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Carbon Dioxide Insufflation and Brain Protection During Open Heart Surgery: A Randomised Controlled Trial

The CO_2 Study

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Glossary / abbreviations

AE	Adverse event - any undesirable event in a subject receiving treatment according to the protocol, including occurrences which are not necessarily caused by or related to administration of the research procedures.
BTC CTEU	Bristol Trials Centre Clinical Trials and Evaluation Unit
CAM	Confusion Assessment Method
CBFV	Cerebral blood flow velocity
CBF	Cerebral blood flow
CDI	Carbon dioxide insufflation
CI	Chief investigator
CO2	Carbon dioxide
CPAP	Continuous positive airway pressure
CPB	Cardiopulmonary bypass - a technique that temporarily takes over the function of the heart and lungs during surgery, maintaining the circulation of blood and the oxygen content of the body whilst allowing surgeons to access static cardiac tissue.
DMSC	Data monitoring and safety committee
DVT	Deep vein thrombosis
DWI	Diffusion weighted imaging
NIHR-EME	National Institute for Health Research – Efficacy and Mechanism Evaluation
GCP	Good Clinical Practice
HRA	Health Research Authority
ISF	Investigator site file
MHRA	Medicines and healthcare products regulatory agency
MRC	Medical Research Council
MRI	Magnetic resonance imaging
NIHSS	National Institutes of Health Stroke Scale
NIRS	Near-infrared spectroscopy
PIL	Patient information leaflet
RCT	Randomised controlled trial
REC	Research ethics committee
SAE	Serious adverse event - events which result in death, are life threatening, require hospitalisation or prolongation of hospitalisation, result in persistent or significant disability or incapacity.
SCTS	Society of Cardiothoracic Surgeons
SOP	Standard operating procedure
SPC	Summary of Product Characteristics
SUSAR	Suspected unexpected serious adverse reaction - an untoward medical occurrence suspected to be related to a medicinal product that is not consistent with the applicable product information and is serious.
TCD	Transcranial doppler ultrasound
TMG	Trial management group

TOI	Tissue oxygen index
TSC	Trial steering committee
UHBW	University Hospitals Bristol and Weston NHS Foundation Trust
UKCRC	UK Clinical Research Collaboration

1. Trial summary

Brain dysfunction and injury is a common complication after heart surgery. It affects about 6 in every 10 people who undergo open heart surgery. It can be severe and permanent, for example stroke, although this is rare. In most people degree of dysfunction and injury is milder, manifesting as problems with memory and thinking. In some patients, these problems can persist for up to one year after heart surgery and may increase the risk of developing dementia.

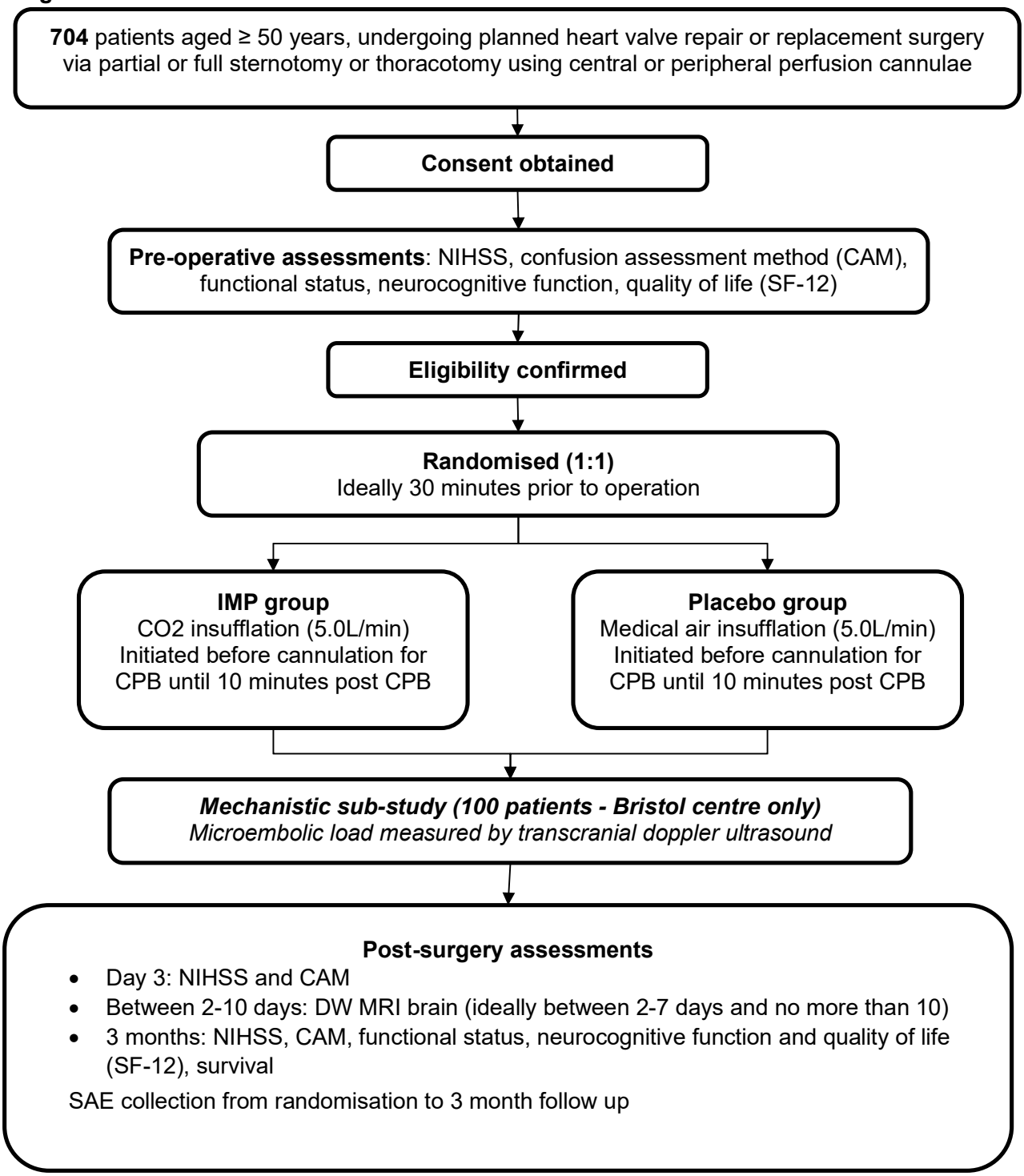
Surgeons believe that brain dysfunction partly results from microscopic air bubbles (microemboli) that enter the bloodstream when the heart is opened during surgery. These air bubbles are carried to the brain where they can get stuck in the small blood vessels and prevent blood from reaching that area of the brain, damaging, and eventually killing, surrounding nerve cells. One way that surgeons think they can reduce the number of air bubbles entering the bloodstream is by gently blowing the gas carbon dioxide into the area they are operating on. Carbon dioxide dissolves in blood much more easily than air, so it can displace air bubbles. However, there is little evidence that this technique, known as carbon dioxide insufflation (CDI), prevents brain injury after cardiac surgery.

The CO2 study will test the hypothesis that CDI prevents brain injury in patients undergoing heart surgery. Patients who are undergoing surgery to repair or replace one or more of their heart valves will be randomised into two groups; one group will receive carbon dioxide gas blowing into the heart and the other group will receive medical air (placebo). Medical air has no effect on the amount of air entering the bloodstream. Neither surgeons nor participants will know which type of gas is being used; only the person operating the cylinder will know. Everything else about the operation will be exactly the same. Participants will have a very sensitive brain scan (diffusion weighted magnetic resonance imaging, or DW MRI) between 2 and 10 days after their surgery. This is safe and should take no more than 30 minutes. Participants will also complete tests and questionnaires to assess brain and physical function and their quality of life before and 3 months after the operation. We will also collect information about how the operation went and any complications that the patients experience during and after the surgery, for example, strokes and kidney damage.

We hope to recruit 704 patients from at least eight UK NHS cardiac surgery centres into the trial. The trial is expected to take about 3 and half years to complete. We also want to do a small sub-study within the trial to determine whether CDI does lead to fewer air bubbles entering the circulation and whether fewer air bubbles means less damage to the brain. We will ask 100 patients taking part at the Bristol centre to have an ultrasound scan of the main artery going to their head during their operation. The ultrasound scan is safe and will not delay the operation. The scan will allow the number of air bubbles entering the blood stream to be counted using a computer. This information can be reviewed against the brain scan to see if patients with fewer bubbles have fewer areas of damage in their brains.

1.1 Trial schema

Figure 1 Trial schema



2. Background & Rationale

2.1 Brain injury and dysfunction after cardiac surgery

Brain injury and dysfunction is a common complication after cardiac surgery and significantly increases the likelihood of patients requiring long-term care. Peri-operative stroke occurs in 2-6% of all patients (1). More than 20% of patients over 65 years of age and 33% of patients over 80 years of age experience post-operative delirium (1, 2). Post-operative cognitive dysfunction is estimated to affect more than 80% of patients at discharge, and persist in 25% of all patients at 1 year (3). Brain injury may also trigger chronic or progressive dementia (4).

