Organ donation after circulatory death with Normothermic Regional Perfusion (NRP)

Dutch National Protocol V3.0

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Towards more donor organs due to functional assessment and immediate correction of oxygen depletion

Revision with extention to all 50+ DCD donors and combination with thoracic cold lung retrieval

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1. Introduction

Normothermal regional perfusion (NRP) during circulatory arrest (DCD) donation restores the circulation of the abdominal organs with the aim of reconditioning them. Oxygenated blood at a temperature of 33-37°C will be perfused through the abdominal compartment for a period of 2 hours. During this period, the organs in the DCD situation can be assessed for function and quality.

This leads to:

1. Less pressure to extract organs quickly, moving from a DCD scenario to a DBD scenario.

2. Replenishing ATP reserves.

3. Possibilities to determine organ function and quality.

NRP will be performed in Maastricht category III and V DCD donors where the kidneys or kidneys and lungs have been accepted for transplantation, and the other abdominal organs initially fall outside the criteria. The expectations are that NRP will nevertheless prove to be transplantable a number of pancreata and livers. This will increase the number of organs transplanted per donor, with the organs being transplanted also expected to be of better quality. NRP is NOT an experimental procedure, but is already being used successfully in the UK, Spain and France. In the UK, NRP is performed in Category III and IV DCD donors. In Spain and France in category II and III DCD donors. In these countries, the number of transplanted kidneys and livers per DCD donor has increased significantly. Furthermore, the risk of postoperative complications after transplantation has decreased.

It is important to note that within the current protocol, protocol-accepted lungs take priority over the NRP liver, should any concerns arise regarding organ preservation. After all, the lungs are primarily accepted for a recipient, the liver only under the premise of a successful NRP procedure. The combination of cold thoracic perfusion with normothermic abdominal perfusion has been discussed, practiced and found to be safe by the supervisors of the thoracic teams of UMCU, UMCG and Erasmus MC and has been used successfully several times without problems.

This protocol describes the technical aspects of the procedure and highlights the changes from the current DCD procedure. It is written using the National UK protocol.

2. Independent Retrieval Team (ZUT) during NRP procedure

When performing NRP during a Multi Organ Donation (MOD), the independent retrieval team (ZUT) will at least consist of:

- 2 surgeons; of which 1 MOD certified
- 2 Operation Assistants
- 1 Transplantation Coordinator
- 1-2 Organ Perfusion Specialist(s)

3. Pre-Retrieval Setup

The preparation for an NRP procedure requires 30 minutes extra time compared to a standard DCD procedure.

The different steps include the following:

● The operating assistant will set up the operating room in a similar way to a DBP procedure. This includes preparing for diathermy and the power saw for sternotomy. Since the Donor Assist (DA), the device that is used for NRP, requires the supply of oxygen, the operating assistant will take into account where the oxygen connections are located (this is the place where the DA will be placed).

● Prepare the aorta and v. cava inferior cannulas. Two 50mL syringes should be filled with heparinized saline (5,000 units of heparin in 500mL NaCl). They will be used to airtightly connect the aorta and v. cava inferior cannulas and the arterial and venous perfusion lines.

● After the NRP circuit has been prepared, the sterile part of the circuit (the "loop") should be handed over to the surgical assistant. This should then be placed on a sterile table and secured. The pressure line is returned to the perfusionist who then connects it to the DA. After asystole is established, the perfusionist and surgical assistant place tubing clamps on the arterial and venous perfusion lines. The circuit bypass line is opened.

a. NRP Setup

The analysis equipment (Skyla and EPOC or i-STAT) must be turned on and the sampling table prepared. The equipment is operated by the perfusionist. The DA is prepared and the heat exchanger must be turned on. The temperature should be set at 37°C. The preparation of the DA is performed according to the Organ Assist protocol. (Appendix 1 for detailed info: Step by Step Guide Donor Assist).

b. Preparation Cold Perfusion

Cold University of Wisconsin Solution (UW) should be prepared as for a standard DCD. The perfusion line is vented so that it can be used immediately if NRP cannot be started or if problems occur during NRP. Heparin will also be kept at hand if NRP cannot be started and a cold perfusion has to be immediately changed. This will be done by the transplant coordinator.

c. Preparation Blood

The transplant coordinator arranges 6 packed cells (erythrocyte concentrate) with cross-matched blood on the donor. Four of these must be present in the operating room before the start of NRP. The other two are kept cool, the transplant coordinator knows where and can get them if necessary. In case of combined thoracic and abdominal retrieval, 8 PC are ordered.

