year, 2 years, 5 years, 10 years, and 20 years.
Biopsy-proven acute rejection (BPAR) within 1 year.
Graft survival at 3 months, 1 year, 2 years, 5 years, 10 years, and 20 years: time from transplantation to death, retransplantation or permanent dialysis.
Death-censored graft survival at 3 months, 1 year, 2 years, 5 years, 10 years, and 20 years: time from transplantation to retransplantation or permanent dialysis, death-censored
Pancreatic allografts
Glycosylated haemoglobin (HbA1c) at 3 months, 1 year, 2 years, 5 years, 10 years and 20 years
Acute rejection in pancreatic allograft, either biopsy-proven (allograft pancreas or duodenal biopsy) acute rejection or clinically treated suspected acute rejection within 1 year
Pancreatic allograft survival at 3 months, 1 year, 2 years, 5 years, 10 years and 20 years: Time from transplantation to death, retransplantation, explantation or daily insulin dependence
Death-censored pancreatic allograft survival at 3 months, 1 year, 2 years, 5 years, 10 years and 20 years: Time from transplantation to death, retransplantation, explantation or daily insulin dependence, death-censored

Liver allografts
MEAF-score at 3rd post-operative day (POD): Model for Early Allograft Function Scoring. MEAF = score ALTmax:3POD + score INRmax:3POD + score bilirubin3POD, score range 0 - 10, higher score indicates worse outcome (18)
Postoperative biliary complications within 1 year: Amount and type of postoperative biliary complications: stricture at anastomosis, bile leak or ischemic type biliary lesions (ITBL) requiring intervention (ERC, PTC, operation) or prolonged drainage within 1 year
Post-transplantation kidney injury (acute kidney injury) within 1 week, at 3 months, 1 year: according to ADQI 2010 criteria (19).
Biopsy proven acute rejection (BPAR) within 1 year.
Graft survival at 1 year, 2 years, 5 years, 10 years, and 20 years: time from transplantation to death, retransplantation or explantation
Heart allografts
Ischemia-reperfusion injury determined by peripheral blood TnI levels at 6 hours after transplantation
Peripheral blood proBNP measurement at 1 week after transplantation
Primary graft dysfunction according to ISHLT definition (20) within 24 hours after transplantation
Biopsy-proven or clinically treated acute rejection within one year after transplantation
Vasculopathy-free survival according to ISHLT definition (21) at 1 year, 2 years, 5 years, 10 years, and 20 years
Graft survival at 1 year, 2 years, 5 years, 10 years, and 20 years, time from transplantation to death, retransplantation or explantation
Lung allografts
Primary graft dysfunction according to ISHLT definition (22) within 72 hours after transplantation
Biopsy proven or clinically treated acute rejection within one year
Chronic Lung Allograft Dysfunction (CLAD) free survival according to ISHLT/ATS/ERS 2014 guideline (23) at 1 year, 2 years, 5 years, 10 years, and 20 years, time from transplantation to death or retransplantation
Graft survival at 1, 2, 5, 10 and 20 years : time from transplantation to death, retransplantation or explantation

Table 1. Secondary outcome measures

Subgroup analyses	Subgroups
Donor cardiopulmonary resuscitation	yes / no
Donor age (years)	Under 60 /over 60
Donor sex	male / female
Organ cold ischemia time (hours, organ specific)	below / above median
Uncompleted study intervention	yes / no
Liver transplantation for acute liver failure	yes / no

Table 2. Subgroup analyses

Statistical analyses

The incidence of DGF in kidney allografts after transplantation from a brain-dead donor in Finland is approximately 25 % (Finnish Transplantation Registry). We aim to show that RIPC reduces the incidence of DGF to 15%. With a 5 % significance level and 80 % power, 496 kidney transplantations are required to show this difference. Sample size is not adjusted for cross-over or loss-of-follow up because the risk of these are considered to be minimal. Usually two kidneys per donor are transplanted. Because a portion of procured kidneys will be transferred to another Nordic country according to ScandiaTransplant rules, are untransplantable, transplanted in a combined organ transplantation, or transplanted to a recipient below 18 years old, we assume 90% of donors will lead to two kidney transplantations and 10% will lead to one kidney transplantation within the study. We set the final sample size to 500 kidney transplantations, for which approximately 260 donors are required to be randomised.

The primary outcome measure and the secondary outcomes for kidney transplantation will be analysed using generalized linear mixed models taking into account that kidneys from a single donor will usually be transplanted to two recipients included in the study. Survival analysis for kidney allografts and transplant recipients are done using Kaplan Meier survival diagrams and the effect size is estimated using Cox proportional hazards regression model similarly taking into account single donor providing kidneys to two recipients.

The categorical outcome variables for liver, pancreas, heart, and lungs are analysed with Chi square test (or Fischer's exact test, if *n* is under 5 in any of the subcategories). The continuous outcome variables for these organs are analysed using independent T-test or Mann Whitney U-test depending on whether the outcome has normal distribution or not. The effect size for categorical variables is calculated with odds ratio and 95 % confidence interval (CI). For continuous variables the effect size is calculated with difference in means with 95 % CI for variables with normal distribution. If a continuous variable can be converted for normal distribution with a logarithmic transformation, will the effect size be reported using the ratio of geometrical means with 95 % CI. Other continuous variables will be calculated using Mann Whitney U-test and the effect size will be reported using $r = Z/\sqrt{N}$ without 95 % CI. Survival analysis for these organs will be described using Kaplan-Meier

survival diagrams and log-rank-test and effect size estimated using Cox proportional hazards regression model.

Subgroup analysis will be made using generalized linear mixed models as univariate analysis by adjusting models by subgroup. A multivariate analysis of subgroups can be done with aforementioned generalized linear model and by selecting the significant subgroups ($p < 0.05$) from univariate analysis as covariates. Subgroup analysis for survival variables will be described with Kaplan-Meier, which will be stratified by subgroup and effect size will be estimated using Cox proportional hazards regression model by adjusting it with the subgroup.

In case that because of missing values more than 5 % of patients would be left out from sensitivity analyses, multiple imputations may be used to conduct sensitivity analyses. Otherwise, the missing data will not be adjusted separately, but these cases will either be left out from the analyses or censored at the last point of follow up.

Data security
All patient data included in the study is confidential and will be concealed on a computer behind an AES 256-bit encryption. Any data stored in a paper from will be held in the study hospitals in locked offices. Only the study personnel will have the access to the trial dataset.

Data availability statement
After the completion of the study the depersonalized data can be requested from the authors.

Schedule and interim safety analyses
The study was conceptualised in June 2017. The study plan was approved by the Helsinki University Hospital's Ethics Committee 9th May 2018. Helsinki University Hospital's Institutional Review Board gave permission to conduct the study 14th August 2018. The study started recruiting in May 13th 2019 in four out of five university hospitals in Finland. The Ethics Committee required a safety analysis after 16 donors had been randomised. The donors and recipients of kidneys from these 16 donors were analysed without unmasking the allocated group. All 16 donors randomised successfully underwent procurement. No adverse events were noted in the recipients. After this safety analysis, the study will be disseminated to non-university donor hospitals. Second interim analysis will be done when half of the target sample size is reached (250 kidney transplantations). In Finland, approximately 230 DBD kidney transplants are being performed annually. We estimate that data for primary outcome would be available in 4 years.

Role of the funding sources and sponsors
The funders or sponsors have had and will have no role in study design, data collection, data analysis, data interpretation, or writing the report, or any other aspect of the work, except for funding.

Ethics and dissemination

Study ethics

This study has been approved by Helsinki University Hospital Ethics Committee and Helsinki University Hospital's Institutional Review Board.

The intervention is performed on a donor, who has been determined brain dead and has given permission to act as a donor according to Finnish legislation, and is determined suitable and scheduled for kidney procurement. The Ethics Committee has approved that donors (or next of kin) do not need to consent to RIPC or sham-procedure because it is a non-invasive procedure, the donor is brain dead and scheduled for procurement already.

The recipients of organs from randomised donors will be recruited in the study and will be required to give written informed consent to participate. The recipient cannot influence whether the donor has been randomised or received the allocated treatment. The recipient has the right to decline participation in the trial, but can still choose to receive the planned allograft. In these cases, the recipient's data is not used in the study analyses. The recipient has also right to decline the offered organ. The recipient does not have the right to know the allocated treatment the donor has received before the study has been completed, data analysed, and blinding unmasked. The donors or recipients do not receive any compensation for their participation in the trial. The recipients have the right to discontinue the trial or withdraw their consent at any point. In these cases, the collected data will be used in the analyses up to the point of discontinuation.

A few additional blood samples (and a urine sample from the kidney recipients) will be taken from the kidney, heart, and lung recipients for the study purposes during and shortly after the transplantation, but otherwise the recipients only give their consent to the study group to observe and collect medical information. These samples are stored maximally for five years after the completion of the study recruitment. The patient informed consent forms are in Finnish and Swedish and will be provided by request made to the study group.

Harms

Earlier studies on RIPC have not indicated any harm (7-16). On the contrary, many earlier studies suggest that RIPC may be beneficial for the function and longevity of the allografts. Before wider adoption of the RIPC in transplantation, it's safety and benefits need to be addressed in a randomised controlled trial such as RIPTRANS. Any possible harmful effects of the intervention will be reported together with the study results. The Finnish patient insurance covers the organ recipients participating in the study.

Monitoring

Helsinki University Hospital Ethics Board monitored the results of the first interim analysis. Initially the Ethics board did not necessitate a separate Data Monitoring Committee (DMC). To provide external validity for the study, a DMC contract was made with Clinical Research Institute HUCH Ltd (HYKS Instituutti) in March 2020. The site monitoring will be performed every three months including review of the Investigator's Trial File, facilities, the equipment at the site, compliance to study protocol and study specific procedures, source document quality and the intervention implementation documentation for all donors. All the study patients will be monitored for: existence, informed consent process and documentation of the Trial outcome measures. A complete review will be conducted for 10 % of the subjects. A close-out visit shall be done after all the data has been collected and the treatment of all

the subjects has been completed. This Monitoring plan and Agreement is made in collaboration with the guideline for coordinated GCP-monitoring of clinical trials in the Nordic countries (version 5/24.10.2017).

Patient and Public involvement

The transplant recipients are informed about the study once they are asked to participate. This study protocol was published in ClinicalTrials.gov before beginning of the study. Patient organizations were not involved in the study design.

Dissemination

The study protocol was be presented at the European Society of Organ Transplantation congress in Copenhagen 14-15th September 2019 and will possibly be presented in other scientific conferences. The study results will be submitted to an international peer-reviewed scientific journal for publication and possibly discussed at scientific meetings. The study is also being made public via social media platforms (Twitter). The International Committee of Medical Journal Editors recommendations (www.ICMJE.org) are applied when considering the authorship of any publications from this trial.

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Author's contributions
Concept: VS, ML
Initial draft of protocol: VS, AU, IH, KL
Critical revision of the protocol: All authors
Implementation: VS, AU, KL, SS, AP
Data collection: AU, SS
Donor recruitment: MB, MS, TL, JR, MaL, JL, JG

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Conflicts of interests statement
The authors have no competing interests that would affect this study.

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Supplement 1. Exploratory outcome measures

Kidney allografts
Peroperative blood samples before and after graft perfusion and urinary sample 6 hours after transplantation. Measurement of ischemia/reperfusion injury in blood and urine samples using following factors
Micro-RNA miR-21
Micro-RNA miR-24
Neutrophil gelatinase associated lipocain NGAL
Kidney injury molecule 1 KIM-1
Fatty acid binding protein 1 FABP-1
secretory leucocyte proteinase inhibitor SLPI
Liver allografts
Early allograft dysfunction at 7 days after transplantation according to Olthoff (24): Bil >100, INR 1.6 or more, ALT or AST > 2000 at 7th POD
Highest ALT within 1 week
Highest INR within 1 week
Highest Bil within 1 week
Heart allografts
Ischemia-reperfusion injury determined by peripheral blood TnI, CK-MBm, lactate, and C-reactive protein levels at 0, 1, 12, and 24 hours
Peripheral blood proBNP at 1, 7, 14 and 21 days
Crea at 1, 7, 14 and 21 days
Urea at 1, 7, 14 and 21 days
eGFR at 1, 7, 14 and 21 days
Left ventricle ejection fraction (LVEF) at 1 day, 7 days, 14 days and 21 days
Left ventricle (LV) wall thickness measurements at 1 day, 7 days, 14 days and 21 days
Tricuspidal valve leak grading at 1 day, 7 days, 14 days and 21 days
The appearance of ischemia-reperfusion injury in routine biopsies at 7, 14 and 21 days

The appearance of fibrosis associated factors in routine biopsies at 7, 14 and 21 days
Long-time follow-up of proBNP at 1, 3, 6 and 12 months
Long-time follow-up of LVEF in cardiac ECHO at 1, 3, 6 and 12 months
Coronary Artery Disease (CAD) in coronary angiography at 1 year
Major Adverse Cardiac Events (MACE, including death because of cardiac cause, graft loss, primary allograft dysfunction, rejection classified as ISHLT G2R or more) at 1, 3, 6 and 12 months
Lung allografts
Factors possibly presenting the severity of ischemia/reperfusion injury after transplantation:
Standardized P/F-ratio during mechanical ventilation at 0 hours, 1, 6 , 12 and 24 hours
Non-standardized P/F-ratio during mechanical ventilation at 0 hours, 1 , 6, 12 and 24 hours
Plasma lactate at 0 hours, 1, 6, 12 and 24 hours
Serum highly sensitive C-reactive protein at 0 hours, 1, 6, 12 and 24 hours
Blood leukocyte count at 0 hours, 1, 6, 12 and 24 hours
Neutrophil count at 0 hours, 1, 6, 12 and 24 hours
Forced lung expiratory volume in one second (FEV1) at 1, 3, 6 and 12 months
Forced vital lung capacity (FVC) at 1, 3, 6 and 12 months
Evaluation of chronic rejection at 1, 3, 6 and 12 months
Exploratory outcomes lung allografts: Infections after transplantation at 1, 3, 6 and 12 months

References for supplement 1

24. Olthoff KM, Kulik L, Samstein B et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. Liver Transpl. 2010;16(8):943-9

Supplement 2. Protocol amendments

Study Protocol version 1.11 (Finnish version), 2019-Jan-16 in use at time when study recruiting started March 13th 2019

Version 1.13 (Finnish version), 2019-Aug-11
Reported the results of the safety analysis the to University of Helsinki Ethics Committee
Changed the practise of taking the 6 h postoperative urinary sample from kidney recipients.
Original: The sample is taken from the urine bag
Updated: The sample is taken from the catheter hose

Version 1.14 (English version), 2020-Mar-6
Donor pre-intervention blood samples are taken in selected centers, not necessarily only in Helsinki.