It is assumed that brain injury is triggered by the release of microemboli (microscopic atherosclerotic particles or air bubbles) in the blood stream, which are carried to the brain. Indeed, intraoperative transcranial doppler (TCD) ultrasound monitoring demonstrates showers of small particulate or air emboli during cardiac and vascular manipulations (5). However, the relationship between intraoperative brain embolic load and brain injury still needs to be clarified. Some studies have reported that the embolic burden detected by TCD monitoring is associated with early cognition deficits while others have not confirmed this finding (6, 7).

2.2 Magnetic resonance imaging (MRI) to detect perioperative brain injury

MRI examination of the brain is the “gold standard” for identifying and quantifying peri-operative brain injury (8) and has been used widely in randomised controlled trials (RCTs) investigating neuroprotective interventions in cardiac surgery (9-11). A variety of imaging techniques are applied to identify markers of such injury, including diffusion weighted imaging (DWI). The main advantage of DWI is that lesions typically appear within 2 hours of surgery and disappear after two weeks, therefore, new acute lesions represent “new” injury, so a baseline scan is not required to confirm that the lesion was not present before surgery. Another advantage is that the new framework for defining stroke proposed by the American Heart Association/American Stroke Association (8) includes neuroimaging (together with clinical or pathological evidence), so lesions found on DWI count as “silent” brain injury even in the absence of obvious clinical findings.

DWI lesions following left heart valve surgery are reported in about 50% of patients (5, 9). These lesions are multiple and very small (1-10mm in diameter and 32-750mm^3 in volume). They are located in all cerebrovascular territories, but more frequently in frontal and watershed border zones and the pattern of distribution confirms an embolic basis. Few (~9%) are associated with overt clinical signs of stroke so they represent “silent” brain injury in most cases (5, 9).

In population-based studies, a strong association has been found between “silent” brain injury identified by MRI and prevalent cognitive dysfunction and dementia (12-14), therefore it is plausible that a similar relationship exists between the appearance of new lesions after cardiac surgery and neurocognitive decline. Some preliminary data has suggested that the appearance of new “silent” brain lesions after cardiac surgery is associated with early post-operative

neurocognitive deterioration (15) although further investigations with longer follow-up are needed.

The use of post-operative cognitive dysfunction as a marker of peri-operative brain injury is problematic because of potential difficulties in ascertainment (5). Multiple factors affect neurocognitive test performance during the first week after surgery; namely treatment of post-operative pain, sedation, and other clinical recovery issues. Many, if not most, patients have some degree of cognitive dysfunction in the immediate post-operative period. Such a nearly universal occurrence is clearly not an appropriate marker of brain injury. Only after this period has passed can an objective assessment of the patient's cognition be performed; although the duration of altered cognition after surgery has not been defined clearly (5).

2.3 Intraoperative carbon dioxide insufflation (CDI)

Most research on cerebral embolisation during cardiac surgery has focused on the issue of atherosclerotic microemboli. Devices designed to remove atherosclerotic microemboli from the bloodstream have been tested but they were not shown to reduce the burden of brain injury. One possible explanation is that the embolic load to the brain is mainly constituted by air (>80%) (16). Air microemboli form when the heart chambers and great vessels are opened, which inevitably fills them with air. The air will stay until it is mobilised into the arterial bed during and after weaning from cardiopulmonary bypass (CPB). Air microemboli are believed to lead to brain injury by occluding small vessels or causing endothelial damage (arterioles with inner diameter of 30-60μm) with relative distal ischemia. Manual de-airing techniques are commonly used to remove air from the heart chambers but, unfortunately, they are unable to fully eliminate air microemboli (17, 18).

Carbon dioxide insufflation (CDI) in the pericardial cavity has been proposed to reduce the formation of air microemboli during cardiac surgery (19, 20). The rationale behind its use is that carbon dioxide gas is more dense and more soluble than air, and can therefore displace air from the heart chambers, leading to fewer air microemboli entering the blood stream (20). However, the evidence that CDI prevents brain injury after cardiac surgery is still scarce (19). There are only four small, low-quality RCTs investigating the neuroprotective effect of CDI. They are all open trials at high risk of bias, are all based on neuro-psychometric tests performed at different time points with no brain imaging assessment and resulted in conflicting conclusions (21).

Lack of definitive evidence has resulted in a wide variability in CDI adoption in the clinical practice. Our survey has shown that surgeons are currently in equipoise, with proponents arguing for continued use of CDI, and opponents arguing that in addition to the increased costs (~£50-100 per patient), CDI may cause significant harm. In fact, CDI induces systemic hypercapnic acidosis (excess carbon dioxide causing the pH of blood and tissues to decrease), cerebral vasodilation and a relative increase in cerebral blood flow, which would counterbalance any protective effects because a higher number of microemboli (particulate or gaseous) could access the cerebral circulation (22). However, one RCT compared CDI vs. medical air insufflation in one hundred and twenty-five patients during open heart surgery and showed no

significant difference in the rate of cardiac complications experienced between the two groups (23).

2.4 Review of existing evidence

We have conducted and recently published a systematic review and meta-analysis of all available RCTs investigating the efficacy of CDI versus standard de-airing techniques (21). Outcomes investigated were post-operative stroke, neurocognitive dysfunction, and in-hospital mortality. Pooled estimates were obtained using random-effects models to account for clinical diversity and methodological variation between studies. Eight RCTs were identified comparing CDI vs. standard de-airing but only four studies reported on the incidence of post-operative neurocognitive decline (23-25); 283 randomised to CDI, 284 randomised to standard de-airing. Most studies were small (<100 patients) and at high risk of bias. Type and timing of neurocognitive function tests varied among studies. Different methods were used for delivery of carbon dioxide. In-hospital mortality was 2.1% vs 3.0% in the CDI and standard de-airing (control) groups, respectively (risk difference (RD) 0%; 95% CI −2% to 2%. Incidence of stroke was 1.0% vs 1.2% (RD 0%; 95% CI −1% to 2%) and neurocognitive dysfunction was 12% vs 21% (RD: −7%; 95% CI −0.22% to 8%) in the CDI and control group, respectively. The results of the meta-analysis were influenced by our decision to include a trial with no events (23), which resulted in a more conservative estimate of treatment effect. Excluding this trial from the analysis suggested a protective effect of CDI on neurocognitive function (OR 0.46, 95%CI 0.22 to 0.96).

3. Rationale

Nearly 40,000 people in the UK (http://bluebook.scts.org/) and many more worldwide have cardiac surgery annually. Ischaemic brain injury represents a common complication and is associated with increased hospital stay, slow recovery, poor quality of life, further health care resource use and reduced long term survival. Cardiac surgery is increasingly being offered to older, higher-risk patients with comorbidities, so the incidence of neurological complications is likely to increase in the future. CDI is a widely used intervention, which many surgeons believe improves neurological outcomes. However, there is little evidence for its efficacy or risk of harm and its routine use is not recommended and surgeons are currently in equipoise regarding its adoption.

The CO2 study will assess the neuroprotective effect of CDI versus placebo (medical air insufflation) in addition to standard de-airing in patients undergoing left side heart valve surgery. In addition, the CO2 study will investigate the relationship between cerebral air embolic load and peri-operative brain injury detected by MRI and the relationship between perioperative brain injury and post-operative neurocognitive function.

4. Aims and objectives

The CO2 study aims to evaluate the efficacy and safety of CDI in patients undergoing planned open left side heart valve surgery.

It is hypothesised that in patients undergoing open heart valve surgery via partial or full sternotomy or thoracotomy, CDI plus standard de-airing is protective against ischaemic brain injury caused by cerebral embolisation (air microemboli) compared with medical air insufflation (placebo) plus standard de-airing.

Specific objectives of the trial are to evaluate:

1. The difference between groups receiving CDI or medical air insufflation in the incidence of acute clinical or radiographic ischemic brain injury between 2 and 10 days post procedure (primary outcome)
2. The difference between groups receiving CDI or medical air insufflation with respect to a range of secondary outcomes (clinical, DWI lesion characteristics, adverse events, and patient-reported)
3. The association between the burden and location with new ischemic brain lesions detected with DWI and post-operative neurocognitive function assessed by standard neurocognitive function tests

A mechanistic sub-study will also be conducted. Specific objectives of the mechanistic sub-study are to determine whether there is a relationship between:

1. Intraoperative air embolic load detected by TCD
2. Cerebral blood flow (CBF) detected by TCD
3. Tissue oxygen index (TOI) of the frontal lobes by Near Infrared Spectroscopy (NIRS)

and ischaemic brain injury detected by MRI, and whether these relationships differ between the CDI and medical air insufflation groups.

5. Primary and secondary outcomes

5.1.1 Primary outcome

The primary outcome is acute ischemic brain injury within 10 days post-surgery based on new brain lesions identified with DW MRI or clinical evidence of permanent brain injury according to the updated definition of stroke for the 21st century: symptoms persisting ≥ 24 hours in the brain, spinal cord or/and retina; not including cases of global ischemia (26).

All patients will be assessed by 1.5 or 3.0 T MRI between 2 and 10 days post-operatively. MRIs will be performed at each participating centre according to a standardised protocol and will be centrally analysed at the Wellcome Centre for Integrative Neuroimaging at the University of Oxford.

We will use the framework for assessing brain injury proposed by the American Heart Association/American Stroke Association for use in RCTs assessing neuroprotection during cardiovascular interventions (8). These guidelines recommend post-procedural brain DWI as the gold standard in studies investigating devices or procedures designed to prevent iatrogenic or spontaneous acute neurological injury.