4. Priming Perfusate

The DA should be primed with liquid and drugs consisting of:

- Sodium Bicarbonate 8.4% - 1mL/kg
- Ringer's lactate - 1500mL
- Albumin (200g/L) - 100mL
- Heparin - 50,000 units

- Fluconazole - 200mg
- Meropenem - 250mg
- Vancomycin - 500 mg
- Methylprednisolone - 1gram

In the case of Erasmus MC:

- Pancuronium - 12mg

In the case of the LUMC:

- Rocuronium - 100mg

Additional packed cells (4-8 units), lactated ringer and albumin are required at a later time, depending on the fluid level in the reservoir.

5. Relationship NRP and time of cessation of treatment (Switch off)

The usual procedure for withdrawal of life supporting treatment is followed, as with a standard DCD procedure. After cessation of treatment, a maximum of 2 hours is waited for circulatory death of the donor, after which the donation procedure is terminated according to protocol.

The acceptable duration of ischemia during the death phase should be discussed with recipient centers for specific organs. Typically, a functional ischemic phase (time with saturation <80% or blood pressure <50 mm Hg) of 30-45 minutes is now accepted for livers and functional ischemia up to 120 minutes for kidneys.

Contrary to the current acceptance protocol for DCD livers, the time between switch-off and the actual occurrence of this functional ischemia is disregarded.

A liver from a donor that dies between 1 and 2 hours after switch-off is accepted for NRP if no functional ischemia has occurred in the first hour.

6. Surgical Protocol

6.1. Pre-NRP phase

Incision: Midline incision (Processus Xiphoideus to Os Pubis).

A tubing clamp is applied to the aortic canula. The distal infrarenal aorta is identified at the aortic bifurcation and clamped or ligated distally. The aortic cannula (22/24 Fr DLP Medtronic cannula) is inserted proximally, checking the position of the tip. The cannula is secured in place using a vascular tourniquet.

A tubing clamp is applied to the vena cava canula. The infrarenal inferior v. cava is then identified at the iliac bifurcation and clamped or ligated. The venous cannula (36/46/46 Fr Edwards Lifesciences Q3 Trim-Flex Triple Stage) is inserted proximally with the tip positioned directly below the diaphragm. The cannula is secured in place using a vascular tourniquet.

The arterial perfusion line of the circuit (the perfusion line with the red line) is then connected to the aortic cannula. Both sides are capped with heparin water to eliminate all air bubbles. The venous perfusion line of the circuit (the perfusion line with the blue line) is then connected to the venous cannula. Again, both sides are capped with heparin water to eliminate air bubbles. After this, the surgeon removes the clamps on the venous and arterial perfusion lines, respectively, and the perfusionist opens the venous perfusion line and checks for venous return.

The perfusion cannot be started yet!

Subsequently, the aorta is clamped, either initially intra-abdominal or intra-thoracic by means of a sternotomy. **Only when the aortic clamp is in place can the perfusion be started.**

If an initial intra-abdominal clamping of the aorta is chosen, a sternotomy is subsequently performed and the abdominal clamp is moved thoracically below the level of the left subclavian artery, to avoid injury to the left hepatic lobe. The suprahepatic inferior v. cava is also clamped to prevent mixing with deoxygenated blood from the thorax and upper limbs.

6.2. Switch to NRP

▪ The heat exchanger temperature should be 33°C during the first 30 minutes of perfusion, 35°C between 30 and 60 minutes and 37°C after 60 minutes.

▪ The O2 is set to a FiO2 of 50% and the gas flow to 1 L/min. Changes in the oxygen/air mixture may be necessary depending on the arterial blood gas analysis.

▪ After clamping the aorta proximally, the surgeon communicates with the perfusionist that the perfusion can be started. After this, the perfusionist opens the arterial perfusion line and closes the bypass line of the circuit.