BNP as an outcome measure changed to proBNP according to a change in Helsinki University Hospital laboratory HUSlab protocol. Blood samples from heart transplant recipients transplanted before this are reanalysed for proBNP as possible.

Added a new secondary outcome measure for the lung recipients:
Lung allograft: graft survival: time from transplantation to death, retransplantation or explantation. The recruitment Lung recipients started later than for other organs because of a conflicting trial, which now has completed recruiting. Only four lung patients have thus far been included in the study and no results for lung recipients have been analysed.

Changed the manner of dealing with the possible missing data in analyses:
Original: Missing data will not be adjusted separately, but these cases will either be left out from the analyses or censored at the last point of follow up.
Updated: In case that because of missing values more than 5 % of patients would be left out from sensitivity analyses, multiple imputations may be used to conduct sensitivity analyses. Otherwise, the missing data will not be adjusted separately, but these cases will either be left out from the analyses or censored at the last point of follow up.

External Study Monitoring Committee was initiated by Clinical Research Institute HUCH Ltd in March 2020.

Supplement 3. Model consent form (in Finnish)

TUTKIMUSTIEDOTE POTILAALLE

Tutkimuksen nimi: **Etäiskeeminen esikäsittely elinsiirteiden toiminnan parantajana**

Hyvä potilas,

Olette tulossa munuaissiirtoon HYKS Vatsakeskuksen elinsiirto- ja maksakirurgian klinikkaan. Pyydämme Teitä osallistumaan tutkimukseen, jossa selvitetään etäiskeemisen esikäsittelyn vaikutusta munuaissiirteen toimintaan.

Kun munuaissiirre on irrotettu elinluovuttajalta, siirre altistuu hapenpuutteelle (iskemialle) kunnes se liitetään vastaanottajan verenkiertoon munuaissiirtoleikkauksessa. Tämä hapenpuute vaikut-taa munuaissiirteen toimintaan, esim. virtsanerityksen käynnistymiseen, ja voi altistaa hyljinnälle. Hapenpuutteen aiheuttamia vaurioita voidaan pyrkiä ennaltaehkäisemään ns. etäiskeemisellä esikäsittelyllä. Etäiskeemisellä esikäsittelyllä tarkoitetaan sitä, että elinluovuttajan jokin muu kudos kuin irrotettavat elimet (tässä tutkimuksessa alaraaja) altistetaan hapenpuutteelle ennen elinirroitusleikkausta. Elinluovuttajan munuaisia ei siis altisteta hapenpuutteelle. Alaraajan hapenpuute aiheuttaa koko elimistössä, myös munuaisissa, hormonaalisia ja hermostollisia muutoksia, joilla elimistö pyrkii suojautumaan hapenpuutteen aiheuttamilta vaurioilta.

Tämä tutkimus on satunnaistettu, eli puolet elinluovuttajista saa etäiskeemisen esikäsittelyn ja puolet ei. Tutkimus on sokkoutettu, tarkoittaen sitä, että Te tai hoitavat lääkärit eivät tiedä onko elinluovuttaja, jolta munuaissiirteenne tulee, saanut etäiskeemisen esikäsittelyn vai ei. Elinsiirto ja hoito sen jälkeen toteutetaan täysin samalla tavalla kuin potilaiden, jotka eivät osallistu tutkimukseen.

Pyydämme Teiltä lupaa ottaa tutkimukseen liittyen kolme verinäytettä munuaissiirtoleikkauksen (nukutuksen) aikana ja yksi virtsanäyte virtsakatetrista leikkauksen jälkeen. Näytteistä tutkitaan erilaisia munuaisvaurion merkkiaineita. Tuloksia verrataan Teistä rutiininomaisesti leikkauksen jälkeen otettuihin munuaisten toimintakokeisiin, dialyysitarpeeseen, mahdolliseen siirteen hyljintään ja siirteen pitkäaikaiseen toimintaan.

Mikäli veri- tai virtsanäytteitä jää tutkimuksesta yli, niistä voidaan etäiskeemisen esikäsittelyn vaikutusten selvittämiseksi määrittää myöhemmin alkuperäisessä tutkimussuunnitelmassa mainittujen lisäksi muitakin analyysejä. Näytteitä säilytetään tutkimusryhmän pakastimessa korkeintaan 5 vuotta, jonka jälkeen ne tuhotaan.

Tutkimustiedon oikeellisuuden varmistamiseksi tutkimustietoja verrataan muun muassa alkuperäisiin sairauskertomuksiin. Tällöin tietoja käsitellään ns. monitoroijan toimesta tutkijalääkärin tai muun tutkimushenkilöstön valvonnassa ja vastuulla. Tämän lisäksi tutkimuksessa henkilöllisyytenne sekä muut tunnistettavat tiedot ovat ainoastaan tutkijalääkäreiden tiedossa, ja he kaikki ovat salassapitovelvollisia. Tutkimusrekisteriin

talletetaan vain tutkimuksen kannalta välttämättömiä tietoja. Tutkittavia seurataan 20 vuotta.

Tämä tutkimus ja siihen kuuluva henkilötietojen käsittely perustuvat seuraaviin lainsäädäntöihin: EU tietosuoja-asetus (2016/679), 6. artikla 1 a), b), c) ja e) ja 9. artikla 3 a), g), i) ja j) kohdat, laki lääketieteellisestä tutkimuksesta (1999/488), terveydenhuoltolaki (1326/2010), laki potilaan asemasta ja oikeuksista (785/1992), laki terveydenhuollon ammattihenkilöistä (559/1994), laki viranomaisten toiminnan julkisuudesta (621/1999), tietosuojalaki (2019) ja arkistolaki (831/1994). Lisäksi huomioidaan EU:n tietosuoja-asetuksen yli kansallisen lainsäädännön menevät määräykset.

Tutkimuksen loputtua tutkimusrekisteri säilytetään hyvän kliinisen tutkimustavan vaatimusten mukaisesti ja hävitetään sen jälkeen. Tutkimusrekisterissä ei käytetä automaattista päätöksentekoa. Tämä ei koske tutkimukseen liittyvää ryhmien randomointia, joka on tieteelliseen tutkimukseen kuuluva metodi.

Tutkimuksen rekisterinpitäjänä toimii Helsingin ja Uudenmaan sairaanhoitopiirin kuntayhtymä.

Osoite:
Helsingin ja Uudenmaan sairaanhoitopiirin kuntayhtymä, Stenbäckinkatu 9
PL 100, 00029 HUS

Yhteystiedot
Puhelinvaihde 09 4711
Kirjaamon telefax 09 471 75500,
Kirjaamon sähköposti keskuskirjaamo@hus.fi
postiosoite: HUS keskuskirjaamo PL 200, 00029 HUS

Voitte toteuttaa tietosuoja-asetuksen mukaisia oikeuksianne vapaamuotoisilla ilmoituksilla, mutta suosittelemme käyttämään näitä tarkoituksia varten laadittuja HUSin lomakkeita. Lomakkeet löydätte HUSin internet-sivuilta:

http://www.hus.fi/potilaalle/potilaan_oikeudet/terveystieteellinen%20tutkimus/Sivut/default.aspx

Teillä on myös oikeus tehdä tietosuoja-asioissa valitus Suomessa tietosuojasta vastaavalle viranomaiselle eli tietosuojavaltuutetulle.

Tietosuojavaltuutetun toimisto
Käyntiosoite: Ratapihantie 9, 6. krs, 00520 Helsinki
Postiosoite: PL 800, 00521 Helsinki
Puhelinvaihde: 029 566 6700
Sähköposti (kirjaamo): tietosuoja@om.fi

Pyydämme Teiltä kirjallista suostumusta tutkimukseen osallistumisesta. Voitte syytä ilmoittamatta keskeyttää tutkimukseen osallistumisen tai peruuttaa suostumuksenne missä

tahansa tutkimuk-sen vaiheessa ennen sen päättymistä ilman, että siitä koituu Teille mitään haittaa. Keskeyttämi-seen tai peruuttamiseen saakka kerättyjä tietoja ja näytteitä käytetään osana tutkimusaineistoa etäiskeemisen esialtistuksen vaikutusten, tehon ja turvallisuuden varmistamiseksi.

Mikäli Teillä on kysyttävää tai haluatte lisätietoja, vastaamme mielellämme.

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SUOSTUMUS LÄÄKETIETEELLISEEN TUTKIMUKSEEN

Tutkimuksen nimi: **Etäiskeeminen esikäsittely elinsiirteiden toiminnan parantajana**

Minua on pyydetty osallistumaan edellä mainittuun HYKS Vatsakeskuksen elinsiirto- ja maksakirurgian klinikan ja sen lääkäreiden suorittamaan tutkimukseen.

Olen saanut, lukenut ja ymmärtänyt tutkimuksesta kertovan tiedotteen (päivätty 27.1.2020). Tiedotteesta olen saanut riittävän selvityksen tutkimuksesta ja sen yhteydessä suoritettavasta tietojen keräämisestä, käsittelystä ja luovuttamisesta. Tiedotteen sisältö on kerrottu minulle suullisesti ja olen saanut riittävän vastauksen kaikkiin tutkimusta koskeviin kysymyksiini.

Minulla on ollut riittävästi aikaa harkita osallistumistani tutkimukseen. Annan luvan itseäni koskevien, tutkimuksen kannalta tarpeellisten tietojen keräämiseen HYKS Elinsiirto- ja maksakirurgian klinikan tutkijoiden ”**Etäiskeeminen esikäsittely elinsiirteiden toiminnan parantajana**” tutkimusrekisteriin. Tietojen keräämistä varten lääkäri saa kirjata henkilötunnukseni sekä käyttää sitä tietojen saamiseksi. Kaikki minusta tutkimuksen aikana kerättävät tiedot käsitellään luottamuksellisina.

Ymmärrän, että osallistumiseni tähän tutkimukseen on täysin vapaaehtoista. Olen tietoinen siitä, että voin keskeyttää osallistumisen tai peruuttaa suostumuksen missä tahansa tutkimuksen vaiheessa ennen sen päättymistä ilman, että siitä koituu minulle mitään haittaa. Tutkimuksesta kieltäytyminen, sen keskeyttäminen tai peruuttaminen ei vaikuta jatkohoitooni. Olen tietoinen siitä, että minusta keskeyttämiseen mennessä kerättyjä tietoja ja näytteitä käytetään osana tutkimusaineistoa etäiskeemisen esialtistuksen vaikutusten, tehon ja turvallisuuden varmistamiseksi.

Olen tietoinen siitä, että henkilötietojani voidaan käsitellä myös kotimaisen ja ulkomaisen viranomaisen suorittaman tarkastuksen, tutkimustiimiin kuulumattoman tutkimuksen säännönmukaista laadunvalvontaa tekevän henkilön (tutkimusmonitorin) suorittaman laadunvarmistustoiminnan yhteydessä.

Allekirjoituksellani vahvistan osallistumiseni tähän tutkimukseen ja suostun vapaaehtoisesti tutkimushenkilöksi.

_____ _____

potilaan allekirjoitus päiväys

_____ _____

nimenselvennys potilaan syntymäaika

_____

potilaan osoite

Suostumus vastaanotettu

_____ _____

lääkärin allekirjoitus päiväys

_____

nimen selvennys

Alkuperäinen allekirjoitettu tutkimushenkilön suostumus sekä kopio tutkimustiedotteesta jäävät tutkijalääkärin arkistoon. Tutkimustiedote ja kopio allekirjoitetusta suostumuksesta annetaan tutkimushenkilölle.

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	1,2,4-10,13
Protocol version	#3	Date and version identifier	1
Funding	#4	Sources and types of financial, material, and other support	9,13
Roles and	#5a	Names, affiliations, and roles of protocol contributors	1,13

responsibilities: contributorship			
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	9,13
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	9
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	9-11
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4
Objectives	#7	Specific objectives or hypotheses	4
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	4-5
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	4-5
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	5-6
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	5-6
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	5-6
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	5-6
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6-7
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	5-6
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	8

Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	8
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	5
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	5
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	5
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	5
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	5
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires,	6-10, Supplement 1

		laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	9
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	8-9
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	8
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	9
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	10
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate	9

		the trial	
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	10
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	9-10
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	9-10
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	16
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	5,10
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	5,10
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	9
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	13
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	9-10
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial	10

		participation	
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	11
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	11
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	11
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Supplement 3
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	10

Notes:

- 2b: 1,2,4-10,13
- 18a: 6-10, Supplement 1
- 32: Supplement 3 The SPIRIT checklist is distributed under the terms of the Creative Commons Attribution License CC-BY-ND 3.0. This checklist was completed on 09. March 2020 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai

BMJ Open

A randomised sham-controlled double-blind trial evaluating remote ischemic preconditioning in solid organ transplantation – A study protocol for the RIPTRANS trial

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Manuscript ID	bmjopen-2020-038340.R1
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Date Submitted by the Author:	17-Aug-2020
Complete List of Authors:	Uutela, Aki; Helsinki University Hospital, Transplantation and Liver Surgery Helanterä, Ilkka; HUS, Transplantation and Liver Surgery Lemström, Karl; Helsinki University Hospital, Cardiothoracic Surgery Passov, Arie; Helsinki University Hospital, Perioperative, Intensive Care and Pain Medicine Syrjälä, Simo; Helsinki University Hospital, Cardiothoracic Surgery Aberg, Fredrik; Helsinki University Hospital, Helsinki University, Transplantation and Liver Surgery; Sahlgrenska University Hospital, The Transplant Institute Mäkisalo, Heikki; Helsinki University Hospital, Transplantation and Liver Surgery Nordin, Arno; Helsinki University Hospital, Transplantation and Liver Surgery Lempinen, Marko; Helsinki University Hospital, Transplantation and Liver Surgery Sallinen, Ville; Helsinki University Hospital, Transplantation and Liver Surgery
Primary Subject Heading:	Surgery
Secondary Subject Heading:	Renal medicine, Urology, Gastroenterology and hepatology, Intensive care, Cardiovascular medicine
Keywords:	TRANSPLANT MEDICINE, TRANSPLANT SURGERY, Renal transplantation < NEPHROLOGY, Adult intensive & critical care < INTENSIVE & CRITICAL CARE, Hepatology < INTERNAL MEDICINE, Cardiac surgery < SURGERY

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A randomised sham-controlled double-blind trial evaluating remote ischemic preconditioning in solid organ transplantation – A study protocol for the RIPTRANS trial

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Protocol version 1.14c

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Protocol amendments are listed in Supplement 1.