5.1.2 Secondary outcomes

Data will be collected to characterise the following secondary outcomes:

1. Number and volume of DWI brain lesions
2. Objective quantification of the impairment caused by new ischemic brain injury assessed using the National Institutes of Health Stroke Scale (NIHSS)
3. Delirium assessed using the 3-minute diagnostic interview for Confusion Assessment Method (CAM)
4. Functional status assessed using the Barthel Index score
5. Neurocognitive function in 6 domains (verbal memory, visual memory, executive functioning, visuospatial or constructional praxis, attention, and information processing speed), assessed using the following tests:
    a. Addenbrooke's Cognitive Examination III (ACE III)
    b. Oral Trail making Tests A and B (oTMT A&B)
6. Quality of life assessed using the physical and mental subscales of the 12-Item Short-Form Health Survey (SF-12)
7. Composite of all-cause mortality, clinical stroke, or acute kidney injury within 30 days of surgery
8. Serious adverse events (SAEs) to 3 months
9. Survival to 3 months

All patients will be consented for passive follow up for up to five years after their surgery from NHS and national health and registration records to determine any association between silent brain lesions and long-term neurological outcomes (e.g. dementia).

5.1.3 Mechanistic sub-study outcomes

1. Cerebral gaseous microembolic load assessed using TCD
2. Cerebral blood flow velocity (CBFV) from both middle cerebral arteries
3. Tissue oxygen index (TOI) of the frontal lobes measured using NIRS

6. Plan of Investigation

6.1 Trial design

The CO2 study is a multicentre, parallel two-group placebo-controlled RCT in which participants, clinical care teams, members of the research team responsible for data collection and imaging assessors will be blinded to the treatment allocation (with the exception of the perfusionist responsible delivering the study intervention).

6.2 Key design features to minimise bias

Selection/allocation bias will be prevented by concealed randomisation. The allocation will not be revealed until sufficient information to uniquely identify the participant has been entered into the allocation database.

Performance bias will be minimised by blinding participants and the healthcare professionals looking after them to treatment allocation. We will also i) define the interventions and the standard protocols for all other aspects of care during the study, ii) define procedures for follow-up, iii) monitor adherence to the protocol. The patient information leaflet (PIL) and the process of obtaining informed consent will describe the uncertainty about the effects of CDI. Therefore, in the event of inadvertent unblinding of a participant, they should not have a strong expectation that any one method should lead to a more favourable result.

Detection bias will be minimised by blinding of outcome assessors and using outcome measures that are defined as far as possible on the basis of objective criteria. MRI will follow a standardised protocol and scans will be analysed centrally. Stroke will be defined using the American Heart Association / American Stroke Association updated definition of stroke (26). TCD ultrasound assessments will be standardised and conducted by personnel blinded to the allocation.

Attrition bias will be minimised by using established methods developed in the Bristol Trials Centre Clinical Trials and Evaluation Unit (BTC CTEU) to maximise the quality and completeness of the data (e.g. regular monitoring of data, detailed querying of data inbuilt into the study database, offering alternative methods for participating in follow-up (e.g. postal, online or telephone). Any instances of non-adherence will be fully documented and reviewed at study meetings and an action plan for maximising compliance drawn up as appropriate. Data will be analysed by intention to treat (i.e. according to the treatment allocation, irrespective of future management and events), and every effort will be made to include all randomised patients. Follow-up for the primary outcome should be complete for all patients.

Reporting bias will be minimised by pre-specifying study outcomes and following a detailed analysis plan which will be prepared in advance of any comparative analyses of the study data.

6.3 Setting

Participants will be recruited from at least eight cardiac surgery centres in UK NHS hospitals.

6.4 Trial population

Adults undergoing planned left side heart valve surgery.

6.4.1 Inclusion criteria

1. Age ≥ 50 years
2. Planned aortic or mitral valve surgical repair or replacement (with or without another procedure, e.g. coronary artery bypass graft) via a partial or full sternotomy or thoracotomy using central (i.e. aortic) or peripheral (i.e. femoral) perfusion cannulae

6.4.2 Exclusion criteria

1. Contraindication to medical carbon dioxide: acquired or genetic of acidosis (i.e. renal tubular acidosis)
2. Contraindication to MRI (e.g. known intolerance, permanent pacemaker in situ or expected implantation of a permanent pacemaker)
3. History of clinical stroke within 3 months prior to randomisation
4. Cardiac catheterisation within 3 days of the planned surgery
5. Cerebral and/or aortic arch arteriography or interventions within 3 days of the planned surgery
6. Active endocarditis at time of randomisation
7. Planned concomitant aortic procedure such as root replacement
8. Clinical signs of cardiogenic shock or treatment with IV inotropic therapy prior to randomisation
9. Previous cardiac surgery
10. Participation in an interventional (drug or device) trial
11. Unable to provide written informed consent
12. Prisoners

6.5 Randomisation

Participants will be randomised as close to the planned operation as possible (ideally 30 minutes before the participant is taken to theatre), after consent has been given, baseline assessments have been completed and eligibility has been confirmed by the Principal Investigator, a delegated medically qualified doctor or Advanced Nurse Practitioner who has undergone suitable training.

Participants will be allocated in a 1:1 ratio to either CDI plus standard de-airing or medical air insufflation (placebo) plus standard de-airing. The allocation will be computer-generated and will be stratified by centre, type of procedure (i.e. valve only or valve combined with another procedure e.g. CABG) and whether planned surgical access is minimally invasive (e.g. thoracotomy), by an independent BTC CTEU statistician, not involved in the study, before recruitment begins.

Randomisation will be performed using a secure internet-based randomisation system ensuring allocation concealment by a member of the research team not involved in data collection or participant follow-up.

6.6 Blinding

Participants, their clinical care team (i.e. the surgeon, anaesthetist and those responsible for their post-operative care), research nurse(s) responsible for participant follow-up and imaging assessors will not be informed of the intervention allocation.

The perfusionist will be unblinded to the trial intervention allocated at randomisation in order to deliver the allocated intervention and monitor the participant throughout the surgery. Either the perfusionist or an unblinded member of the team will be responsible for carrying out the randomisation and preparing the unblinded case report form (CRF) with the treatment allocation. If an unblinded member of the team randomises the participant, they will hand documentation containing the treatment allocation and the relevant CRF in a sealed envelope to the perfusionist.

Following surgery the completed CRF will be entered into the database by either the perfusionist or the unblinded member of the team. This documentation will be stored separately to the rest of the CRFs to ensure blinding is maintained throughout the trial.

Any member of staff who accesses the sealed envelope containing the allocation details will have to record the reason for accessing the documentation on the relevant case report form.

Cylinders of carbon dioxide and medical air can be purchased in similar physical sizes, both are grey in colour and only the collars differ. The cylinder will be brought into theatre by the Perfusionist. The cylinder and flowmeter will be covered with a sterile surgical drape/sleeve to maintain blinding. The insufflation flow rate will be standardised for both groups (5L/min).

There are no obvious clinical or other cues as to whether CDI or medical air was used, so we do not expect participants to be unblinded. Research Nurses responsible for data collection and participant follow-up will not randomise patients and will not be in the operating theatre. MRI scans will be analysed centrally. We will assess the success of blinding by asking the Research Nurse undertaking the assessments, after the three month follow up, and the Imaging Assessor, following review of the DW-MRI brain, which treatment they think was received (Bang blinding index (27)).

6.7 Unblinding

Requests to unblind on clinical grounds, e.g. to treat a complication, are not anticipated. However, if unblinding is requested during surgery on safety grounds, this will be facilitated by the Perfusionist. Any such request will be fully documented including who requested the unblinding and the reason for unblinding.

Requests to unblind after leaving the operating theatre should not occur; given that the effect of CDI/medical air insufflation is strictly limited to the period of delivery in the operating room. Therefore, the management of any subsequent SAE would not be altered by knowledge of the allocation. Unblinding rates will be monitored throughout the trial by the study team and by the independent Data Monitoring and Safety Committee (DMSC) that will be established to oversee participant safety in the trial (see Section 11 for further details).

Participants will be made aware before entering the study that they will not be told which treatment they will receive until after the trial has completed.

6.8 Research procedures

Participants will be required to do, or undergo, the following tasks or investigations specifically for the study which are also outlined in table 1.

Pre-surgery:

- Read the CO2 study PIL (can be sent to the patient's home before the patient attends the hospital for their surgery)
- Provide informed consent to participate, if willing to do so
- Assessment of NIHSS, CAM, functional status (Barthel Index) and neurocognitive function (ACE III and TMT A&B)
  Complete quality of life questionnaire (SF-12)

During surgery:

- Receive the intervention allocated at randomisation during open heart valve surgery
- *If participating in the mechanistic sub-study (Bristol centre only), a transcranial doppler ultrasound of the middle cerebral artery and near infrared spectroscopy of the frontal lobe will be conducted during surgery*
- Assessment and reporting of any AEs and SAEs experienced

Post-surgery inpatient stay:

- **Day 3** (between 3-5 days post-surgery): Assessment of NIHSS and CAM
- DW MRI brain scan ideally between 2 and 7, but no more than 10, days after surgery before hospital discharge
- Assessment and reporting of any AEs and SAEs experienced

Post-discharge:

- **Three months post-surgery (conducted remotely):** Assessment of NIHSS, CAM, functional status (Barthel Index) and neurocognitive function (ACE III and TMT A&B)
  Complete quality of life questionnaire (SF-12).
  Collection and assessment of any SAEs experienced after discharge from hospital

6.8.1 Neurocognitive function

Neurocognitive function tests, assessing six domains; visual and verbal memory, executive functioning, visuospatial or constructional praxis, attention and information processing speed, will be performed by a member of the research team who has undergone suitable training.