Manuscript word count without Title page, Trial Registration data, Abstract, Strengths and limitations of this study, Tables, References, Author's contributions, Funding statement, Sponsorship statement, Conflicts of interests statement, Acknowledgements and Supplements: 3160 words

Keywords: remote ischemic preconditioning, ischaemia–reperfusion; organ protection; transplantation; delayed graft function

Trial registration data

Data category	Information
Primary registry and trial identifying number	NCT03855722 (ClinicalTrials.gov)
Date of registration in primary registry	February 27th, 2019
Source(s) of monetary or material support	Academy of Finland, Finska Läkaresällskapet, Helsinki University Hospital's research funds
Primary sponsor	Helsinki University Hospital
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Public title	Remote Ischaemic Preconditioning in Transplantation (RIPTRANS)
Scientific title	A randomised sham-controlled double-blind trial evaluating remote ischemic preconditioning in solid organ transplantation (RIPTRANS)
Countries of recruitment	Finland
Health condition(s) or problem(s) studied	Solid organ donation and transplantation
Intervention(s)	Remote ischemic preconditioning of brain dead donors vs. sham procedure
Key inclusion and exclusion criteria	Ages eligible for study: over 18 years Sexes eligible for study: all Accepts healthy volunteers: no Inclusion: includes brain dead kidney and multi-organ donors and their transplant recipients Exclusion for adult donors: not a kidney donor, severe hemodynamic instability, other conflicting clinical trial

Study type	Interventional Allocation: Randomized, parallel assignment, masking double-blind (participant, care provider, investigator, outcomes assessor) Primary purpose: organ preservation
Date of first enrolment	March 13th, 2019
Target sample size	500 kidney transplant recipients
Recruitment status	Recruiting
Primary outcome(s)	Delayed graft function in kidney allografts
Key secondary outcomes	Short-term functional outcomes of transplanted organs, rejections, and graft survival in various time points up to 20 years

Abstract

Introduction

Remote ischemic preconditioning (RIPC) using a non-invasive pneumatic tourniquet is a potential method for reducing ischemia-reperfusion injury. RIPC has been extensively studied in animal models and cardiac surgery, but scarcely in solid organ transplantation. RIPC could be an inexpensive and simple method to improve function of transplanted organs. Accordingly, we aim to study whether RIPC performed in brain-dead organ donors improves function and longevity of transplanted organs.

Methods and analyses

RIPTRANS is a multi-center, sham-controlled, parallel group, randomised superiority trial comparing RIPC intervention versus sham-intervention in brain-dead organ donors scheduled to donate at least one kidney. Recipients of the organs (kidney, liver, pancreas, heart, lungs) from a randomised donor will be included provided that they give written informed consent. The RIPC intervention is performed by inflating a thigh tourniquet to 300 mmHg 4 times for 5 minutes. The intervention is done twice: firstly right after the declaration of brain death and secondly immediately before transferring the donor to the operating theatre. The sham group receives the tourniquet, but it is not inflated. The primary endpoint is delayed graft function (DGF) in kidney allografts. Secondary endpoints include short-term functional outcomes of transplanted organs, rejections, and graft survival in various time points up to 20 years. We aim to show that RIPC reduces the incidence of DGF from 25 % to 15%. According to this, the sample size is set to 500 kidney transplant recipients.

Ethics and dissemination

This study has been approved by Helsinki University Hospital Ethics Committee and Helsinki University Hospital's Institutional Review Board. The study protocol was be presented at the European Society of Organ Transplantation congress in Copenhagen 14-15th September 2019. The study results will be submitted to an international peer-reviewed scientific journal for publication.

Trial registration number

NCT03855722 (ClinicalTrials.gov)

Strengths and limitations of this study

- The study method, a multi-center, double-blinded, sham-controlled, randomised superiority trial, is the best available method to investigate the effects of remote ischemic preconditioning (RIPC) performed in the donor on the function and longevity of transplanted organs in the recipient

-Remote ischemic preconditioning is an extremely simple, reproducible, and inexpensive method

-The sample size, 500 kidney transplant recipients, is large enough to provide confidence in the estimates of outcomes.

-Primary outcome, delayed graft function of kidney allograft, is clinically highly relevant, easy to measure, and objective.

-As the sample size is calculated for kidney transplantation, outcomes of other organ recipients might be underpowered.

Introduction

Solid organ transplantation is an established standard of care for end-stage dysfunction of different organs, but the availability of the treatment is greatly limited globally by the shortage of organ donors. On the other hand, the lifetime of a transplanted organ is often limited and there is a number of patients waiting for a second or subsequent transplant[1-6]. A transplanted organ is exposed to ischemia-reperfusion injury during the transplantation process[7]. Alleviating this injury could improve the function and lifetime of transplanted organs.

Remote ischemic preconditioning (RIPC) is an old concept where remotely produced ischemia induces protective changes in distant organs or tissues and renders them less susceptible for future ischemia via hormonal, metabolic, and neuronal mechanisms[8]. As an intervention, RIPC is easy and cheap to perform – an inflatable tourniquet is used to occlude upper or lower limb. RIPC has been extensively studied in animal models[9-11], and in human clinical trials of cardiac surgery. The largest of these clinical trials - RIPHeart[12] ERICCA[13], and CONDI-2/ERIC-PPCI[14] - have not been successful to show benefit from RIPC, but this might be due to the fact that the patients suffering from chronic myocardial ischemia already have maximal compensatory mechanisms in use. This could also partially explain the results of RenalRIP trial, in which RIPC reduced acute kidney injury associated with cardiac surgery without affecting cardiac parameters [15].

One of the postulated reasons for negative results in RIPHeart and ERICCA trials is the use of propofol instead of the volatile anesthetics, even though this has not been fully verified[16]. In the DBD transplantation setting, when the RIPC intervention is done to a brain dead donor, propofol is not used and should not prevent the effect of RIPC. Propofol may be used in the recipient surgery, but there is at least preliminary small animal data, that this may not prevent effectiveness of RIPC[17].

Organ transplantation is a lucrative field to study RIPC, as the donor organs are healthy, and do not suffer from chronic ischemia, but face invariable acute ischemia of various durations. RIPC has been studied little in clinical transplantation and results have been controversial. A RIPC intervention done to heart transplant recipients together with post conditioning 20 minutes after aortic declamping reduced cTnI levels at 6 hours after transplantation[18]. In a recent trial RIPC done to living liver donors reduced postoperative aspartate aminotransferase (AST) levels in liver recipients[19], whereas a pilot study of the RIPCOLT trial with RIPC done on liver transplant recipients demonstrated no short term benefits [20].

Direct ischemic preconditioning with clamping of liver hilum in donation after brain death (DBD) was not was not beneficial and could even induce excessive ischemic damage[21]. A retrospective *post hoc* analysis of 2 such trials showed that liver ischemic conditioning had no RIPC effect for kidneys[22]. The liver-RIPC may provide an insufficient stimulus for the kidneys and the authors speculated that limb ischemia could be a better method for RIPC. In a study of 29 kidney transplant patients RIPC done on DBD donors resulted in lower creatinine levels at 15 and 30 days after transplantation, but the change in GFR did not reach statistical significance[23]. As far as we know, no larger randomized controlled trial with limb-RIPC on DBD donors have been published.

A small study with 20 living donor kidney recipients per group found no difference in kidney function whether RIPC was done on donor or recipient[24]. A larger trial of 170 living kidney donor – recipient pairs with RIPC done on donors reported lower postoperative creatinine values on donors after RIPC but no long term benefits for donors or recipients[25].

The largest kidney transplant RIPC trial to date, the REPAIR trial, showed that a RIPC performed in both donor and recipient immediately before a living-donor kidney surgery improved the estimated glomerular filtration for the whole follow-up period of 5 years[26 27]. The kidney allografts from living donors are subjected to very short ischemia (in Finland this is typically less than two hours) and even greater benefits could be obtained if RIPC is performed in deceased donors, where ischemia times are much longer (median 15 hours for kidney allografts in Finland, even longer in other countries). RIPC intervention performed to the recipients of deceased donor kidneys during the transplantation surgery did not improve kidney function in CONTEXT trial[28]. This study can be criticized for performing RIPC in the recipients instead of donors, because the ischemic injury has already taken place before RIPC.

The aim of this study is to show that RIPC performed in brain-dead donors (DBD) can be used to improve function and longevity of transplanted organs.

Methods and analysis

Study design

The RIPTRANS trial is a multi-center, double-blinded, parallel group, individual donor randomised superiority trial comparing RIPC with a sham-procedure performed in brain-dead donors. There is only one transplantation centre (Helsinki University Hospital) in Finland that covers the whole country and procurement team travels to all donor hospitals in Finland. This protocol was drafted in accordance with the SPIRIT (Standard Protocol Items: recommendations for Interventional Trials) statement[29]. This trial is registered in ClinicalTrials.gov (NCT03855722), the first registration date was February 27th, 2019.

Participants

RIPC or sham procedure will be performed on a brain-dead donor fulfilling inclusion and exclusion criteria. All brain-dead donors in participating hospitals scheduled for at least one kidney procurement will be included. Donors with significant hemodynamic instability (assessed by the intensive care physician responsible for the treatment of the donor) and under the age of 18 years will be excluded. Donors (or potential recipients of organ from this donor), who are participating in a trial with conflicting interventions or outcomes, will also be excluded. Although the donors are randomised and the intervention is carried out in donors, the recipients are the actual participants of this trial. All patients receiving a kidney, liver, pancreas, heart, or lungs from a donor randomised in the trial will be included in the trial provided that they give a written informed consent (Supplement 2, in Finnish) to participate in the trial and are at least 18 years old. The informed consent will be presented to the patient by a study nurse or physician. As based on the previous studies, RIPC is supposedly not harmful for the donor, and the lack of consent from any of the transplant recipients does not exclude the donor from the study, nor the possible inclusion of the other

recipients. There are no other exclusion criteria for recipients who receive abovementioned organs from a randomised donor.

Randomisation and masking

Eligible donors will be randomly allocated in a 1:1 ratio to either RIPC or sham-procedure group. The randomisation sequence was generated using a web-based commercial service (Sealed Envelope) with randomly variable block size (4, 6, or 8) and stratified according to donor age (under / over 60 years of age), planned organ to be procured (kidneys only / abdominal organs only / both thoracic and abdominal organs), and donor cardiopulmonary resuscitation (yes / no). The randomisation and allocation to either RIPC or sham-intervention is done by a transplant coordinator, who is not blinded to the allocated treatment, using the same web-based service. Once the donor is allocated, the transplant coordinator sends electronically or via fax written instructions on how to perform the allocated treatment to the intensive care team responsible for the treatment of the donor, who also are not blinded to the treatment. This intensive care team will collect data regarding the actual timing of the allocated procedure and whether this caused any noticeable changes in the donor hemodynamics. All researchers and all other treating personnel are blinded, such as procuring surgeons, transplant surgeons, treating physicians, data collectors, and data analysts as well as recipients. After the trial recruitment has been closed and data collected, the allocated group will be named as A and B before the data is analysed. Once the data analyses for primary and secondary outcomes are completed, the full blinding will be removed. No emergency unblinding is planned, but incidents of possible breaches in blinding will be recorded.

Procedures

RIPC will be performed as follows: Donor's thigh will be occluded 4 times for 5 minutes using tourniquet inflated to 300 mmHg each followed by 5 minutes of deflation. The intervention will be performed twice (once in both thighs). Once as soon as possible after brain death is determined, and once right before transferring the donor to the operation room for procurement. Sham-intervention will be performed by putting the inflatable tourniquet in place similarly, but not inflating it. Apart from the RIPC or sham-intervention, the treatment of donors will be according to normal routine. Study blood samples will be acquired from donors before (selected centers) and after the intervention.

Outcomes

The primary outcome measure is delayed graft function (DGF) of kidney allografts, which is defined as the need for dialysis within the first week after transplantation. Secondary outcome measures are different for different organs (Table 1). Outcomes are assessed during the primary hospital stay, and thereafter at the routine follow-up visits. Helsinki University Hospital has a legal requirement to maintain a registry of all patients receiving a transplant in Finland, and data regarding visits in other hospitals are submitted to Helsinki University Hospital for registry purposes. Secondary outcomes are assessed directly from the registry, from the data provided by other hospitals, or at routine follow-up visits at Helsinki University Hospital. Survival status is automatically updated to the registry from the National Population Centre, which is an exact, complete, and up-to-date source for causes of death in Finland. Prespecified subgroup analyses are planned for characteristics that may potentially affect the results (Table 2). Further exploratory outcome measures will be done

according to Supplement 3. In the informed consent, the patients are also asked to give their permission for using the excess study blood, urine and tissue samples in possible ancillary analysis.

Table 1. Secondary outcome measures

Kidney allografts
Estimated glomerular filtration rate (eGFR) at 3 months, 1 year, 2 years, 5 years, 10 years, and 20 years.
Biopsy-proven acute rejection (BPAR) within 1 year.
Graft survival at 3 months, 1 year, 2 years, 5 years, 10 years, and 20 years: time from transplantation to death, retransplantation or permanent dialysis.
Death-censored graft survival at 3 months, 1 year, 2 years, 5 years, 10 years, and 20 years: time from transplantation to retransplantation or permanent dialysis, death-censored
Pancreatic allografts
Glycosylated haemoglobin (HbA1c) at 3 months, 1 year, 2 years, 5 years, 10 years and 20 years
Acute rejection in pancreatic allograft, either biopsy-proven (allograft pancreas or duodenal biopsy) acute rejection or clinically treated suspected acute rejection within 1 year
Pancreatic allograft survival at 3 months, 1 year, 2 years, 5 years, 10 years and 20 years: Time from transplantation to death, retransplantation, explantation or daily insulin dependence
Death-censored pancreatic allograft survival at 3 months, 1 year, 2 years, 5 years, 10 years and 20 years: Time from transplantation to death, retransplantation, explantation or daily insulin dependence, death-censored
Liver allografts
MEAF-score at 3rd post-operative day (POD): Model for Early Allograft Function Scoring. MEAF = score ALTmax:3POD + score INRmax:3POD + score bilirubin3POD, score range 0 - 10, higher score indicates worse outcome[30]
Postoperative biliary complications within 1 year: Amount and type of postoperative biliary complications: stricture at anastomosis, bile leak or ischemic type biliary lesions (ITBL) requiring intervention (ERC, PTC, operation) or prolonged drainage within 1 year
Post-transplantation kidney injury (acute kidney injury) within 1 week, at 3 months, 1 year: according to ADQI 2010 criteria[31].
Biopsy proven acute rejection (BPAR) within 1 year.

Graft survival at 1 year, 2 years, 5 years, 10 years, and 20 years: time from transplantation to death, retransplantation or explantation
Heart allografts
Ischemia-reperfusion injury determined by peripheral blood TnI levels at 6 hours after transplantation
Peripheral blood proBNP measurement at 1 week after transplantation
Primary graft dysfunction according to ISHLT definition[32] within 24 hours after transplantation
Biopsy-proven or clinically treated acute rejection within one year after transplantation
Vasculopathy-free survival according to ISHLT definition[33] at 1 year, 2 years, 5 years, 10 years, and 20 years
Graft survival at 1 year, 2 years, 5 years, 10 years, and 20 years, time from transplantation to death, retransplantation or explantation
Lung allografts
Primary graft dysfunction according to ISHLT definition[34] within 72 hours after transplantation
Biopsy proven or clinically treated acute rejection within one year
Chronic Lung Allograft Dysfunction (CLAD) free survival according to ISHLT/ATS/ERS 2014 guideline [35] at 1 year, 2 years, 5 years, 10 years, and 20 years, time from transplantation to death or retransplantation
Graft survival at 1, 2, 5, 10 and 20 years : time from transplantation to death, retransplantation or explantation

Table 2. Subgroup analyses**Subgroup analyses**	**Subgroups**
Donor cardiopulmonary resuscitation	yes / no
Donor age (years)	Under 60 /over 60
Donor sex	male / female
Organ cold ischemia time (hours, organ specific)	below / above median
Uncompleted study intervention	yes / no
Liver transplantation for acute liver failure	yes / no

Statistical analyses

The incidence of DGF in kidney allografts after transplantation from a brain-dead donor in Finland is approximately 25 % (Finnish Transplantation Registry). We aim to show that RIPC reduces the incidence of DGF to 15%. With a 5 % significance level and 80 % power, 496 kidney transplantations are required to show this difference. Sample size is not adjusted for cross-over or loss-of-follow up because the risk of these are considered to be minimal. Usually two kidneys per donor are transplanted. Because a portion of procured kidneys will be transferred to another Nordic country according to ScandiaTransplant rules, are untransplantable, transplanted in a combined organ transplantation, or transplanted to a recipient below 18 years old, we assume 90% of donors will lead to two kidney transplantations and 10% will lead to one kidney transplantation within the study. We set the final sample size to 500 kidney transplantations, for which approximately 260 donors are required to be randomised.

The primary outcome measure and the secondary outcomes for kidney transplantation will be analysed using generalized linear mixed models taking into account that kidneys from a single donor will usually be transplanted to two recipients included in the study. Survival analysis for kidney allografts and transplant recipients are done using Kaplan Meier survival diagrams and the effect size is estimated using Cox proportional hazards regression model similarly taking into account single donor providing kidneys to two recipients.

The categorical outcome variables for liver, pancreas, heart, and lungs are analysed with Chi square test (or Fischer's exact test, if *n* is under 5 in any of the subcategories). The continuous outcome variables for these organs are analysed using independent T-test or Mann Whitney U-test depending on whether the outcome has normal distribution or not. The effect size for categorical variables is calculated with odds ratio and 95 % confidence interval (CI). For continuous variables the effect size is calculated with difference in means with 95 % CI for variables with normal distribution. If a continuous variable can be converted for normal distribution with a logarithmic transformation, will the effect size be reported using the ratio of geometrical means with 95 % CI. Other continuous variables will be calculated using Mann Whitney U-test and the effect size will be reported using $r = Z/\sqrt{N}$ without 95 % CI. Survival analysis for these organs will be described using Kaplan-Meier survival diagrams and log-rank-test and effect size estimated using Cox proportional hazards regression model.

Subgroup analysis will be made using generalized linear mixed models as univariate analysis by adjusting models by subgroup. A multivariate analysis of subgroups can be done with aforementioned generalized linear model and by selecting the significant subgroups ($p < 0.05$) from univariate analysis as covariates. Subgroup analysis for survival variables will be described with Kaplan-Meier, which will be stratified by subgroup and effect size will be estimated using Cox proportional hazards regression model by adjusting it with the subgroup.

In case that because of missing values more than 5 % of patients would be left out from sensitivity analyses, multiple imputations may be used to conduct sensitivity analyses. Otherwise, the missing data will not be adjusted separately, but these cases will either be left out from the analyses or censored at the last point of follow up.

Data security
All patient data included in the study is confidential and will be concealed on a computer behind an AES 256-bit encryption. Any data stored in a paper from will be held in the study hospitals in locked offices. Only the study personnel will have the access to the trial dataset.

Data availability statement
After the completion of the study the depersonalized data can be requested from the authors.

Schedule and interim safety analyses
The study was conceptualised in June 2017. The study plan was approved by the Helsinki University Hospital's Ethics Committee 9th May 2018. Helsinki University Hospital's Institutional Review Board gave permission to conduct the study 14th August 2018. The study started recruiting in May 13th 2019 in four out of five university hospitals in Finland. The Ethics Committee required a safety analysis after 16 donors had been randomised. The donors and recipients of kidneys from these 16 donors were analysed without unmasking the allocated group. All 16 donors randomised successfully underwent procurement. No adverse events were noted in the recipients. After this safety analysis, the study will be disseminated to non-university donor hospitals. Second interim analysis will be done when half of the target sample size is reached (250 kidney transplantations). In Finland, approximately 230 DBD kidney transplants are being performed annually. We estimate that data for primary outcome would be available in 4 years.

Role of the funding sources and sponsors
The funders or sponsors have had and will have no role in study design, data collection, data analysis, data interpretation, or writing the report, or any other aspect of the work, except for funding.

Ethics and dissemination

Study ethics
This study has been approved by Helsinki University Hospital Ethics Committee and Helsinki University Hospital's Institutional Review Board.

The intervention is performed on a donor, who has been determined brain dead and has given permission to act as a donor according to Finnish legislation, and is determined suitable and scheduled for kidney procurement. The Ethics Committee has approved that donors (or next of kin) do not need to consent to RIPC or sham-procedure because it is a non-invasive procedure, the donor is brain dead and scheduled for procurement already.

The recipients of organs from randomised donors will be recruited in the study and will be required to give written informed consent to participate. The recipient cannot influence whether the donor has been randomised or received the allocated treatment. The recipient has the right to decline participation in the trial, but can still choose to receive the planned allograft. In these cases, the recipient's data is not used in the study analyses. The recipient has also right to decline the offered organ. The recipient does not have the right to know the allocated treatment the donor has received before the study has been completed, data

analysed, and blinding unmasked. The donors or recipients do not receive any compensation for their participation in the trial. The recipients have the right to discontinue the trial or withdraw their consent at any point. In these cases, the collected data will be used in the analyses up to the point of discontinuation.

A few additional blood samples (and a urine sample from the kidney recipients) will be taken from the kidney, heart, and lung recipients for the study purposes during and shortly after the transplantation, but otherwise the recipients only give their consent to the study group to observe and collect medical information. These samples are stored maximally for five years after the completion of the study recruitment. The patient informed consent forms are in Finnish and Swedish and will be provided by request made to the study group.

Harms

Earlier studies on RIPC have not indicated any harm (7-16). On the contrary, many earlier studies suggest that RIPC may be beneficial for the function and longevity of the allografts. Before wider adoption of the RIPC in transplantation, its safety and benefits need to be addressed in a randomised controlled trial such as RIPTRANS. Any possible harmful effects of the intervention will be reported together with the study results. The Finnish patient insurance covers the organ recipients participating in the study.

Monitoring

Helsinki University Hospital Ethics Board monitored the results of the first interim analysis. Initially the Ethics board did not necessitate a separate Data Monitoring Committee (DMC). To provide external validity for the study, a DMC contract was made with Clinical Research Institute HUCH Ltd (HYKS Instituutti) in March 2020. The site monitoring will be performed every three months including review of the Investigator's Trial File, facilities, the equipment at the site, compliance to study protocol and study specific procedures, source document quality and the intervention implementation documentation for all donors. All the study patients will be monitored for: existence, informed consent process and documentation of the Trial outcome measures. A complete review will be conducted for 10 % of the subjects. A close-out visit shall be done after all the data has been collected and the treatment of all the subjects has been completed. This Monitoring plan and Agreement is made in collaboration with the guideline for coordinated GCP-monitoring of clinical trials in the Nordic countries (version 5/24.10.2017).

Patient and Public involvement

The transplant recipients are informed about the study once they are asked to participate. This study protocol was published in ClinicalTrials.gov before beginning of the study. Patient organizations were not involved in the study design.

Dissemination

The study protocol was be presented at the European Society of Organ Transplantation congress in Copenhagen 14-15th September 2019 and will possibly be presented in other scientific conferences. The study results will be submitted to an international peer-reviewed scientific journal for publication and possibly discussed at scientific meetings. The study is also being made public via social media platforms (Twitter). The International Committee of

Medical Journal Editors recommendations (www.ICMJE.org) are applied when considering the authorship of any publications from this trial.

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Author's contributions
Concept: VS, ML
Initial draft of protocol: VS, AU, IH, KL
Critical revision of the protocol: All authors
Implementation: VS, AU, KL, SS, AP
Data collection: AU, SS
Donor recruitment: MB, MS, TL, JR, MaL, IL, JL, JG, PL

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Sponsorship statement
The study sponsor is Helsinki University Hospital.

Conflicts of interests statement
The authors have no competing interests that would affect this study.

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The authors wish to thank all the collaborators of this study, and in particular Helsinki University Hospital transplant coordinators Siv Ansa, Carola Schauman, Leena Toivonen, Eero Hartikka and Heikki Norio, the personnel of the participating intensive care units and the personnel of the Meilahti Hospital operating theatre and the transplantation wards.

Supplement 1. Protocol amendments

Study Protocol version 1.11 (Finnish version), 2019-Jan-16 in use at time when study recruiting started March 13th 2019

Version 1.13 (Finnish version), 2019-Aug-11
Reported the results of the safety analysis the to University of Helsinki Ethics Committee
Changed the practise of taking the 6 h postoperative urinary sample from kidney recipients.
Original: The sample is taken from the urine bag
Updated: The sample is taken from the catheter hose

Version 1.14 (English version), 2020-Mar-6
Donor pre-intervention blood samples are taken in selected centers, not necessarily only in Helsinki.

BNP as an outcome measure changed to proBNP according to a change in Helsinki University Hospital laboratory HUSlab protocol. Blood samples from heart transplant recipients transplanted before this are reanalysed for proBNP as possible.

Added a new secondary outcome measure for the lung recipients:
Lung allograft: graft survival: time from transplantation to death, retransplantation or explantation. The recruitment Lung recipients started later than for other organs because of a conflicting trial, which now has completed recruiting. Only four lung patients have thus far been included in the study and no results for lung recipients have been analysed.

Changed the manner of dealing with the possible missing data in analyses:
Original: Missing data will not be adjusted separately, but these cases will either be left out from the analyses or censored at the last point of follow up.
Updated: In case that because of missing values more than 5 % of patients would be left out from sensitivity analyses, multiple imputations may be used to conduct sensitivity analyses. Otherwise, the missing data will not be adjusted separately, but these cases will either be left out from the analyses or censored at the last point of follow up.

External Study Monitoring Committee was initiated by Clinical Research Institute HUCH Ltd in March 2020.

Version 1.14c (Finnish version), 2020-May-15
Clarified the Finnish version to equal the English one. The informed consent of the transplant recipient can either be asked before (preferable) or after the transplantation.

Supplement 2. Model consent form (in Finnish)

TUTKIMUSTIEDOTE POTILAALLE

Tutkimuksen nimi: **Etäiskeeminen esikäsittely elinsiirteiden toiminnan parantajana**

Hyvä potilas,

Olette tulossa munuaissiirtoon HYKS Vatsakeskuksen elinsiirto- ja maksakirurgian klinikkaan. Pyydämme Teitä osallistumaan tutkimukseen, jossa selvitetään etäiskeemisen esikäsittelyn vaikutusta munuaissiirteen toimintaan.

Kun munuaissiirre on irrotettu elinluovuttajalta, siirre altistuu hapenpuutteelle (iskemialle) kunnes se liitetään vastaanottajan verenkiertoon munuaissiirtoleikkauksessa. Tämä hapenpuute vaikuttaa munuaissiirteen toimintaan, esim. virtsanerityksen käynnistymiseen, ja voi altistaa hyljinnälle. Hapenpuutteen aiheuttamia vaurioita voidaan pyrkiä ennaltaehkäisemään ns. etäiskeemisellä esikäsittelyllä. Etäiskeemisellä esikäsittelyllä tarkoitetaan sitä, että elinluovuttajan jokin muu kudos kuin irrotettavat elimet (tässä tutkimuksessa alaraaja) altistetaan hapenpuutteelle ennen elinirroitusleikkausta. Elinluovuttajan munuaisia ei siis altisteta hapenpuutteelle. Alaraajan hapenpuute aiheuttaa koko elimistössä, myös munuaisissa, hormonaalisia ja hermostollisia muutoksia, joilla elimistö pyrkii suojautumaan hapenpuutteen aiheuttamilta vaurioilta.

Tämä tutkimus on satunnaistettu, eli puolet elinluovuttajista saa etäiskeemisen esikäsittelyn ja puolet ei. Tutkimus on sokkoutettu, tarkoittaen sitä, että Te tai hoitavat lääkärit eivät tiedä onko elinluovuttaja, jolta munuaissiirteenne tulee, saanut etäiskeemisen esikäsittelyn vai ei. Elinsiirto ja hoito sen jälkeen toteutetaan täysin samalla tavalla kuin potilaiden, jotka eivät osallistu tutkimukseen.

Pyydämme Teiltä lupaa ottaa tutkimukseen liittyen kolme verinäytettä munuaissiirtoleikkauksen (nukutuksen) aikana ja yksi virtsanäyte virtsakatetrista leikkauksen jälkeen. Näytteistä tutkitaan erilaisia munuaisvaurion merkkiaineita. Tuloksia verrataan Teistä rutiininomaisesti leikkauksen jälkeen otettuihin munuaisten toimintakokeisiin, dialyysitarpeeseen, mahdolliseen siirteen hyljintään ja siirteen pitkäaikaiseen toimintaan.

Mikäli veri- tai virtsanäytteitä jää tutkimuksesta yli, niistä voidaan etäiskeemisen esikäsittelyn vaikutusten selvittämiseksi määrittää myöhemmin alkuperäisessä tutkimussuunnitelmassa mainittujen lisäksi muitakin analyysejä. Näytteitä säilytetään tutkimusryhmän pakastimessa korkeintaan 5 vuotta, jonka jälkeen ne tuhotaan.

Tutkimustiedon oikeellisuuden varmistamiseksi tutkimustietoja verrataan muun muassa alkuperäisiin sairauskertomuksiin. Tällöin tietoja käsitellään ns. monitoroijan toimesta tutkijalääkärin tai muun tutkimushenkilöstön valvonnassa ja vastuulla. Tämän lisäksi tutkimuksessa henkilöllisyytenne sekä muut tunnistettavat tiedot ovat ainoastaan tutkijalääkäreiden tiedossa, ja he kaikki ovat salassapitovelvollisia. Tutkimusrekisteriin

talletetaan vain tutkimuksen kannalta välttämättömiä tietoja. Tutkittavia seurataan 20 vuotta.