The tests to assess neurocognitive function are:

- Addenbrooke's Cognitive Examination III (28)
- Oral trail making test part A and B (29, 30)

Tests will be performed prior to surgery and at three-month follow up and should take around 30 minutes to conduct.

6.8.2 National Institutes of Health Stroke Scale

National Institutes of Health Stroke Scale (NIHSS) will be used to assess any impairment that may have been caused by ischemic brain injury. The scale will be completed at the specified timepoints by a member of research team who has undergone suitable training. The scale should take around 15 minutes to conduct.

6.8.3 Confusion Assessment Method

Delirium will be assessed using the confusion assessment method algorithm three minute interview (31). The interview will be performed at the specified timepoints by a member of research team who has undergone suitable training. The interview should take around three minutes to conduct.

6.8.4 Functional status

Functional status will be assessed using the Barthel Index (32). The index will be performed at the specified timepoints by a member of the research team who has undergone suitable training. The score should take around 10 minutes to conduct.

6.8.5 Patient reported outcomes

Quality of life (QoL) will be assessed using the physical and mental subscales of the 12-item Short-Form Health Survey (SF-12) (33). The patient should complete the questionnaire prior to surgery and at three-month follow up. Participants will be able to choose to receive the QoL questionnaire at three months by post (sent by BTC CTEU) or complete online via a secure website.

6.8.6 Diffusion Weighted Magnetic Resonance Imaging (DW MRI)

All participants will undergo a 1.5 or 3.0 T diffusion weighted MRI (DW MRI) scan of their brain post-surgery. The DW MRI scan should be conducted in accordance with the CO2 MRI protocol. The scan will take approximately 30 minutes and will be completed as an inpatient as soon as possible after their surgery, ideally between 2-7 but no more than 10 days following surgery.

Scans will be transferred to the Wellcome Centre for Integrative Neuroimaging at the University of Oxford, who will perform a blinded review to assess any radiographic evidence of brain injury following surgery. Instructions on the transfer of images will be outlined in the imaging manual.

Participants will be asked to consent to the transfer of their DW MRI brain scans for review at entry to the trial.

6.8.7 Mechanistic sub-study – Bristol centre only

One-hundred study participants from the Bristol centre will be asked to take part in the CO2 mechanistic sub-study. Consent to the sub-study is optional.

Sub-study participants will undergo a transcranial doppler (TCD) ultrasound of the middle cerebral artery during their surgery to monitor intraoperative air microemboli load and CBF. Participants will also undergo near-infrared spectroscopy (NIRS) to monitor the TOI of the frontal lobes during surgery.

The TCD and NIRS probes will be placed on the participant's head and secured before insufflation is initiated, until the procedure has been completed. Sub-study assessments will be performed by a Surgeon and Research Fellow who have undergone suitable training.

6.8.8 Non completion of research procedures

Reasons for not completing any research procedure will be recorded and coded where possible. Missing items or errors on questionnaires will be dealt with according to the scoring manual or via imputation methods. Adherence rates will be reported in the results, including the numbers of patient who have withdrawn from the study, been lost to follow up or died. Causes of death will be recorded.

6.8.9 Abnormal findings

Any abnormal findings identified because of the study assessments will be escalated to the relevant care teams for further investigation.

7. Trial intervention

7.1 Trial interventions

Study participants will be randomly allocated on a 1:1 ratio to receive insufflation with either:

- **IMP:** Medical carbon dioxide (carbon dioxide purity 99.5% v/v min)
- **Placebo:** Medical air (oxygen content 21%)

7.1.1 Dosing schedule

Insufflation, with either carbon dioxide or medical air, will be initiated at a flow rate of 5 litres per minute (5.0L/min) before cannulation for cardiopulmonary bypass (CPB) and continued until 10 minutes post-CPB.

The allocated gas will be insufflated into the cardiothoracic wound through a gas diffuser that provides an almost 100% carbon dioxide / medical air atmosphere in the wound, as per the centre's local procedure.

The diffuser will be placed in the inferior part of the surgical incision.

When applied, the cardiotomy suction will be limited to a maximum rate of 1.5 L/min and the rough suction will be set to 10 L/min to avoid affecting the carbon dioxide/air concentrations in the field.

7.1.2 Surgical procedure

Operations will be performed through a complete or limited median sternotomy or thoracotomy with CPB with either a roller or centrifugal pump and hollowfibre membrane oxygenator maintaining non-pulsatile perfusion at 2 to 3L/(min m2), an arterial line filter will be used, mean arterial pressure maintenance at 70 and 90 mm Hg, and patient temperature to be between 37°-32°C. Gas sweep flows will be adjusted to maintain PaCO2 at 35-40 mmHg (4.6-5.2KPa) with the alpha stat method.

Standard cannulation will consist of arterial cannulation in the distal part of the ascending aorta and a 2-stage venous cannula inserted into the right atrium and the inferior venae cava. Mitral valve operations will be an exception, for this surgery bicaval cannulation will be used.

Peripheral cannulation will be carried out as per local procedure in minimally invasive procedures i.e. using the femoral artery.

Myocardial preservation will be achieved with intermittent antegrade and retrograde cold blood cardioplegia.

7.1.3 De-airing manoeuvres

Surgeons should perform standard de-airing manoeuvres such as the following.

Following completion of the surgical procedure, the heart is passively de-aired across the aortic or left atrial suture line as follows:

- With the LV and root vents clamped, and any caval tapes unsnugged, the heart is filled with blood by occlusion of the venous return and the lungs are ventilated.
- The LV and LAA are then massaged, and the left heart de-aired across the aortic/left atrial suture line before the suture is tied.

After the suture line has been tied, and in Trendelenburg position, the heart is again filled and the lungs ventilated whilst maintaining suction on the root vent. Complimentary deairing of the apex is achieved by brief and low suction on the LV vent, which is stopped before removal of the aortic clamp. After removal of the cross clamp, the aortic suction is maintained at 200-300ml/min. The LV vent can be opened for brief periods if the LV distends, but prolonged suction should be avoided when the heart is empty to prevent air from entering the LA around the vent. Gravity drainage of the LV vent (rather than suction) will prevent air being entrained and is acceptable when the heart is empty.

Additional deairing during the rewarming period is by maintaining aortic root vent suction.

When the trans-oesophageal echo (TOE) shows that all microbubbles have been evacuated, the LV vent can be removed.

The aortic root vent is maintained until the patient is totally weaned from cardiopulmonary bypass.

All other aspects of the participant's pre, intra and post-operative management will be in accordance with existing local protocols.

7.1.4 Dose modifications

Dose modifications are not expected to occur.

7.1.5 Regulatory status

Medical carbon dioxide and medical air are UK-licensed and commercially available on the UK market. For this trial it is acceptable to use any licenced supply of medical carbon dioxide or medical air.

In this trial, medical carbon dioxide and medical air will be used 'off label' as part of the open-heart valve repair or replacement surgery.

7.1.6 Preparation and labelling

It is recommended that cylinders of both trial interventions are ordered in the same size to prevent unblinding. If using BOC ltd supplied gas, cylinder size E is recommended.

Two cylinders of the allocated trial intervention should be taken into the operating theatre to ensure sufficient supply is available for the procedure. Green tubing can be used to connect to both cylinders with a Y connector ensure flow can be continuously delivered without unblinding, should both cylinders be required.

Surgical drapes should be placed over the cylinders before they are brought into the operating theatre to prevent unblinding.

All cylinders will be labelled, in the cylinder storage area, under the supervision of the site's trial pharmacist with annex 13 compliant labels and in accordance with Good Clinical Practice (GCP) and pharmacy department SOPs.

The contents of the label will be submitted and approved for use by the MHRA.

7.1.7 Drug storage and supply

Both carbon dioxide and medical air will be sourced locally by the research site pharmacy (or as per local pharmacy delegated arrangements) at market price. Supply levels should be monitored and re-ordered as applicable.

Carbon dioxide and medical air cylinders will be stored and handled as per the current SPC storage precautions, in accordance with GCP, Good Manufacturing Practice and pharmacy department SOPs.

7.1.8 Reference Safety Information

Reference safety information, listed in the medical carbon dioxide SPC, section 4.8 undesirable events, are not applicable to the trial population given that these patients will be unconscious due to anaesthesia and on CPB at the time the trial intervention is received and therefore, cannot experience the listed side effects.

No expected events are listed in the medical air SPC section 4.8, 'undesirable events'. Section 4.8 of the medical carbon dioxide and medical air SPC will be regularly reviewed for updates to the undesirable events listed. If there are any updates these will be reviewed by the Chief Investigator and, in consultation with the Sponsor, a decision made whether the updated document will be submitted to the MHRA for use as the RSI in the trial.

7.1.9 Contraindications

Participants who are known to have acquired or genetic acidosis (i.e. renal tubular acidosis) will be excluded from the trial.

7.1.10 Treatment adherence

Withdrawals during treatment should not occur due to the nature and duration of the intervention; the duration of intervention is from before cannulation for CPB, until 10 minutes

after CPB has stopped. Problems with adherence (e.g. failure to follow randomisation allocation) are also expected to be low. The Perfusionist will document whether the allocated treatment was given, if there were any deviations from the allocated intervention and the reason.

7.2 Duration of treatment period

The duration of the treatment commences when the patient enters the operating room and concludes when the patient leaves the operating room after their surgery.

The duration of the procedure is expected to be between 2 hours and 4 hours.

Table 1 Schedule of assessments

	Baseline	Intraoperative	Post-surgery				
			Day 3 (between 3-5 days post op)	Day 2 – 7 (but no more than 10 days)	Discharge	Routine post-op follow-up	Three-month follow up
National Institutes of Health Stroke Scale	✓		✓				✓
Confusion Assessment Method	✓		✓				✓
Functional status: Barthel Index score	✓						✓
Neurocognitive function tests: Addenbrookes Cognitive Examination III and oral Trail Making Tests A&B	✓						✓
Quality of life questionnaire (SF-12)	✓						✓
Assessment of post-operative complications					✓		
DW MRI brain				✓			
Assessment of serious adverse events (from randomisation to three month follow up), including readmissions			✓	✓	✓	✓	✓
Assessment of blinding success							✓
Transcranial doppler ultrasound*		✓					
Near-infrared spectroscopy*		✓					

**100 patients at the Bristol centre only*

8. Data collection

Each patient will be assigned a unique study number. All data recorded on paper relating to the participant will be located in CRF folders, which will be stored securely. Staff with authorisation to make changes to the study records, including the study database, will be listed on the study delegation log maintained at each centre.

Screening data will be collected for all patients undergoing open heart valve surgery considered for the study. Age, sex and Index of Multiple Deprivation (IMD) will be collected for all patients irrespective if they decline to participate. This information is collected to assess any difference in the patients that do not take part compared to patients that do, to establish if there are any socioeconomic barriers to participation.

Baseline data will be collected after consent. Consenting patients will be contacted by an authorised member of the local research team (as specified in the delegation log) who will provide the opportunity to understand the nature, significance, implications and risks of the trial so that they may make an informed decision if they should take part. If the patient decides to take part the member of the local research team will obtain informed consent.

Patients who choose to consent using electronic consent methods will verbally provide their email address to the local research team to receive a link to the electronic consent form.

Data collection will include the following elements:

a) A screening log of all patients undergoing open heart valve surgery via partial or full sternotomy including age, sex and Index of Multiple Deprivation (IMD) for place of residence derived from the patient's residential postcode, as recorded in their hospital records.
b) Patients approached and assessed against the eligibility criteria and, if ineligible, reasons for ineligibility.
c) Consent information collected prior to randomisation for all participating patients.
d) Baseline information (e.g. medical history and assessments) and neurocognitive function and quality of life (collected via questionnaire) collected for all participating patients.
e) Data relating to surgery and hospital stay collected for all participating patients.
f) Images from the post-operative DW MRI brain scan will be collected for all patients.
g) Data on neurocognitive function and quality of life (collected via questionnaire), adverse events and resource use will be collected at three months post-surgery for all participating patients. Participants will be able to choose to receive the quality of life questionnaire by post or complete via an online secure website.

8.1 Definition of end of trial

The definition of the end of the trial is the date when all patients have completed the three-month post-surgery follow-up, or are lost to follow-up, the database has been locked and all data queries have been resolved.

The end of the trial for an individual patient is defined as completion of the three-month post-surgery follow-up assessments.

Patients will be asked to consent to the collection of long term follow up data (via Government bodies NHS Digital, ISD Scotland or NHS Wales Informatics Service) for up to five years post-surgery for future ethically approved research.

9. Safety reporting

9.1 Overview

Serious and adverse events (AEs) will be recorded and reported in accordance with GCP guidelines and the BTC CTEU's Serious Adverse Events and Safety Reporting Standard Operating Procedure (SOP-GE-012) (see Figure 2).

9.2 Definitions

Adverse event (AE) is any undesirable event in a subject receiving treatment according to the protocol, including occurrences which are not necessarily caused by or related to administration of the research procedures.

Adverse reaction (AR) is any undesirable experience that has happened to a subject while taking a drug that is suspected to be caused by the drug or drugs.

Serious adverse event (SAE) is any event which results in death, is life threatening, requires hospitalisation or prolongs hospitalisation, results in persistent or significant disability or incapacity.

Suspected serious adverse reaction (SSAR) is any serious adverse event that is suspected to be related to the drug or drugs being taken.

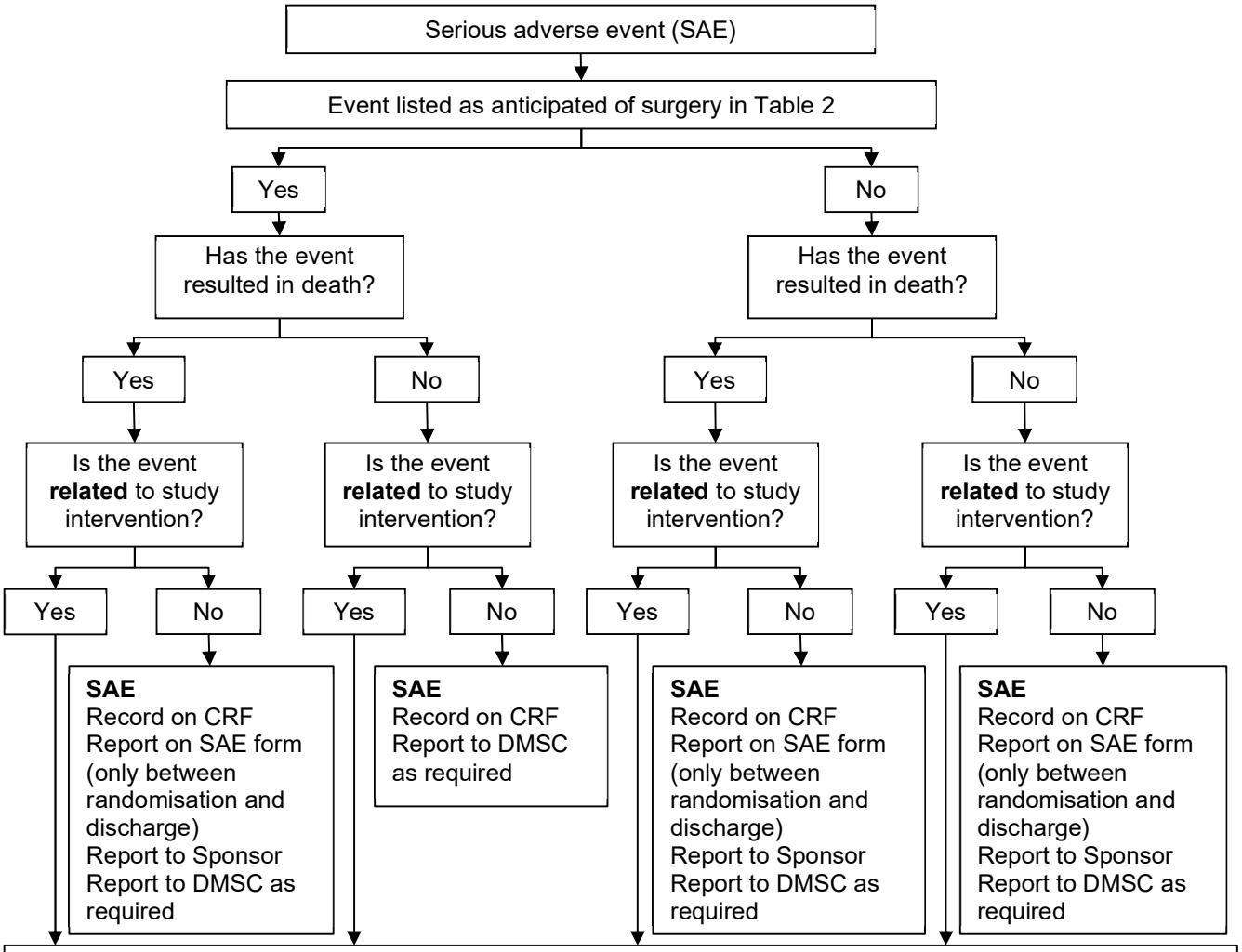
Suspected unexpected serious adverse reaction (SUSAR) is an untoward medical occurrence suspected to be related to the drug or drugs being taken that is not consistent with the applicable product information and is serious.

All AEs must be reviewed, and causality must be assessed by the Principal Investigator or delegated individual.

If an event meets any of the 'serious' criteria listed below it is classified as an SAE:

a) Results in death
b) Life threatening
c) Requires hospitalisation (unless hospitalisation is pre-planned)
d) Prolongation of existing hospitalisation
e) Results in persistent or significant disability or incapacity
f) Congenital anomaly / birth defect
g) Any other event which may jeopardise the patient or require intervention to prevent one of the other outcomes listed above

Figure 2. Serious adverse event reporting flow chart



Note: ALL adverse events that result in DEATH must be reported on SAE forms to the Sponsor

9.3 Period for recording adverse events

Data on adverse events will only be collected for the period of the participant's post-operative hospital stay.