Tämä tutkimus ja siihen kuuluva henkilötietojen käsittely perustuvat seuraaviin lainsäädäntöihin: EU tietosuoja-asetus (2016/679), 6. artikla 1 a), b), c) ja e) ja 9. artikla 3 a), g), i) ja j) kohdat, laki lääketieteellisestä tutkimuksesta (1999/488), terveydenhuoltolaki (1326/2010), laki potilaan asemasta ja oikeuksista (785/1992), laki terveydenhuollon ammattihenkilöistä (559/1994), laki viranomaisten toiminnan julkisuudesta (621/1999), tietosuojalaki (2019) ja arkistolaki (831/1994). Lisäksi huomioidaan EU:n tietosuoja-asetuksen yli kansallisen lainsäädännön menevät määräykset.

Tutkimuksen loputtua tutkimusrekisteri säilytetään hyvän kliinisen tutkimustavan vaatimusten mukaisesti ja hävitetään sen jälkeen. Tutkimusrekisterissä ei käytetä automaattista päätöksentekoa. Tämä ei koske tutkimukseen liittyvää ryhmien randomointia, joka on tieteelliseen tutkimukseen kuuluva metodi.

Tutkimuksen rekisterinpitäjänä toimii Helsingin ja Uudenmaan sairaanhoitopiirin kuntayhtymä.

Osoite:
Helsingin ja Uudenmaan sairaanhoitopiirin kuntayhtymä, Stenbäckinkatu 9
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Kirjaamon telefax 09 471 75500,
Kirjaamon sähköposti keskuskirjaamo@hus.fi
postiosoite: HUS keskuskirjaamo PL 200, 00029 HUS

Voitte toteuttaa tietosuoja-asetuksen mukaisia oikeuksianne vapaamuotoisilla ilmoituksilla, mutta suosittelemme käyttämään näitä tarkoituksia varten laadittuja HUSin lomakkeita. Lomakkeet löydätte HUSin internet-sivuilta:
http://www.hus.fi/potilaalle/potilaan_oikeudet/terveystieteellinen%20tutkimus/Sivut/default.aspx

Teillä on myös oikeus tehdä tietosuoja-asioissa valitus Suomessa tietosuojasta vastaavalle viranomaiselle eli tietosuojavaltuutetulle.

Tietosuojavaltuutetun toimisto
Käyntiosoite: Ratapihantie 9, 6. krs, 00520 Helsinki
Postiosoite: PL 800, 00521 Helsinki
Puhelinvaihde: 029 566 6700
Sähköposti (kirjaamo): tietosuoja@om.fi

Pyydämme Teiltä kirjallista suostumusta tutkimukseen osallistumisesta. Voitte syytä ilmoittamatta keskeyttää tutkimukseen osallistumisen tai peruuttaa suostumuksenne missä tahansa tutkimuksen vaiheessa ennen sen päättymistä ilman, että siitä koituu Teille mitään

haittaa. Keskeyttämiseen tai peruuttamiseen saakka kerättyjä tietoja ja näytteitä käytetään osana tutkimusaineistoa etäiskeemisen esialtistuksen vaikutusten, tehon ja turvallisuuden varmistamiseksi.

Mikäli Teillä on kysyttävää tai haluatte lisätietoja, vastaamme mielellämme.

Aki Uutela	Marko Lempinen	Ville Sallinen
LL, osastonlääkäri	Dosentti, LT, os.ylilääk	Dosentti, LKT, erikoislääkäri
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SUOSTUMUS LÄÄKETIETEELLISEEN TUTKIMUKSEEN

Tutkimuksen nimi: **Etäiskeeminen esikäsittely elinsiirteiden toiminnan parantajana**

Minua on pyydetty osallistumaan edellä mainittuun HYKS Vatsakeskuksen elinsiirto- ja maksakirurgian klinikan ja sen lääkäreiden suorittamaan tutkimukseen.

Olen saanut, lukenut ja ymmärtänyt tutkimuksesta kertovan tiedotteen (päivätty 27.1.2020). Tiedotteesta olen saanut riittävän selvityksen tutkimuksesta ja sen yhteydessä suoritettavasta tietojen keräämisestä, käsittelystä ja luovuttamisesta. Tiedotteen sisältö on kerrottu minulle suullisesti ja olen saanut riittävän vastauksen kaikkiin tutkimusta koskeviin kysymyksiini.

Minulla on ollut riittävästi aikaa harkita osallistumistani tutkimukseen. Annan luvan itseäni koskevien, tutkimuksen kannalta tarpeellisten tietojen keräämiseen HYKS Elinsiirto- ja maksakirurgian klinikan tutkijoiden ”**Etäiskeeminen esikäsittely elinsiirteiden toiminnan parantajana**” tutkimusrekisteriin. Tietojen keräämistä varten lääkäri saa kirjata henkilötunnukseni sekä käyttää sitä tietojen saamiseksi. Kaikki minusta tutkimuksen aikana kerättävät tiedot käsitellään luottamuksellisina.

Ymmärrän, että osallistumiseni tähän tutkimukseen on täysin vapaaehtoista. Olen tietoinen siitä, että voin keskeyttää osallistumisen tai peruuttaa suostumuksen missä tahansa tutkimuksen vaiheessa ennen sen päättymistä ilman, että siitä koituu minulle mitään haittaa. Tutkimuksesta kieltäytyminen, sen keskeyttäminen tai peruuttaminen ei vaikuta jatkohoitooni. Olen tietoinen siitä, että minusta keskeyttämiseen mennessä kerättyjä tietoja ja näytteitä käytetään osana tutkimusaineistoa etäiskeemisen esialtistuksen vaikutusten, tehon ja turvallisuuden varmistamiseksi.

Olen tietoinen siitä, että henkilötietojani voidaan käsitellä myös kotimaisen ja ulkomaisen viranomaisen suorittaman tarkastuksen, tutkimustiimiin kuulumattoman tutkimuksen säännönmukaista laadunvalvontaa tekevän henkilön (tutkimusmonitorin) suorittaman laadunvarmistustoiminnan yhteydessä.

Allekirjoituksellani vahvistan osallistumiseni tähän tutkimukseen ja suostun vapaaehtoisesti tutkimushenkilöksi.

_____ _____

potilaan allekirjoitus päiväys

_____ _____

nimenselvennys potilaan syntymäaika

_____

potilaan osoite

Suostumus vastaanotettu

_____ _____

lääkärin allekirjoitus päiväys

_____

nimen selvennys

Alkuperäinen allekirjoitettu tutkimushenkilön suostumus sekä kopio tutkimustiedotteesta jäävät tutkijalääkärin arkistoon. Tutkimustiedote ja kopio allekirjoitetusta suostumuksesta annetaan tutkimushenkilölle.

Supplement 3. Exploratory outcome measures

Kidney allografts
Peroperative blood samples before and after graft perfusion and urinary sample 6 hours after transplantation. Measurement of ischemia/reperfusion injury in blood and urine samples using following factors
Micro-RNA miR-21
Micro-RNA miR-24
Neutrophil gelatinase associated lipocain NGAL
Kidney injury molecule 1 KIM-1
Fatty acid binding protein 1 FABP-1
secretory leucocyte proteinase inhibitor SLPI
Liver allografts
Early allograft dysfunction at 7 days after transplantation according to Olthoff[1]: Bil >100, INR 1.6 or more, ALT or AST > 2000 at 7th POD
Highest ALT within 1 week
Highest INR within 1 week
Highest Bil within 1 week
Heart allografts
Ischemia-reperfusion injury determined by peripheral blood TnI, CK-MBm, lactate, and C-reactive protein levels at 0, 1, 12, and 24 hours
Peripheral blood proBNP at 1, 7, 14 and 21 days
Crea at 1, 7, 14 and 21 days
Urea at 1, 7, 14 and 21 days
eGFR at 1, 7, 14 and 21 days
Left ventricle ejection fraction (LVEF) at 1 day, 7 days, 14 days and 21 days
Left ventricle (LV) wall thickness measurements at 1 day, 7 days, 14 days and 21 days
Tricuspidal valve leak grading at 1 day, 7 days, 14 days and 21 days
The appearance of ischemia-reperfusion injury in routine biopsies at 7, 14 and 21 days

The appearance of fibrosis associated factors in routine biopsies at 7, 14 and 21 days
Long-time follow-up of proBNP at 1, 3, 6 and 12 months
Long-time follow-up of LVEF in cardiac ECHO at 1, 3, 6 and 12 months
Coronary Artery Disease (CAD) in coronary angiography at 1 year
Major Adverse Cardiac Events (MACE, including death because of cardiac cause, graft loss, primary allograft dysfunction, rejection classified as ISHLT G2R or more) at 1, 3, 6 and 12 months
Lung allografts
Factors possibly presenting the severity of ischemia/reperfusion injury after transplantation:
Standardized P/F-ratio during mechanical ventilation at 0 hours, 1, 6 , 12 and 24 hours
Non-standardized P/F-ratio during mechanical ventilation at 0 hours, 1 , 6, 12 and 24 hours
Plasma lactate at 0 hours, 1, 6, 12 and 24 hours
Serum highly sensitive C-reactive protein at 0 hours, 1, 6, 12 and 24 hours
Blood leukocyte count at 0 hours, 1, 6, 12 and 24 hours
Neutrophil count at 0 hours, 1, 6, 12 and 24 hours
Forced lung expiratory volume in one second (FEV1) at 1, 3, 6 and 12 months
Forced vital lung capacity (FVC) at 1, 3, 6 and 12 months
Evaluation of chronic rejection at 1, 3, 6 and 12 months
Exploratory outcomes lung allografts: Infections after transplantation at 1, 3, 6 and 12 months

References for supplement 3

1. Olthoff KM, Kulik L, Samstein B, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. *Liver Transpl* 2010;16(8):943-9. doi: 10.1002/lt.22091 [published Online First: 2010/08/03]

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Altman DG, Laupacis A, Gøtzsche PC, Krleža-Jerić K, Hróbjartsson A, Mann H, Dickersin K, Berlin J, Doré C, Parulekar W, Summerskill W, Groves T, Schulz K, Sox H, Rockhold FW, Rennie D, Moher D. SPIRIT 2013 Statement: Defining standard protocol items for clinical trials. Ann Intern Med. 2013;158(3):200-207

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1,2
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2,4
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	2-3
Protocol version	#3	Date and version identifier	1
Funding	#4	Sources and types of financial, material, and other support	2,12, 17
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1,17

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	2, 17
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	12
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	12-13
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6-7
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	6-7
Objectives	#7	Specific objectives or hypotheses	6-7
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be	1,7,17

		obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7-8
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	8
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	7-8
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7-8
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8-10, Suppl 1
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7-8
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	12

Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	8
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	8
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the	8-11, Suppl 1

		protocol	
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	8-9,12-13
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12-13
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10-11
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10-11
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	13
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	12
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	13
Auditing	#23	Frequency and procedures for auditing trial conduct, if	12-13

		any, and whether the process will be independent from investigators and the sponsor	
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	12-13
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	Suppl 2
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7-8
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	7-8,12-13
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12-13
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	17
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	12
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	13
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	13
Dissemination policy:	#31b	Authorship eligibility guidelines and any intended use of	13-14

authorship		professional writers	
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	13
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Suppl 3
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	13

Notes:

- 12: 8-10, Suppl 1
- 18a: 8-11, Suppl 1 This checklist was completed on 14. August 2020 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai

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A randomised sham-controlled double-blind trial evaluating remote ischemic preconditioning in solid organ transplantation – A study protocol for the RIPTRANS trial

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A randomised sham-controlled double-blind trial evaluating remote ischemic preconditioning in solid organ transplantation – A study protocol for the RIPTRANS trial

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Keywords: remote ischemic preconditioning, ischaemia–reperfusion; organ protection; transplantation; delayed graft function

Trial registration data

Data category	Information
Primary registry and trial identifying number	NCT03855722 (ClinicalTrials.gov)
Date of registration in primary registry	February 27th, 2019
Secondary identifying numbers	1411/2018 (Helsinki University Ethical Board numer)
Source(s) of monetary or material support	Academy of Finland, Finska Läkaresällskapet, Helsinki University Hospital's research funds
Primary sponsor	Helsinki University Hospital
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Public title	Remote Ischaemic Preconditioning in Transplantation (RIPTRANS)
Scientific title	A randomised sham-controlled double-blind trial evaluating remote ischemic preconditioning in solid organ transplantation (RIPTRANS)
Countries of recruitment	Finland
Health condition(s) or problem(s) studied	Solid organ donation and transplantation
Intervention(s)	Remote ischemic preconditioning of brain dead donors vs. sham procedure
Key inclusion and exclusion criteria	Ages eligible for study: over 18 years Sexes eligible for study: all Accepts healthy volunteers: no Inclusion: includes brain dead kidney and multi-organ donors and their transplant recipients

	Exclusion for adult donors: not a kidney donor, severe hemodynamic instability, other conflicting clinical trial
Study type	Interventional Allocation: Randomized, parallel assignment, masking double-blind (participant, care provider, investigator, outcomes assessor) Primary purpose: organ preservation
Date of first enrolment	March 13th, 2019
Target sample size	500 kidney transplant recipients
Recruitment status	Recruiting
Primary outcome(s)	Delayed graft function in kidney allografts
Key secondary outcomes	Short-term functional outcomes of transplanted organs, rejections, and graft survival in various time points up to 20 years

Abstract

Introduction

Remote ischemic preconditioning (RIPC) using a non-invasive pneumatic tourniquet is a potential method for reducing ischemia-reperfusion injury. RIPC has been extensively studied in animal models and cardiac surgery, but scarcely in solid organ transplantation. RIPC could be an inexpensive and simple method to improve function of transplanted organs. Accordingly, we aim to study whether RIPC performed in brain-dead organ donors improves function and longevity of transplanted organs.

Methods and analyses

RIPTRANS is a multi-center, sham-controlled, parallel group, randomised superiority trial comparing RIPC intervention versus sham-intervention in brain-dead organ donors scheduled to donate at least one kidney. Recipients of the organs (kidney, liver, pancreas, heart, lungs) from a randomised donor will be included provided that they give written informed consent. The RIPC intervention is performed by inflating a thigh tourniquet to 300 mmHg 4 times for 5 minutes. The intervention is done twice: firstly right after the declaration of brain death and secondly immediately before transferring the donor to the operating theatre. The sham group receives the tourniquet, but it is not inflated. The primary endpoint is delayed graft function (DGF) in kidney allografts. Secondary endpoints include short-term functional outcomes of transplanted organs, rejections, and graft survival in various time points up to 20 years. We aim to show that RIPC reduces the incidence of DGF from 25 % to 15%. According to this, the sample size is set to 500 kidney transplant recipients.