9.4 Period for recording serious adverse events

Data will be collected on all SAEs that occur from randomisation until the three-month follow up visit using the relevant CRFs.

Centres should expedite reporting of all fatal and 'unexpected' non-fatal SAEs that occur between randomisation and discharge to BTC CTEU within 24 hours of becoming aware, using an SAE report form.

The BTC CTEU will report all fatal and 'unexpected' non-fatal SAEs to the Sponsor. Unexpected events are those not listed in the trial protocol or on the CRFs. The Sponsor will report SUSARs to the MHRA and copy all reports to the BTC CTEU. BTC CTEU will report SUSARS to the REC.

9.5 Expected adverse event association with trial intervention

There are no known expected adverse events from the trial intervention in this trial population. Any SAEs deemed related to the trial intervention are considered to be SUSARs and must be reported as per Figure 2.

9.6 Anticipated adverse events associated with surgery

In cardiac surgery, post-operative transient complications are expected and can be frequent. These are classified as 'anticipated adverse events of surgery'.

The AEs 'anticipated' in patients undergoing cardiac surgery are listed in Table 2.

Table 2 Anticipated adverse events of surgery

Body system	Anticipated adverse event
Cardiovascular	Myocardial Infarction
	Cardiac arrest, requiring: • Resuscitation involving ventricular defibrillation / direct current (DC) shock • Chest reopening • External/internal cardiac massage
	Haemodynamic support, including use of: • Any inotropes • Intra-aortic balloon pump (IABP) • Pulmonary artery catheter • Vasodilator
	Arrhythmias, including:

Body system	Anticipated adverse event
	• Supraventricular tachycardia / atrial fibrillation / atrial flutter • Ventricular tachycardia • Requirement for pacing • Requirement for implantable cardioverter defibrillator (ICD)
	Chest pain
	Bleeding (needing re-operation or not)
	Deep vein thrombosis (DVT)
	Pulmonary embolus (PE)
	Pericardial effusion / tamponade
	Congestive heart failure requiring treatment
	Pericarditis requiring treatment
	Blood clots
	Haematoma
	Peripheral thrombophlebitis
Blood & lymphatic disorders	Thrombocytopenia
Pulmonary	Intubation / re-intubation and ventilation
	Respiratory depression
	Pneumonia
	Dyspnoea
	Cough
	Intra-thoracic collection/abscess (requiring drainage or not)
	Acute respiratory failure
	Tracheostomy
	Initiation of mask continuous positive airway pressure (CPAP) or non-invasive ventilation
	Acute respiratory distress syndrome (ARDS)
	Pneumothorax
	Pleural effusion
	Requirement for high flow oxygen
	Atelectasis / pulmonary collapse
	Surgical emphysema requiring intervention
	Bronchopleural fistula
	Prolonged air leak
	Chylothorax
	Acute aspiration
	Tracheobronchial injury
Renal / Urology	New haemofiltration / dialysis
	Acute kidney injury
	Urinary retention requiring reinsertion of urinary catheter, delaying discharge, or discharge with urinary catheter
	Incontinence
Infective	Sepsis
	Wound infection
	Respiratory infection
	Mediastinitis

Body system	Anticipated adverse event
	Urinary Tract Infection (UTI)
	Central venous catheter infection requiring removal or antibiotics
	Unspecified infection
	Other infections requiring antibiotics
Gastrointestinal (GI)	Peptic ulcer / GI bleed /
	Diagnostic laparotomy / laparoscopy
	Ischaemic bowel requiring treatment
	Pancreatitis
	Vomiting
	Nausea
	Diarrhoea
	Abdominal pain
	Dyspepsia
	Constipation
	Dry mouth or throat
	Flatulence
	Intestinal ischaemia
	Delayed gastric emptying requiring intervention or delaying discharge or requiring maintenance of nasogastric drainage >7 days post-operatively
	Dysphagia
	New onset diabetes
Hepatobiliary disorders	Hepatitis
	Jaundice
Metabolism and nutrition disorders	Anorexia
	Increased appetite
	Hyperglycaemia
	Hypoglycaemia
	Hyponatraemia
Neurological / Psychiatric	Acute delirium (including hostility, confusion and emotional lability, depression, anxiety, nervousness, thinking abnormal)
	Agitation
	Hallucinations
	Somnolence
	Dizziness
	Insomnia
	Mental impairment
	Permanent stroke
	Transient ischaemic attack (TIA)
	Fitting/seizure
	Recurrent laryngeal nerve damage
	Acute psychosis
	Delirium tremens
	Other neurological injury

Body system	Anticipated adverse event
Eye disorders	Visual disturbances such as amblyopia, diplopia
Musculoskeletal	Arthralgia
	Myalgia
	Back pain
	Twitching
General disorders & administration site conditions	Fatigue
	Fever
	Peripheral oedema
	Generalised oedema
	Pain
Investigations	Elevated liver function tests: • Aspartate aminotransferase (AST) • Alanine aminotransferase (ALT) • Bilirubin
	Weight gain
Re-operation	Re-operation due to any cause
Injury, poisoning, procedural complication	Incisional hernia
	Acute diaphragmatic hernia
	Bronchoscopy for any cause
	Wound dehiscence
	Conversion from minimal access surgery to open surgery, for any reason
	Recurrent laryngeal nerve damage
	Genital/renal tract injury
	Bronchoscopy for any cause
	Chyle leak / chylous ascites
	Surgical complications, including anatomical/surgical damage (e.g. aortic rupture)

10. Trial methods

To minimise bias, outcome measures are defined as far as possible on the basis of objective criteria. All personnel carrying out outcome assessments will be blinded; this will minimise detection and performance bias.

10.1 Source data

Clinical outcome data will be collected using purpose-designed case report forms (CRFs), which will be completed at baseline, during the participant's surgery and post-operative inpatient stay until discharge, routine post-operative follow-up appointment and the three-month post-surgery follow up (see Table 1~~Table 1~~ for schedule of data collection). The primary data source for these data will be the participant's hospital notes.

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10.2 Planned recruitment rate

CDI can, in principle, be applied to most adults undergoing left side heart valve surgery. The 704 participants will be recruited over 24 months from at least eight high-volume UK NHS cardiac centres. Potential participants will be identified prospectively from lists of patients having cardiac surgery. Research staff considered part of the clinical care team are permitted to access this information.

Based on an average of 250 procedures per year per centre (http://bluebook.scts.org/), we estimate that 70% of patients will be eligible and approached and that 30% of those approached will consent to take part which is in line with the average 35% consent rate achieved across five other adult cardiac surgery trials run in Bristol (n=3055 eligible patients, 1114 consented) that required similar participant involvement. This equates to projected recruitment rate of 35 patients/month when all centres are open to recruitment allowing for a staggered opening of the sites.

10.3 Participant recruitment

Patients undergoing planned left side heart valve surgery will be invited to participate. All potential participants will be sent or given an invitation letter and PIL (approved by the local Research Ethics Committee (REC)) describing the study. The patient will have time to read the PIL and to discuss their participation with others outside the research team (e.g. relatives or friends) if they wish. Most patients will have at least 24 hours to consider whether to participate. In a few cases, this time interval may be as little as 12 hours, for example for patients admitted for urgent planned surgery without prior notification to the waiting list co-ordinator. Despite the short notice, it is important to include these patients for the applicability of the trial findings since about 40% of patients having cardiac surgery are admitted as urgent cases.

Potential participants will be identified and approached with information about the trial prior to their surgery. An initial eligibility check will be performed prior to giving any details about the trial to the patient.

Potential participants will be seen or contacted by a member of the local research team who will answer any questions and take informed consent if the patient decides to participate. Consent will be obtained either by face to face at a clinic appointment or remotely by telephone/video call or electronically using a purposed designed electronic database. The consent process will be described in detail in the study manual.

Details of all patients approached for the trial and reason(s) for non-participation (e.g. reason for ineligibility or patient decline) will be documented. Participant eligibility will be assessed in full as close to randomisation as possible.

10.4 Discontinuation/withdrawal of participants

Each participant has the right to withdraw at any time.

Withdrawals during treatment should not occur due to the nature and duration of the intervention; the duration of the intervention is from before cannulation for CPB and continued until 10 minutes post-CPB. However, a clinician may withdraw a participant at any time if they feel it is in the participant's best interests.

Participants withdrawn from their allocated intervention but willing to continue completing follow-up schedules will be encouraged to do so. If a participant withdraws, data collected up until that point will be included in the analyses.

Reasons for all discontinuations and withdrawals will be captured in the study database and reported.

10.5 Frequency and duration of follow up

After discharge from hospital, participants are followed up at three months after surgery. Data concerning health status will be collected by questionnaires.

10.6 Likely rate of loss to follow-up

Until discharge from hospital, the only losses to follow-up will be due to death or participant withdrawal; these losses are expected to be very few (<2%). We expect loss to follow-up at three months to be minimal (less than 5%); the Titre-2 trial (which randomised over 2000 cardiac surgery patients across 16 centres), achieved >95% follow-up to three months (34).

10.7 Expenses

Any documents that need to be returned by the participant will be given with a pre-paid envelope, so that no expenses are incurred.

If the post-operative DW MRI brain cannot be completed before discharge but the participant is willing to return within the 2-10 day timeframe to undergo the scan, then participants may claim for travel expenses. However, every attempt must be made to complete the DW MRI before the patient is discharged.

11. Statistics

11.1 Sample size calculation

11.1.1 Main trial

The target sample size is 704 participants, 352 per group, which will provide 90% power at a 5% significance level (2-sided) to detect a 25% relative reduction in the incidence of post-operative cerebral infarcts with CDI, assuming a 48% rate in the placebo group. Missing data for the primary outcome is expected to be minimal as the DW MRI brain and assessment of stroke will be completed prior to the participant's discharge home.

Data from our systematic review and meta-analysis (21) showed a 43% relative risk (from 21% to 12%) reduction in the incidence of neurocognitive decline with CDI. It also showed a 47% relative risk reduction in the incidence of stroke over time following the introduction of CDI in Bristol in 2006 from 3.4% in 2005 to 1.6% in 2008. This was estimated from research data on 1754 valve surgeries and 42 strokes. This provided the basis for the target effect size; it is less than the effect observed in our systematic review, which is likely to have overestimated the treatment effect based on small RCTs at unclear or high risk of bias.

The anticipated event rate in the placebo group was chosen based on a meta-analysis of all available observational studies where brain MRI was routinely performed early after open heart valve surgery. Eight studies were identified with sample sizes ranging from 15 to 129 participants and rates of new ischemic lesions at MRI ranging from 38% to 72%. The pooled random effects estimate of new ischemic lesions was 55% (95% CI 48% to 63%). Assuming a 48% event rate (the lower limit of the 95% CI) leads to a larger sample size (compared to assuming a 55% event rate).

11.1.2 Mechanistic sub-study

There are no previous studies investigating the association between embolic load and brain injury post-cardiac surgery, but embolic load detected by TCD shows a strong correlation with DWI positive lesions (r=0.70) during coil embolization of unruptured intracranial aneurysms (35). Assuming a weaker association during open heart surgery the sample size for sub-study has been set at 100 participants which will provide 90% power at a 5% significance level (2-sided) to detect a correlation of 0.32 (linear regression r-squared of 0.1).

11.2 Stopping rules (phase 1)

There are three conditions that might lead the Trial Steering Committee (TSC) to recommend stopping the trial early:

1. A failure to recruit sufficient patients to meet the target sample size within the proposed duration of the grant and refusal of the funder to extend the duration of recruitment
2. A failure to deliver the intervention as allocated

3. A failure to complete the MRI scan according to the protocol before discharge after surgery

With respect to 1., it is planned to recruit the target sample size of 704 randomised participants over 24 months. NIHR-EME will formally review progress against this target after six months of recruitment, with the option of a contract break to minimise financial risk to the NIHR if recruitment does not reach a predefined criterion. After six months of active recruitment 92 participants should have been recruited, if the target recruitment rate is being met. Accepting recruitment typically starts slowly and increases over time as the trial gets established and that there is some variability from one month to the next (e.g. recruitment is typically lower over Christmas and in the summer holiday period than at other times of year). Therefore, it is proposed that trial recruitment will be halted if;

a) fewer than 69 have been recruited within six months and
b) we are unable to provide a plan that satisfies the NIHR-EME to make up the shortfall of 23 participants (i.e. the target recruitment of 4-5 participants per centre per month is not being exceeded and all eligible surgeons are participating)

The BTC CTEU will prepare a report for the Trial Steering Committee (TSC) to consider and make a recommendation to the NIHR-EME.

With respect to 2. and 3., the BTC CTEU will monitor adherence to the protocol throughout the trial and will investigate all cases of non-adherence. The BTC CTEU will prepare a report for the TSC to consider and propose halting the trial if the reasons for non-adherence cannot be addressed satisfactorily.

In addition to monitoring recruitment and adherence, the Data Monitoring and Safety Committee (DMSC) will monitor safety outcomes and the assumptions underpinning the sample size calculation (i.e. the event rate in the placebo group). The DMSC may recommend stopping the trial if the accrued data suggest that the trial is unsafe for one or both groups of participants or that a study of 704 participants will be inadequate, and a larger trial is not feasible.

11.3 Plan of analysis – primary and secondary outcomes

Analyses will be performed on the intention-to-treat basis and will be directed by a pre-specified statistical analysis plan. Analyses will use data from all patients randomised. Primary and secondary outcomes will be compared using logistic (binary variables), poisson (count variables), Cox proportional hazards (time to event variables), or linear mixed model (continuous variables measured at multiple time points) regression, with placebo as the reference group. Mixed models allow all participants with data to be included in the analysis, i.e. partial missing data (assumed missing at random) is permitted. Interactions between treatment and time will be examined and if significant at the 10% level, results will be reported separately for each post-operative time point; otherwise overall treatment effects will be reported. Model validity will be checked using standard methods; if a model is a poor fit, alternative models or transformations will be explored. Outcomes analysed on a logarithmic scale were transformed

back to the original scale after analysis and results presented as geometric mean ratios. Analyses will be adjusted for baseline values, and stratification variables (centre and type of surgery). Findings will be reported as effect sizes with 95% CIs.

11.4 Plan of analysis - mechanistic studies

The analyses of the association between new ischaemic brain injury detected by MRI and each of; intraoperative air embolic load, CBF and TOI will be exploratory. Correlations between measures will be investigated, both overall and by treatment group. Comparisons of measures between treatment groups will follow the analysis plan outlined above.

11.5 Frequency of analyses

The primary analysis will take place when follow-up is complete for all recruited patients. The value of including an interim will be discussed with the DMSC. Safety data will be reported to the DMSC approximately every six months, together with any additional analyses the committee request.

11.6 Criteria for the termination of the trial

The trial may be terminated early by the TSC. A decision to terminate may arise from a recommendation by the DMSC to stop the trial, for example based on *an interim analysis of the data from the trial* or if the results of another study make the completion of the trial unnecessary.

12. Trial management

University Hospitals Bristol and Weston NHS Foundation Trust will act as Sponsor. Responsibility for running the CO2 study will be established via a collaboration agreement with the University of Bristol. Agreements between the Sponsor and participating centres will be required, as well as standard site initiation documents, before recruitment commences. The study will be conducted in accordance with GCP guidelines, the European Union Directive 2001/20/EC on clinical trials, the Data Protection Act and the UK Policy Framework for Health and Social Care Research. The trial will be registered on an open access clinical trial database (ISRCTN). Clinical trial documents will be archived and held by the Sponsor for 15 years after study closure in accordance with the standard operating procedures of the Sponsor and in compliance with the principles of GCP.

The study will be managed by the Chief Investigator (CI), with mentoring and support from senior members of the research team who will provide experience of implementing large scale clinical trials, and the Trial Manager, with full support from the wider BTC CTEU, which is a UK Clinical Research Collaboration registered clinical trial unit (UKCRC Reg. No 11). The BTC CTEU has an established track record of designing, conducting, managing and reporting multi-centre clinical trials. The BTC CTEU has experience in building study database systems and providing randomisation services.

The CI and BTC CTEU team will work with the co-applicants to prepare the final protocol and submit the REC, MHRA and associated HRA applications. The BTC CTEU will prepare the study manual, provide the randomisation service and design and implement the data management system.

The CI, BTC CTEU team and Sponsor will endeavour to ensure that the trial runs according to the agreed timetable, recruitment targets are met, the CRFs are completed accurately, the trial complies with relevant ethical and other regulatory standards, and that all aspects of the study are performed to the highest quality. The CI and BTC CTEU team will also train investigators at participating centres, check that centre are ready to start ("green light") and monitor their progress during the study. The Trial Manager will be the contact point to provide support and guidance to the participating centres throughout the study.

12.1 Day-to-day management

The CO2 study will be managed by a Trial Management Group (TMG), which will meet face-to-face or by teleconference approximately monthly. The TMG will be chaired by the Chief Investigator and members of the named research team will be invited as appropriate (see Chief Investigator & Research Team Contact Details).

An appropriately qualified person by training will be responsible at each site for identifying potential trial participants, seeking informed participant consent, randomising participants, liaising with pharmacy, collecting trial data and ensuring the trial protocol is adhered to.

12.2 Monitoring of sites

12.2.1 Initiation visit

Before the study commences, training session(s) will be organised by the BTC CTEU. These sessions will ensure that personnel at each site involved fully understand the protocol, CRFs, the operational requirements of the study and the assessments to be conducted within the trial e.g. neurocognitive function tests.

12.2.2 Site monitoring

The trial coordinating centre will carry out regular monitoring and audit of compliance of centres with GCP and data collection procedures described in section 7.3. Monitoring of data collection will be via the study database (checks for data completeness and routine data query review), which will be carried out on a regular basis. The Investigator Site File and CRFs will be monitored by site self-completed checklists at least once in the lifecycle of the trial. The TMG will review accumulating data on, including but not limited to, screening, eligibility, recruitment, data completeness, adherence to trial visits and procedures, adverse events and protocol deviations in the form of central monitoring reports generated approximately monthly.