Ethics and dissemination

This study has been approved by Helsinki University Hospital Ethics Committee and Helsinki University Hospital's Institutional Review Board. The study protocol was be presented at the European Society of Organ Transplantation congress in Copenhagen 14-15th September 2019. The study results will be submitted to an international peer-reviewed scientific journal for publication.

Trial registration number

NCT03855722 (ClinicalTrials.gov)

Strengths and limitations of this study

- The study method, a multi-center, double-blinded, sham-controlled, randomised superiority trial, is the best available method to investigate the effects of remote ischemic preconditioning (RIPC) performed in the donor on the function and longevity of transplanted organs in the recipient

-Remote ischemic preconditioning is an extremely simple, reproducible, and inexpensive method

-The sample size, 500 kidney transplant recipients, is large enough to provide confidence in the estimates of outcomes.

-Primary outcome, delayed graft function of kidney allograft, is clinically highly relevant, easy to measure, and objective.

-As the sample size is calculated for kidney transplantation, outcomes of other organ recipients might be underpowered.

Introduction

Solid organ transplantation is an established standard of care for end-stage dysfunction of different organs, but the availability of the treatment is greatly limited globally by the shortage of organ donors. On the other hand, the lifetime of a transplanted organ is often limited and there is a number of patients waiting for a second or subsequent transplant[1-6]. A transplanted organ is exposed to ischemia-reperfusion injury during the transplantation process[7]. Alleviating this injury could improve the function and lifetime of transplanted organs.

Remote ischemic preconditioning (RIPC) is an old concept where remotely produced ischemia induces protective changes in distant organs or tissues and renders them less susceptible for future ischemia via hormonal, metabolic, and neuronal mechanisms[8]. As an intervention, RIPC is easy and cheap to perform – an inflatable tourniquet is used to occlude upper or lower limb. RIPC has been extensively studied in animal models[9-11], and in human clinical trials of cardiac surgery. The largest of these clinical trials - RIPHeart[12] ERICCA[13], and CONDI-2/ERIC-PPCI[14] - have not been successful to show benefit from RIPC, but this might be due to the fact that the patients suffering from chronic myocardial ischemia already have maximal compensatory mechanisms in use. This could also partially explain the results of RenalRIP trial, in which RIPC reduced acute kidney injury associated with cardiac surgery without affecting cardiac parameters [15].

One of the postulated reasons for negative results in RIPHeart and ERICCA trials is the use of propofol instead of the volatile anesthetics, even though this has not been fully verified[16]. In the DBD transplantation setting, when the RIPC intervention is done to a brain dead donor, propofol is not used and should not prevent the effect of RIPC. Propofol may be used in the recipient surgery, but there is at least preliminary small animal data, that this may not prevent effectiveness of RIPC[17].

Organ transplantation is a lucrative field to study RIPC, as the donor organs are healthy, and do not suffer from chronic ischemia, but face invariable acute ischemia of various durations. RIPC has been studied little in clinical transplantation and results have been controversial. A RIPC intervention done to heart transplant recipients together with post conditioning 20 minutes after aortic declamping reduced cTnI levels at 6 hours after transplantation[18]. In a recent trial RIPC done to living liver donors reduced postoperative aspartate aminotransferase (AST) levels in liver recipients[19], whereas a pilot study of the RIPCOLT trial with RIPC done on liver transplant recipients demonstrated no short term benefits [20].

Direct ischemic preconditioning with clamping of liver hilum in donation after brain death (DBD) was not was not beneficial and could even induce excessive ischemic damage[21]. A retrospective *post hoc* analysis of 2 such trials showed that liver ischemic conditioning had no RIPC effect for kidneys[22]. The liver-RIPC may provide an insufficient stimulus for the kidneys and the authors speculated that limb ischemia could be a better method for RIPC. In a study of 29 kidney transplant patients RIPC done on DBD donors resulted in lower creatinine levels at 15 and 30 days after transplantation, but the change in GFR did not reach statistical significance[23]. As far as we know, no larger randomized controlled trial with limb-RIPC on DBD donors have been published.

A small study with 20 living donor kidney recipients per group found no difference in kidney function whether RIPC was done on donor or recipient[24]. A larger trial of 170 living kidney donor – recipient pairs with RIPC done on donors reported lower postoperative creatinine values on donors after RIPC but no long term benefits for donors or recipients[25].

The largest kidney transplant RIPC trial to date, the REPAIR trial, showed that a RIPC performed in both donor and recipient immediately before a living-donor kidney surgery improved the estimated glomerular filtration for the whole follow-up period of 5 years[26 27]. The kidney allografts from living donors are subjected to very short ischemia (in Finland this is typically less than two hours) and even greater benefits could be obtained if RIPC is performed in deceased donors, where ischemia times are much longer (median 15 hours for kidney allografts in Finland, even longer in other countries). RIPC intervention performed to the recipients of deceased donor kidneys during the transplantation surgery did not improve kidney function in CONTEXT trial[28]. This study can be criticized for performing RIPC in the recipients instead of donors, because the ischemic injury has already taken place before RIPC.

The aim of this study is to show that RIPC performed in brain-dead donors (DBD) can be used to improve function and longevity of transplanted organs.

Methods and analysis

Study design

The RIPTRANS trial is a multi-center, double-blinded, parallel group, individual donor randomised superiority trial comparing RIPC with a sham-procedure performed in brain-dead donors. There is only one transplantation centre (Helsinki University Hospital) in Finland that covers the whole country and procurement team travels to all donor hospitals in Finland. This protocol was drafted in accordance with the SPIRIT (Standard Protocol Items: recommendations for Interventional Trials) statement[29]. This trial is registered in ClinicalTrials.gov (NCT03855722), the first registration date was February 27th, 2019.

Participants

RIPC or sham procedure will be performed on a brain-dead donor fulfilling inclusion and exclusion criteria. All brain-dead donors in participating hospitals scheduled for at least one kidney procurement will be included. Donors with significant hemodynamic instability (assessed by the intensive care physician responsible for the treatment of the donor) and under the age of 18 years will be excluded. Donors (or potential recipients of organ from this donor), who are participating in a trial with conflicting interventions or outcomes, will also be excluded. Although the donors are randomised and the intervention is carried out in donors, the recipients are the actual participants of this trial. All patients receiving a kidney, liver, combined pancreas-kidney, heart, or lungs from a donor randomised in the trial will be included in the trial provided that they give a written informed consent (Supplement 2) to participate in the trial and are at least 18 years old. The informed consent will be presented to the patient by a study nurse or physician. As based on the previous studies, RIPC is supposedly not harmful for the donor, and the lack of consent from any of the transplant recipients does not exclude the donor from the study, nor the possible inclusion of the other

recipients. There are no other exclusion criteria for recipients who receive abovementioned organs from a randomised donor.

Randomisation and masking

Eligible donors will be randomly allocated in a 1:1 ratio to either RIPC or sham-procedure group. The randomisation sequence was generated using a web-based commercial service (Sealed Envelope) with randomly variable block size (4, 6, or 8) and stratified according to donor age (under / over 60 years of age), planned organ to be procured (kidneys only / abdominal organs only / both thoracic and abdominal organs), and donor cardiopulmonary resuscitation (yes / no). The randomisation and allocation to either RIPC or sham-intervention is done by a transplant coordinator, who is not blinded to the allocated treatment, using the same web-based service. Once the donor is allocated, the transplant coordinator sends electronically or via fax written instructions on how to perform the allocated treatment to the intensive care team responsible for the treatment of the donor, who also are not blinded to the treatment. This intensive care team will collect data regarding the actual timing of the allocated procedure and whether this caused any noticeable changes in the donor hemodynamics. All researchers and all other treating personnel are blinded, such as procuring surgeons, transplant surgeons, treating physicians, data collectors, and data analysts as well as recipients. After the trial recruitment has been closed and data collected, the allocated group will be named as A and B before the data is analysed. Once the data analyses for primary and secondary outcomes are completed, the full blinding will be removed. No emergency unblinding is planned, but incidents of possible breaches in blinding will be recorded.

Procedures

RIPC will be performed as follows: Donor's thigh will be occluded 4 times for 5 minutes using tourniquet inflated to 300 mmHg each followed by 5 minutes of deflation. The intervention will be performed twice (once in both thighs). Once as soon as possible after brain death is determined, and once right before transferring the donor to the operation room for procurement. Sham-intervention will be performed by putting the inflatable tourniquet in place similarly, but not inflating it. Apart from the RIPC or sham-intervention, the treatment of donors will be according to normal routine. Study blood samples will be acquired from donors before (selected centers) and after the intervention.

Outcomes

The primary outcome measure is delayed graft function (DGF) of kidney allografts, which is defined as the need for dialysis within the first week after transplantation. Secondary outcome measures are different for different organs (Table 1). Outcomes are assessed during the primary hospital stay, and thereafter at the routine follow-up visits. Helsinki University Hospital has a legal requirement to maintain a registry of all patients receiving a transplant in Finland, and data regarding visits in other hospitals are submitted to Helsinki University Hospital for registry purposes. Secondary outcomes are assessed directly from the registry, from the data provided by other hospitals, or at routine follow-up visits at Helsinki University Hospital. Survival status is automatically updated to the registry from the National Population Centre, which is an exact, complete, and up-to-date source for causes of death in Finland. Prespecified subgroup analyses are planned for characteristics that may potentially affect the results (Table 2). Further exploratory outcome measures will be done

according to Supplement 3. In the informed consent, the patients are also asked to give their permission for using the excess study blood, urine and tissue samples in possible ancillary analysis.

Table 1. Secondary outcome measures

Kidney allografts
Estimated glomerular filtration rate (eGFR) at 3 months, 1 year, 2 years, 5 years, 10 years, and 20 years.
Biopsy-proven acute rejection (BPAR) within 1 year.
Graft survival at 3 months, 1 year, 2 years, 5 years, 10 years, and 20 years: time from transplantation to death, retransplantation or permanent dialysis.
Death-censored graft survival at 3 months, 1 year, 2 years, 5 years, 10 years, and 20 years: time from transplantation to retransplantation or permanent dialysis, death-censored
Pancreatic allografts
Glycosylated haemoglobin (HbA1c) at 3 months, 1 year, 2 years, 5 years, 10 years and 20 years
Acute rejection in pancreatic allograft, either biopsy-proven (allograft pancreas or duodenal biopsy) acute rejection or clinically treated suspected acute rejection within 1 year
Pancreatic allograft survival at 3 months, 1 year, 2 years, 5 years, 10 years and 20 years: Time from transplantation to death, retransplantation, explantation or daily insulin dependence
Death-censored pancreatic allograft survival at 3 months, 1 year, 2 years, 5 years, 10 years and 20 years: Time from transplantation to death, retransplantation, explantation or daily insulin dependence, death-censored
Liver allografts
MEAF-score at 3rd post-operative day (POD): Model for Early Allograft Function Scoring. MEAF = score ALTmax:3POD + score INRmax:3POD + score bilirubin3POD, score range 0 - 10, higher score indicates worse outcome[30]
Postoperative biliary complications within 1 year: Amount and type of postoperative biliary complications: stricture at anastomosis, bile leak or ischemic type biliary lesions (ITBL) requiring intervention (ERC, PTC, operation) or prolonged drainage within 1 year
Post-transplantation kidney injury (acute kidney injury) within 1 week, at 3 months, 1 year: according to ADQI 2010 criteria[31].
Biopsy proven acute rejection (BPAR) within 1 year.

Graft survival at 1 year, 2 years, 5 years, 10 years, and 20 years: time from transplantation to death, retransplantation or explantation
Heart allografts
Ischemia-reperfusion injury determined by peripheral blood TnI levels at 6 hours after transplantation
Peripheral blood proBNP measurement at 1 week after transplantation
Primary graft dysfunction according to ISHLT definition[32] within 24 hours after transplantation
Biopsy-proven or clinically treated acute rejection within one year after transplantation
Vasculopathy-free survival according to ISHLT definition[33] at 1 year, 2 years, 5 years, 10 years, and 20 years
Graft survival at 1 year, 2 years, 5 years, 10 years, and 20 years, time from transplantation to death, retransplantation or explantation
Lung allografts
Primary graft dysfunction according to ISHLT definition[34] within 72 hours after transplantation
Biopsy proven or clinically treated acute rejection within one year
Chronic Lung Allograft Dysfunction (CLAD) free survival according to ISHLT/ATS/ERS 2014 guideline [35] at 1 year, 2 years, 5 years, 10 years, and 20 years, time from transplantation to death or retransplantation
Graft survival at 1, 2, 5, 10 and 20 years : time from transplantation to death, retransplantation or explantation

Table 2. Subgroup analyses

Subgroup analyses	**Subgroups**
Donor cardiopulmonary resuscitation	yes / no
Donor age (years)	Under 60 /over 60
Donor sex	male / female
Organ cold ischemia time (hours, organ specific)	below / above median
Uncompleted study intervention	yes / no
Liver transplantation for acute liver failure	yes / no

Statistical analyses

The incidence of DGF in kidney allografts after transplantation from a brain-dead donor in Finland is approximately 25 % (Finnish Transplantation Registry). We aim to show that RIPC reduces the incidence of DGF to 15%. With a 5 % significance level and 80 % power, 496 kidney transplantations are required to show this difference. Sample size is not adjusted for cross-over or loss-of-follow up because the risk of these are considered to be minimal. Usually two kidneys per donor are transplanted. Because a portion of procured kidneys will be transferred to another Nordic country according to ScandiaTransplant rules, are untransplantable, transplanted in a combined organ transplantation, or transplanted to a recipient below 18 years old, we assume 90% of donors will lead to two kidney transplantations and 10% will lead to one kidney transplantation within the study. We set the final sample size to 500 kidney transplantations, for which approximately 260 donors are required to be randomised.

The primary outcome measure and the secondary outcomes for kidney transplantation will be analysed using generalized linear mixed models taking into account that kidneys from a single donor will usually be transplanted to two recipients included in the study. Survival analysis for kidney allografts and transplant recipients are done using Kaplan Meier survival diagrams and the effect size is estimated using Cox proportional hazards regression model similarly taking into account single donor providing kidneys to two recipients.

The categorical outcome variables for liver, pancreas, heart, and lungs are analysed with Chi square test (or Fischer's exact test, if *n* is under 5 in any of the subcategories). The continuous outcome variables for these organs are analysed using independent T-test or Mann Whitney U-test depending on whether the outcome has normal distribution or not. The effect size for categorical variables is calculated with odds ratio and 95 % confidence interval (CI). For continuous variables the effect size is calculated with difference in means with 95 % CI for variables with normal distribution. If a continuous variable can be converted for normal distribution with a logarithmic transformation, will the effect size be reported using the ratio of geometrical means with 95 % CI. Other continuous variables will be calculated using Mann Whitney U-test and the effect size will be reported using $r = Z/\sqrt{N}$ without 95 % CI. Survival analysis for these organs will be described using Kaplan-Meier survival diagrams and log-rank-test and effect size estimated using Cox proportional hazards regression model.

Subgroup analysis will be made using generalized linear mixed models as univariate analysis by adjusting models by subgroup. A multivariate analysis of subgroups can be done with aforementioned generalized linear model and by selecting the significant subgroups ($p < 0.05$) from univariate analysis as covariates. Subgroup analysis for survival variables will be described with Kaplan-Meier, which will be stratified by subgroup and effect size will be estimated using Cox proportional hazards regression model by adjusting it with the subgroup.

In case that because of missing values more than 5 % of patients would be left out from sensitivity analyses, multiple imputations may be used to conduct sensitivity analyses. Otherwise, the missing data will not be adjusted separately, but these cases will either be left out from the analyses or censored at the last point of follow up.

Data security
All patient data included in the study is confidential and will be concealed on a computer behind an AES 256-bit encryption. Any data stored in a paper from will be held in the study hospitals in locked offices. Only the study personnel will have the access to the trial dataset.

Data availability statement
After the completion of the study the depersonalized data can be requested from the authors.

Schedule and interim safety analyses
The study was conceptualised in June 2017. The study plan was approved by the Helsinki University Hospital's Ethics Committee 9th May 2018. Helsinki University Hospital's Institutional Review Board gave permission to conduct the study 14th August 2018. The study started recruiting in May 13th 2019 in four out of five university hospitals in Finland. The Ethics Committee required a safety analysis after 16 donors had been randomised. The donors and recipients of kidneys from these 16 donors were analysed without unmasking the allocated group. All 16 donors randomised successfully underwent procurement. No adverse events were noted in the recipients. After this safety analysis, the study will be disseminated to non-university donor hospitals. Second interim analysis will be done when half of the target sample size is reached (250 kidney transplantations). In Finland, approximately 230 DBD kidney transplants are being performed annually. We estimate that data for primary outcome would be available in 4 years.

Role of the funding sources and sponsors
The funders or sponsors have had and will have no role in study design, data collection, data analysis, data interpretation, or writing the report, or any other aspect of the work, except for funding.

Patient and Public involvement
Patient organizations were not involved in the study design.

Ethics and dissemination

Study ethics
This study has been approved by Helsinki University Hospital Ethics Committee and Helsinki University Hospital's Institutional Review Board.

The intervention is performed on a donor, who has been determined brain dead and has given permission to act as a donor according to Finnish legislation, and is determined suitable and scheduled for kidney procurement. The Ethics Committee has approved that donors (or next of kin) do not need to consent to RIPC or sham-procedure because it is a non-invasive procedure, the donor is brain dead and scheduled for procurement already.

The recipients of organs from randomised donors will be recruited in the study and will be required to give written informed consent to participate. The recipient cannot influence whether the donor has been randomised or received the allocated treatment. The recipient

has the right to decline participation in the trial, but can still choose to receive the planned allograft. In these cases, the recipient's data is not used in the study analyses. The recipient has also right to decline the offered organ. The recipient does not have the right to know the allocated treatment the donor has received before the study has been completed, data analysed, and blinding unmasked. The donors or recipients do not receive any compensation for their participation in the trial. The recipients have the right to discontinue the trial or withdraw their consent at any point. In these cases, the collected data will be used in the analyses up to the point of discontinuation.

A few additional blood samples (and a urine sample from the kidney recipients) will be taken from the kidney, heart, and lung recipients for the study purposes during and shortly after the transplantation, but otherwise the recipients only give their consent to the study group to observe and collect medical information. These samples are stored maximally for five years after the completion of the study recruitment. The patient informed consent forms are in Finnish and Swedish and will be provided by request made to the study group.

Harms

Earlier studies on RIPC have not indicated any harm (7-16). On the contrary, many earlier studies suggest that RIPC may be beneficial for the function and longevity of the allografts. Before wider adoption of the RIPC in transplantation, its safety and benefits need to be addressed in a randomised controlled trial such as RIPTRANS. Any possible harmful effects of the intervention will be reported together with the study results. The Finnish patient insurance covers the organ recipients participating in the study.

Monitoring

Helsinki University Hospital Ethics Board monitored the results of the first interim analysis. Initially the Ethics board did not necessitate a separate Data Monitoring Committee (DMC). To provide external validity for the study, a DMC contract was made with Clinical Research Institute HUCH Ltd (HYKS Instituutti) in March 2020. The site monitoring will be performed every three months including review of the Investigator's Trial File, facilities, the equipment at the site, compliance to study protocol and study specific procedures, source document quality and the intervention implementation documentation for all donors. All the study patients will be monitored for: existence, informed consent process and documentation of the Trial outcome measures. A complete review will be conducted for 10 % of the subjects. A close-out visit shall be done after all the data has been collected and the treatment of all the subjects has been completed. This Monitoring plan and Agreement is made in collaboration with the guideline for coordinated GCP-monitoring of clinical trials in the Nordic countries (version 5/24.10.2017).

Dissemination

The study protocol was be presented at the European Society of Organ Transplantation congress in Copenhagen 14-15th September 2019 and will possibly be presented in other scientific conferences. The study results will be submitted to an international peer-reviewed scientific journal for publication and possibly discussed at scientific meetings. The study is also being made public via social media platforms (Twitter). The International Committee of Medical Journal Editors recommendations (www.ICMJE.org) are applied when considering the authorship of any publications from this trial.

Discussion

We formed our study protocol based on earlier RIPC studies. The RIPC intervention on transplant recipients has already been thoroughly studied by Krogstrup et al[28] and we saw more potential in performing the intervention prior to organ procurement, in deceased donors. Our RIPC tourniquet protocol is similar to several earlier trials[12-14 28]. Our intervention is performed on thigh (instead of upper extremity), which has a larger mass and could thus produce larger effect, and donor arms are usually used for cannulas which could cause interference with RIPC. A constant tourniquet pressure of 300 mmHg was selected because it ensures that the circulation to the lower extremity is ceased and ischemia introduced. Also, a static pressure protocol instead of changing the pressure according to systolic blood pressure is simpler and easier to reproduce.

The aim of our study is to be able to show a decrease in DGF rate from 25% to 15%. The largest kidney transplant RIPC trial thus far, the REPAIR trial, recruited 406 kidney recipients and the reported a positive long term outcome for kidney function, but not in DGF[26 27]. A DGF difference in living donor setting is hard to show because of very low incidence of DGF compared to deceased donation. A DGF reduction of ten percentage points was chosen because there were several reasons assume that the effect of RIPC would be higher in our study. First, the intervention is performed on thigh with larger mass compared to arm as noted above. Second, intervention is performed on DBD donors, in which both warm and cold ischemia times are longer than in living donors, where the earlier trials have mostly been conducted. Further, we think that the effect of RIPC could be larger because of the systemic inflammatory cascade in DBD donors, which RIPC might be able to alleviate. All in all, these are hypotheses, which are now being tested in a novel double-blind RCT. An interim analysis will be made when half of the sample size is recruited (250 kidney transplant recipients) and at that point, we will acquire a more precise estimate of the effect.

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Author's contributions
Concept: VS, ML
Initial draft of protocol: VS, AU, IH, KL
Critical revision of the protocol: VS, AU, IH, KL, AP, SS, FÅ, HM, AN, ML
Implementation: VS, AU, KL, SS, AP
Data collection: AU, SS
Donor recruitment: MB, MS, TL, JR, MaL, JL, JG

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Sponsorship statement
The study sponsor is Helsinki University Hospital.

Conflicts of interests statement
The authors have no competing interests that would affect this study.

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Supplement 1. Protocol amendments

Study Protocol version 1.11 (Finnish version), 2019-Jan-16 in use at time when study recruiting started March 13th 2019

Version 1.13 (Finnish version), 2019-Aug-11
Reported the results of the safety analysis the to University of Helsinki Ethics Committee
Changed the practise of taking the 6 h postoperative urinary sample from kidney recipients.
Original: The sample is taken from the urine bag
Updated: The sample is taken from the catheter hose

Version 1.14 (English version), 2020-Mar-6
Donor pre-intervention blood samples are taken in selected centers, not necessarily only in Helsinki.

BNP as an outcome measure changed to proBNP according to a change in Helsinki University Hospital laboratory HUSlab protocol. Blood samples from heart transplant recipients transplanted before this are reanalysed for proBNP as possible.

Added a new secondary outcome measure for the lung recipients:
Lung allograft: graft survival: time from transplantation to death, retransplantation or explantation. The recruitment Lung recipients started later than for other organs because of a conflicting trial, which now has completed recruiting. Only four lung patients have thus far been included in the study and no results for lung recipients have been analysed.

Changed the manner of dealing with the possible missing data in analyses:
Original: Missing data will not be adjusted separately, but these cases will either be left out from the analyses or censored at the last point of follow up.
Updated: In case that because of missing values more than 5 % of patients would be left out from sensitivity analyses, multiple imputations may be used to conduct sensitivity analyses. Otherwise, the missing data will not be adjusted separately, but these cases will either be left out from the analyses or censored at the last point of follow up.

External Study Monitoring Committee was initiated by Clinical Research Institute HUCH Ltd in March 2020.

Version 1.14c (Finnish version), 2020-May-15
Clarified the Finnish version to equal the English one. The informed consent of the transplant recipient can either be asked before (preferable) or after the transplantation.

Version 1.14d (Finnish version), 2020-September-24
Clarified the exact data that will be collected form the patients' medical reports.

Supplement 2. Model Patient Consent Form for Kidney Transplant Recipients
Unofficial English translation

Name of the project: **Remote Ischemic Preconditioning in Transplantation**

Dear patient,

You have arrived in Helsinki University Hospital Clinic of Transplantation and Liver Surgery to receive a kidney transplant. We ask you to participate in a study where evaluate the effects of remote ischemic preconditioning in solid organ transplantation.

When a kidney transplant has been procured, the organ is suspect to lack of oxygen until it has been transplanted to the recipient. This ischemia affects the function of the transplant, e.g. the beginning of urine production, and can predispose the transplant for rejection. The damage caused by ischemia could possibly be alleviated with remote ischemic preconditioning. This means that a donor tissue other than the one to be transplanted (here lower extremity) is exposed to lack of oxygen before the organ procurement. Thus, the transplanted kidneys will not directly suffer from the lack of oxygen. A lower extremity ischemia will cause hormonal and neural changes in whole body, including the kidneys, which aim to protect the body form ischemic damage.

This study is randomized, which means that half of the donors will receive the study intervention and the other half will not. The study is blinded, which means that neither you nor the physicians treating you will know, whether your kidney transplant has received the study intervention or not. The transplantation operation and all postoperative treatment will be carried out in the same way as for the patients not participating in the study.

We ask for your permission to take three blood samples during the kidney transplantation (during the general anesthesia) and one urine sample from the urinary catheter after the operation. These samples will be studied for different markers of kidney injury and the results are compared to routinely taken tests for kidney function, need of dialysis, possible transplant rejection and long-time transplant function.

Possible excess samples can be used to study effects of remote ischemic preconditioning, also other than originally mentioned in the study plan. The samples are stored in the study group freeze maximally for five years, after which they will be disposed.

To verify the quality of the study, the collected data will be compared to the original patient records. This will be done by a study monitor under the surveillance and responsibility of study physician or other study personnel. Otherwise your personality and other identifiable information is only known for the study physicians and they all have an obligation of confidentiality. The study registry contains only information needed for the study. The study subjects will be followed for 20 years.

This study and processing of personal information within it are based in EU General Data Protection Regulation (GDPR 2016/679), Article 6 section 1 a), b) and e) and Article 9 section 3 a), g), i) and j), and following Finnish Laws: Law for Medical Research (1999/488), Law for

Health Care (1326/2010), Law for Patients Standing and Rights (785/1992), Law for Health Care Professionals (559/1994), Law for Publicity of the Actions of Authorities (621/1999), Law for Personal Data Protection (2019) and Law for Archives (831/1994). In addition to this will GDPR regulation be regarded as primary legislation over the national laws.

After the completion of this study the study registry will be stored according to Good Clinical Practice Guidelines and disposed thereafter. No automated decision making will be used in the study registry other than the study randomization, which is part of the study scientific methodology.

The study registry holder is Joint Hospital District of Helsinki and Uusimaa (HUS).
Address: Joint Hospital District of Helsinki and Uusimaa, Stenbäckinkatu 9
PL 100, 00029 HUS, Finland

Contact information:
Telephone +358 (0)9 4711
Registry telefax +358 (0)9 471 75500
Registry e-mail keskuskirjaamo@hus.fi
Registry mail address HUS keskuskirjaamo PL 200, 00029 HUS, Finland

If you wish to execute your rights according to GDPR, we recommend the use of spesified hospital forms, which can be found in our internet pages:
http://www.hus.fi/potilaalle/potilaan_oikeudet/terveystieteellinen%20tutkimus/Sivut/default.aspx

You also have a right to make a data protection claim to national responsible data protection authority (tietosuojavaltuutettu).

Address:
Tietosuojavaltuutetun toimisto
Visiting address: Ratapihantie 9, 6. krs, 00520 Helsinki, Finland
Mail address: PL 800, 00521 Helsinki, Finland
Telephone: +358 (0)29 566 6700
e-mail (registry): tietosuoja@om.fi

We ask for your written permission to participate in the study. You can, whenever you choose, during the study interrupt your participation or cancel your permission without any consequences. The data and samples collected before interruption or cancelling will be used as a part of the study to ensure the effects, efficacy and safety of remote ischemic preconditioning.

Should you have any further questions, we will be happy to answer.

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Consent Form for a Medical Study

Name of the project: **Remote Ischemic Preconditioning in Transplantation**

I have been asked to participate in aforementioned study conducted by physicians in Helsinki University Hospital Clinic of Transplantation and Liver Surgery.
I have received, read and understood the Study Consent Form, in which I have received sufficient information regarding the study and data gathering, processing and disclosure within the study. I have been informed about the contents of the Consent Form orally and I have received sufficient answers to all of my questions regarding the study.
I have had enough time to consider my participation in the study. I give my permission to gather information essential for this study by the researchers of the Clinic of Transplantation and Liver Surgery in the study registry "**Etäiskeeminen esikäsittely elinsiirteiden toiminnan parantajana**". To gather the information the physician may document my personal national health insurance registration number and use it to obtain data. All the information gathered during the study is confidential.
I understand that my participation in the study is totally voluntary. I am aware that I can whenever I choose, during the study interrupt my participation or cancel my permission without any consequences. Interruption or cancelling will not affect my care. I am aware that the data and samples collected before interruption or cancelling will be used as a part of the study to ensure the effects, efficacy and safety of remote ischemic preconditioning.
I am aware that my personal information can also be processed by a national or international regular study monitoring authority outside the study group to ensure the quality of the study.
With my signature I confirm my participation in the study and I give my permission to become a study subject.