12.3 Trial Steering Committee and Data Monitoring and Safety Committee

An independent TSC will be established to oversee the conduct of the study. It is anticipated that the TSC will comprise the CI, an independent chair and at least two additional independent members, at least one of whom will be a patient/public representative. The TSC will develop terms of reference outlining their responsibilities and operational details. The TSC will meet before recruitment in the trial begins and then approximately every six months during the course of the study.

An independent DMSC will be established to review safety data during the course of the study and will advise on interim analyses. The DMSC will develop a charter outlining their responsibilities and operational details. The DMSC will meet jointly with the TSC, before recruitment in the trial begins and they will meet approximately every six months after recruitment has begun.

Stopping rules for the trial will be discussed at the first joint TSC/DMSC meeting, and decisions documented in the DMSC Charter.

13. Ethical considerations

13.1 Review by an NHS Research Ethics Committee

The research will be performed subject to a favourable opinion from an NHS REC and Health Research Authority (HRA) approval, including any provisions of a non-NHS site assessment form. Ethics review of the protocol for the trial and other trial related essential documents (e.g. PIL and consent form) will be carried out by a UK NHS REC. Any subsequent amendments to these documents will be submitted to the REC and HRA for approval prior to implementation.

13.2 Risks and anticipated benefits

The conduct of this study will allow us to determine which of the treatments is the most clinically effective, as such, this study will allow us to make evidence-based recommendations for the treatment of this patient population.

There are no known risks or benefits in either intervention in this trial population; this will be quantified as part of this study.

13.3 Informing potential study participants of possible benefits and known risks

Information about possible benefits and risks of participation will be described in the PIL.

13.4 Obtaining informed consent from participants

All participants will be required to give written informed consent. This process, including the information about the trial given to patients in advance of recruitment, is described above in section 10.3.

The PI or members of the team delegated by the PI will be responsible for obtaining informed consent. The consent process will be described in detail in the study manual. Research personnel authorised to obtain consent will be recorded on the Delegation of Responsibilities Log. All individuals obtaining informed consent will have received GCP training.

13.5 Co-enrolment

Subject to agreement with the Chief Investigator, a participant may be co-enrolled to a non-intervention study as well as to the CO2 study. A participant must not be co-enrolled to another intervention study while they are actively participating (up to the three-month follow up visit) in the CO2 study.

14. Research governance

This study will be conducted in accordance with:

- The Medicine for Human Use (Clinical Trial) Regulations 2004
- Good Clinical Practice (GCP) guidelines
- UK Policy Framework for Health and Social Care Research

14.1 Sponsor approval

Any amendments to the trial documents must be approved by the sponsor prior to submission to the HRA, REC and MHRA as applicable.

14.2 NHS confirmation of capacity and capability

Confirmation of capacity and capability is required from each participating site prior to their participation in the trial.

Any amendments to the trial documents approved by the REC, HRA and MHRA (if applicable) will be submitted to participating sites for information and implementation, as required.

14.3 Investigators' responsibilities

Investigators will be required to ensure that local research approvals have been obtained and that any contractual agreements required have been signed off by all parties before recruiting any participant. Investigators will be required to ensure compliance to the protocol and study manual and with completion of the CRFs. Investigators will be required to allow access to study documentation or source data on request for monitoring visits and audits performed by the Sponsor or the BTC CTEU or any regulatory authorities.

Investigators will be required to read, acknowledge and inform their trial team of any amendments to the trial documents approved by the REC that they receive and ensure that the changes are complied with.

14.4 Monitoring by sponsor

The study will be monitored and audited in accordance with University Hospitals Bristol and Weston's Monitoring and Oversight of Research Activity SOP, which is consistent with the UK Policy Framework for Health and Social Care Research and the Medicines for Human Use (Clinical Trials) Regulations 2004. All study related documents will be made available on request for monitoring and audit by the sponsor (or the BTC CTEU if they have been delegated to monitor see 7.2.2), the relevant REC and for inspection by the MHRA or other licensing bodies. A monitoring plan will be prepared by the Sponsor.

14.5 Indemnity

This is an NHS-sponsored research study. For NHS sponsored research if there is negligent harm during the clinical trial when the NHS body owes a duty of care to the person harmed, NHS Indemnity covers NHS staff, medical academic staff with honorary contracts, and those conducting the trial. NHS Indemnity does not offer no-fault compensation and is unable to agree in advance to pay compensation for non-negligent harm. Ex-gratia payments may be considered in the case of a claim.

14.6 Clinical Trial Authorisation

CDI is classed as an investigational medicinal product and a Clinical Trial Authorisation (CTA) from the MHRA must be in place before starting the trial.

15. Data protection and participant confidentiality

15.1 Data protection

Data will be collected and retained in accordance with the UK Data Protection Act 2018 and UK General Data Protection Regulation (UK-GDPR) 2020.

15.2 Data handling, storage and sharing

15.2.1 Data handling

The CO2 study team will provide the Sponsor with a Data Management Plan prior to the study opening to recruitment.

Data will be entered into a purpose-designed server database hosted on the NHS network. Information capable of identifying participants and the nature of treatment received will be held in the database with passwords restricted to CO2 study staff at the participating site and the co-ordinating centre.

The database and randomisation system will be designed to protect patient information in line with data protection legislation. Trial staff will ensure that the participants' anonymity is maintained through protective and secure handling and storage of patient information at

participating sites and in accordance with ethics approval. All documents will be stored securely and only accessible by trial staff and authorised personnel. Data will be collected and retained in accordance with data protection legislation.

Access to the database will be via a secure password-protected web-interface. Study data transferred electronically to the University of Bristol network for statistical analyses will contain the participant's unique study identifier only and will not include any personal identifiable information. with the unique study identifier and transferred via a secure network. Participants will be identified by their initials and unique study identifier on the secure NHS hosted database. Data will be entered promptly, and data validation and cleaning will be carried out throughout the trial.

Each recruiting centre will have access to the study manual, which will cover database use, data validation and data cleaning. The BTC CTEU will maintain and update the study manual as required.

15.2.2 Data storage

All study documentation will be retained in a secure location during the conduct of the study and for 15 years after the end of the study, when all patient identifiable paper records will be destroyed by confidential means. Where trial related information is documented in the medical records, these records will be identified by a label bearing the name and duration of the trial and clearly stating the 'do not destroy before' date. Where electronic records are in use, local site policy will be followed. In compliance with the MRC Policy on Data Sharing, relevant 'meta'-data about the trial and the full dataset, but without any participant identifiers other than the unique participant identifier, will be held indefinitely (University server).

15.2.3 Data sharing

Data will not be made available for sharing until after publication of the main results of the study. Thereafter, anonymised individual patient data will be made available for secondary research, conditional on assurance from the secondary researcher that the proposed use of the data is compliant with the MRC Policy on Data Sharing regarding scientific quality, ethical requirements and value for money. A minimum requirement with respect to scientific quality will be a publicly available pre-specified protocol describing the purpose, methods and analysis of the secondary research, e.g. a protocol for a Cochrane systematic review. A second file containing patient identifiers would be retained for record linkage or a similar purpose, subject to confirmation that the secondary research protocol has been approved by a UK REC or other similar, approved ethics review body. Patient identifiers would not be passed on to any third party.

16. Dissemination of findings

A full report of the trial findings will be provided to the EME. Findings of the trial will be presented at national and international conferences and published in peer-reviewed journals. We will link with the British Heart Foundation, with the valve group of the British Society of

Cardiothoracic Surgeons (SCTS) to highlight the importance of effective neuroprotection during open heart surgery. In view of the current variation in clinical practice, we will also link to guidelines committees of the European Society of Cardiothoracic Surgeons, of which the CI is a member, to produce a consensus paper and recommendations on the use of carbon dioxide insufflation for neuroprotection. Social networking media will be used to disseminate and publicise the trial via our website, Facebook and Twitter streams. PPI groups will be consulted to identify how we can best publicise the trial findings.

Expected outputs include publication of the trial results, informing clinicians and patients on the efficacy of carbon dioxide insufflation for preventing neurocognitive dysfunction after heart surgery. The results of the trial will inform national and international guidelines on optimising the care pathway for patients undergoing valve surgery.

17. References

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18. Amendments to protocol

Amendment number (i.e. REC and/or MHRA amendment number)	Previous version	Previous date	New version	New date	Brief summary of change	Date of ethical approval (NA if non-substantial)
SA1	1.0	29/04/2020	2.0	28/09/2020	Updates to the participant pathway (including remote consent and follow up), surgical guidance, and addition of background safety data.	19/10/2020
SA2	2.0	28/09/2020	3.0	24/11/2020	Updates to the inclusion criteria and randomisation strata.	05/01/2021
SA3	3.0	24/11/2020	4.0	25/05/2021	Update to Chief Investigator details on front page	16/06/2021
SA4	4.0	25/05/2021	5.0	23/09/2021	Updates to eligibility criteria, addition of Advance Nurse Practitioners confirming eligibility. Updates to surgical procedures to allow for wider range of equipment and flow rates to be	17/11/2021

					used, which do not affect the primary outcome data. Updates throughout to add clarity to procedures.	
SA5	5.0	23/09/2021	6.0	Xx/11/2021	Updated research team. Updated terminology in the dosing schedule from lower to inferior. Updated surgical procedures to include normothermic cardiopulmonary bypass procedures and PaCO2 levels in kilopascals.	