_____ _____
Patient signature Date

_____ _____
Patient name Patient date of birth

_____
Patient address

Permission received

_____ _____
Physician signature Date

_____
Physician name

The original signed Consent Form will be stored by the study physician. A copy will be given to the study subject.

The official Finnish version

TUTKIMUSTIEDOTE POTILAALLE

Tutkimuksen nimi: **Etäiskeeminen esikäsittely elinsiirteiden toiminnan parantajana**

Hyvä potilas,

Olette tulossa munuaissiirtoon HYKS Vatsakeskuksen elinsiirto- ja maksakirurgian klinikkaan. Pyydämme Teitä osallistumaan tutkimukseen, jossa selvitetään etäiskeemisen esikäsittelyn vaikutusta munuaissiirteen toimintaan.

Kun munuaissiirre on irrotettu elinluovuttajalta, siirre altistuu hapenpuutteelle (iskemialle) kunnes se liitetään vastaanottajan verenkiertoon munuaissiirtoleikkauksessa. Tämä hapenpuute vaikut-taa munuaissiirteen toimintaan, esim. virtsaneritykseen käynnistymiseen, ja voi altistaa hyljinnälle. Hapenpuutteen aiheuttamia vaurioita voidaan pyrkiä ennaltaehkäisemään ns. etäiskeemisellä esikäsittelyllä. Etäiskeemisellä esikäsittelyllä tarkoitetaan sitä, että elinluovuttajan jokin muu kudos kuin irrotettavat elimet (tässä tutkimuksessa alaraaja) altistetaan hapenpuutteelle ennen elinirroitusleikkausta. Elinluovuttajan munuaisia ei siis altisteta hapenpuutteelle. Alaraajan hapenpuute aiheuttaa koko elimistössä, myös munuaisissa, hormonaalisia ja hermostollisia muutoksia, joilla elimistö pyrkii suojautumaan hapenpuutteen aiheuttamilta vaurioilta.

Tämä tutkimus on satunnaistettu, eli puolet elinluovuttajista saa etäiskeemisen esikäsittelyn ja puolet ei. Tutkimus on sokkoutettu, tarkoittaen sitä, että Te tai hoitavat lääkärit eivät tiedä onko elinluovuttaja, jolta munuaissiirteenne tulee, saanut etäiskeemisen esikäsittelyn vai ei. Elinsiirto ja hoito sen jälkeen toteutetaan täysin samalla tavalla kuin potilaiden, jotka eivät osallistu tutkimukseen.

Pyydämme Teiltä lupaa ottaa tutkimukseen liittyen kolme verinäytettä munuaissiirtoleikkauksen (nukutuksen) aikana ja yksi virtsanäyte virtsakatetrista leikkauksen jälkeen. Näytteistä tutkitaan erilaisia munuaisvaurion merkkiaineita. Tuloksia verrataan Teistä rutiininomaisesti leikkauksen jälkeen otettuihin munuaisten toimintakokeisiin, dialyysitarpeeseen, mahdolliseen siirteen hyljintään ja siirteen pitkäaikaiseen toimintaan.

Mikäli veri- tai virtsanäytteitä jää tutkimuksesta yli, niistä voidaan etäiskeemisen esikäsittelyn vaikutusten selvittämiseksi määrittää myöhemmin alkuperäisessä tutkimussuunnitelmassa mainittujen lisäksi muitakin analyysejä. Näytteitä säilytetään tutkimusryhmän pakastimessa korkeintaan 5 vuotta, jonka jälkeen ne tuhotaan.

Tutkimustiedon oikeellisuuden varmistamiseksi tutkimustietoja verrataan muun muassa alkuperäisiin sairauskertomuksiin. Tällöin tietoja käsitellään ns. monitoroijan toimesta tutkijalääkärin tai muun tutkimushenkilöstön valvonnassa ja vastuulla. Tämän lisäksi tutkimuksessa henkilöllisyytenne sekä muut tunnistettavat tiedot ovat ainoastaan tutkijalääkäreiden tiedossa, ja he kaikki ovat salassapitovelvollisia. Tutkimusrekisteriin talletetaan vain tutkimuksen kannalta välttämättömiä tietoja. Tutkittavia seurataan 20 vuotta.

Tämä tutkimus ja siihen kuuluva henkilötietojen käsittely perustuvat seuraaviin lainsäädäntöihin: EU tietosuoja-asetus (2016/679), 6. artikla 1 a), b), c) ja e) ja 9. artikla 3 a), g), i) ja j) kohdat, laki lääketieteellisestä tutkimuksesta (1999/488), terveydenhuoltolaki (1326/2010), laki potilaan asemasta ja oikeuksista (785/1992), laki terveydenhuollon ammattihenkilöistä (559/1994), laki viranomaisten toiminnan julkisuudesta (621/1999), tietosuojalaki (2019) ja arkistolaki (831/1994). Lisäksi huomioidaan EU:n tietosuoja-asetuksen yli kansallisen lainsäädännön menevät määräykset.

Tutkimuksen loputtua tutkimusrekisteri säilytetään hyvän kliinisen tutkimustavan vaatimusten mukaisesti ja hävitetään sen jälkeen. Tutkimusrekisterissä ei käytetä automaattista päätöksentekoa. Tämä ei koske tutkimukseen liittyvää ryhmien randomointia, joka on tieteelliseen tutkimukseen kuuluva metodi.

Tutkimuksen rekisterinpitäjänä toimii Helsingin ja Uudenmaan sairaanhoitopiirin kuntayhtymä.

Osoite:
Helsingin ja Uudenmaan sairaanhoitopiirin kuntayhtymä, Stenbäckinkatu 9
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Voitte toteuttaa tietosuoja-asetuksen mukaisia oikeuksianne vapaamuotoisilla ilmoituksilla, mutta suosittelemme käyttämään näitä tarkoituksia varten laadittuja HUSin lomakkeita. Lomakkeet löydätte HUSin internet-sivuilta:

http://www.hus.fi/potilaalle/potilaan_oikeudet/terveystieteellinen%20tutkimus/Sivut/default.aspx

Teillä on myös oikeus tehdä tietosuoja-asioissa valitus Suomessa tietosuojasta vastaavalle viranomaiselle eli tietosuojavaltuutetulle.

Tietosuojavaltuutetun toimisto
Käyntiosoite: Ratapihantie 9, 6. krs, 00520 Helsinki
Postiosoite: PL 800, 00521 Helsinki
Puhelinvaihde: 029 566 6700
Sähköposti (kirjaamo): tietosuoja@om.fi

Pyydämme Teiltä kirjallista suostumusta tutkimukseen osallistumisesta. Voitte syytä ilmoittamatta keskeyttää tutkimukseen osallistumisen tai peruuttaa suostumuksenne missä tahansa tutkimuk-sen vaiheessa ennen sen päättymistä ilman, että siitä koituu Teille mitään haittaa. Keskeyttämi-seen tai peruuttamiseen saakka kerättyjä tietoja ja näytteitä käytetään

osana tutkimusaineistoa etäiskeemisen esialtistuksen vaikutusten, tehon ja turvallisuuden varmistamiseksi.

Mikäli Teillä on kysyttävää tai haluatte lisätietoja, vastaamme mielellämme.

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SUOSTUMUS LÄÄKETIETEELLISEEN TUTKIMUKSEEN

Tutkimuksen nimi: **Etäiskeeminen esikäsittely elinsiirteiden toiminnan parantajana**

Minua on pyydetty osallistumaan edellä mainittuun HYKS Vatsakeskuksen elinsiirto- ja maksakirurgian klinikan ja sen lääkäreiden suorittamaan tutkimukseen.

Olen saanut, lukenut ja ymmärtänyt tutkimuksesta kertovan tiedotteen (päivätty 3.3.2020). Tiedotteesta olen saanut riittävän selvityksen tutkimuksesta ja sen yhteydessä suoritettavasta tietojen keräämisestä, käsittelystä ja luovuttamisesta. Tiedotteen sisältö on kerrottu minulle suullisesti ja olen saanut riittävän vastauksen kaikkiin tutkimusta koskeviin kysymyksiini.

Minulla on ollut riittävästi aikaa harkita osallistumistani tutkimukseen. Annan luvan itseäni koskevien, tutkimuksen kannalta tarpeellisten tietojen keräämiseen HYKS Elinsiirto- ja maksakirurgian klinikan tutkijoiden ”**Etäiskeeminen esikäsittely elinsiirteiden toiminnan parantajana**” tutkimusrekisteriin. Tietojen keräämistä varten lääkäri saa kirjata henkilötunnukseni sekä käyttää sitä tietojen saamiseksi. Kaikki minusta tutkimuksen aikana kerättävät tiedot käsitellään luottamuksellisina.

Ymmärrän, että osallistumiseni tähän tutkimukseen on täysin vapaaehtoista. Olen tietoinen siitä, että voin keskeyttää osallistumisen tai peruuttaa suostumuksen missä tahansa tutkimuksen vaiheessa ennen sen päättymistä ilman, että siitä koituu minulle mitään haittaa. Tutkimuksesta kieltäytyminen, sen keskeyttäminen tai peruuttaminen ei vaikuta jatkohoitooni. Olen tietoinen siitä, että minusta keskeyttämiseen mennessä kerättyjä tietoja ja näytteitä käytetään osana tutkimusaineistoa etäiskeemisen esialtistuksen vaikutusten, tehon ja turvallisuuden varmistamiseksi.

Olen tietoinen siitä, että henkilötietojani voidaan käsitellä myös kotimaisen ja ulkomaisen viranomaisen suorittaman tarkastuksen, tutkimustiimiin kuulumattoman tutkimuksen säännönmukaista laadunvalvontaa tekevän henkilön (tutkimusmonitorin) suorittaman laadunvarmistustoiminnan yhteydessä.

Allekirjoituksellani vahvistan osallistumiseni tähän tutkimukseen ja suostun vapaaehtoisesti tutkimushenkilöksi.

_____ _____

potilaan allekirjoitus päiväys

_____ _____

nimenselvennys potilaan syntymäaika

_____

potilaan osoite

Suostumus vastaanotettu

_____ _____

lääkärin allekirjoitus päiväys

_____

nimen selvennys

Alkuperäinen allekirjoitettu tutkimushenkilön suostumus sekä kopio tutkimustiedotteesta jäävät tutkijalääkärin arkistoon. Tutkimustiedote ja kopio allekirjoitetusta suostumuksesta annetaan tutkimushenkilölle.

Supplement 3. Exploratory outcome measures

Kidney allografts
Peroperative blood samples before and after graft perfusion and urinary sample 6 hours after transplantation. Measurement of ischemia/reperfusion injury in blood and urine samples using following factors
Micro-RNA miR-21
Micro-RNA miR-24
Neutrophil gelatinase associated lipocain NGAL
Kidney injury molecule 1 KIM-1
Fatty acid binding protein 1 FABP-1
secretory leucocyte proteinase inhibitor SLPI
Liver allografts
Early allograft dysfunction at 7 days after transplantation according to Olthoff[1]: Bil >100, INR 1.6 or more, ALT or AST > 2000 at 7th POD
Highest ALT within 1 week
Highest INR within 1 week
Highest Bil within 1 week
Heart allografts
Ischemia-reperfusion injury determined by peripheral blood TnI, CK-MBm, lactate, and C-reactive protein levels at 0, 1, 12, and 24 hours
Peripheral blood proBNP at 1, 7, 14 and 21 days
Crea at 1, 7, 14 and 21 days
Urea at 1, 7, 14 and 21 days
eGFR at 1, 7, 14 and 21 days
Left ventricle ejection fraction (LVEF) at 1 day, 7 days, 14 days and 21 days
Left ventricle (LV) wall thickness measurements at 1 day, 7 days, 14 days and 21 days
Tricuspidal valve leak grading at 1 day, 7 days, 14 days and 21 days
The appearance of ischemia-reperfusion injury in routine biopsies at 7, 14 and 21 days

The appearance of fibrosis associated factors in routine biopsies at 7, 14 and 21 days
Long-time follow-up of proBNP at 1, 3, 6 and 12 months
Long-time follow-up of LVEF in cardiac ECHO at 1, 3, 6 and 12 months
Coronary Artery Disease (CAD) in coronary angiography at 1 year
Major Adverse Cardiac Events (MACE, including death because of cardiac cause, graft loss, primary allograft dysfunction, rejection classified as ISHLT G2R or more) at 1, 3, 6 and 12 months
Lung allografts
Factors possibly presenting the severity of ischemia/reperfusion injury after transplantation:
Standardized P/F-ratio during mechanical ventilation at 0 hours, 1, 6 , 12 and 24 hours
Non-standardized P/F-ratio during mechanical ventilation at 0 hours, 1 , 6, 12 and 24 hours
Plasma lactate at 0 hours, 1, 6, 12 and 24 hours
Serum highly sensitive C-reactive protein at 0 hours, 1, 6, 12 and 24 hours
Blood leukocyte count at 0 hours, 1, 6, 12 and 24 hours
Neutrophil count at 0 hours, 1, 6, 12 and 24 hours
Forced lung expiratory volume in one second (FEV1) at 1, 3, 6 and 12 months
Forced vital lung capacity (FVC) at 1, 3, 6 and 12 months
Evaluation of chronic rejection at 1, 3, 6 and 12 months
Exploratory outcomes lung allografts: Infections after transplantation at 1, 3, 6 and 12 months

References for supplement 1

1. Olthoff KM, Kulik L, Samstein B, et al. Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. *Liver Transpl* 2010;16(8):943-9. doi: 10.1002/lt.22091 [published Online First: 2010/08/03]

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1,2
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	2,4
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	2-3
Protocol version	#3	Date and version identifier	1
Funding	#4	Sources and types of financial, material, and other support	2,12, 17
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1,17

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	2, 17
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	12
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	12-13
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	6-7
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	6-7
Objectives	#7	Specific objectives or hypotheses	6-7
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be	1,7,17

		obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7-8
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	8
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	7-8
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	7-8
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	8-10, Suppl 3
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7-8
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11, 14
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	12

Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	8
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	8
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	8
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	8
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the	8-11, Suppl 3

		protocol	
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	8-9,12-13
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	12-13
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	10-11
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	10-11
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	11
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	13
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	12, 14
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	13
Auditing	#23	Frequency and procedures for auditing trial conduct, if	12-13

		any, and whether the process will be independent from investigators and the sponsor	
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	12-13
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	Suppl 1
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7-8
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	7-8,12-13
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	12-13
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	17
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	12
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	13
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	13
Dissemination policy:	#31b	Authorship eligibility guidelines and any intended use of	13-14

authorship		professional writers	
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	13
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Suppl 2
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	13

Notes:

- 12: 8-10, Suppl 3
- 18a: 8-11, Suppl 3 This checklist was completed on 12. October 2020 using https://www.goodreports.org/, a tool made by the EQUATOR Network in collaboration with Penelope.ai