▪ The NRP is intentionally continued for 2 hours before proceeding to organ removal.

If for any reason NRP cannot be started, then cold perfusion should be done immediately as with a standard DCD.

6.3. Hemodynamic and Biochemical Targets

Based on international experience, the following goals are pursued during the NRP:

● Pump flow > 1.7 L/min

● Temperature 33-37°C (0-30min: 33°C, 30-60min: 35°C, 60-120min: 37°C)

● Gas flow 1-4 L/min (air/O2 mix depending on the PaO2)

●PaO2: 110-150mmHg

● blood pH 7.35-7.45

● Hematocrit > 20%

6.4. During NRP

a. Limit blood loss

Immediately after the start of the NRP, the sternum is opened and the aorta clamped in the thorax, if this was not done initially. This is followed by a round of hemostasis through the abdomen. Special points of attention are: lumbar arteries and veins that bleed back through the insertion openings of the cannulas, or from the ligated distal side. This can be remedied by placing an extra slim clamp laterally along the cannula. In the thorax, the lungs are mobilized, if no lung extraction takes place, to find and bind the azygos and hemiazygos veins. This greatly limits blood loss in the thorax and prevents refilling of the heart with venous blood from the top of the body. Specific attention from the thoracic surgical team is requested to clamp the small bronchial arteries when mobilizing tissues from the aorta to prevent additional blood loss. In addition, it has been agreed in the LORUT that in the case of combined NRP lung removal, the procedure will not be performed by a less experienced lung removal surgeon as a teaching moment, in order to keep the pace high and the blood loss minimal, as also applies with regular combined DCD abdominal lung removal. See also in detail Appendix 3.

b. Cannulate common bile duct

After initiation of NRP, the common bile duct can be transected distally and cannulated with an IV extension line for collection of bile. The cannula can be fixed with a snugger. The infusion extension line is extended with a pressure line, this line will be given to the perfusionist, who will connect it to a reservoir with a three-way valve. For example, the amount of bile production can be evaluated and samples can be taken.

c. volume management

Careful hemostasis of the abdominal wound margins, retroperitoneal tissues and sternum should be performed during NRP. Bleeding occurs frequently during the first 60 minutes. Significant blood loss can potentially occur during perfusion, partly due to the administration of 50,000 units of heparin in the perfusion fluid. For this reason, sufficient volume correcting fluids must be present.

In case of volume loss, the perfusionist will deliver the following “volume replacement bolus” of approximately 800 ml:

1 unit packed cells, 100mL albumin (200g/L) and 500mL Ringer lactate

d. Correcting pH

Bicarbonate may be added depending on the results of the arterial blood gas analysis. The pH can also be influenced by the perfusionist by changing settings on the gas mixture. Based on international experience, it is preferable not to perform artificial correction of the pH in the first half hour after starting NRP, because a low pH initially protects against ischemic injury and rapid correction can be counterproductive.

e. Biochemical Assessment

An arterial blood sample is taken every 30 minutes to assess liver and kidney quality and function, as described in 6.6.

f. Assessment of abdominal organs

The surgeon should initially perform a rapid macroscopic evaluation of the abdominal organs to determine whether the NRP perfusion is leading to adequate organ perfusion. Extensive dissection of the organs at this stage is not recommended because it takes approximately one hour for arterial spasms to resolve and bleeding can occur during dissection that prevents further NRP. After one hour the dissection of the organs to be transplanted can be started, as during a DBD procedure and described in the Model Protocol for Organ Removal. This is not necessary, but can save a lot of time in the extraction phase at the end of the perfusion and prevents extensive dissection on the backtable with extension of the cold ischemia time.

g. Evaluation of the organs

Kidney

- Macroscopic aspect.
- Urine production.
- Creatinine increase.

Liver

- Macroscopic aspect before, during and after perfusion.
- Presence of bile production and volume.

▪ Transaminase evaluation. A sharp rise in ALT is an indication of a damaged liver. During NRP, the ALAT at the end of the procedure should not be more than 500 IU/ml. Also, there should not be a significant upward trend in the ALT values during NRP, but a plateau phase.

▪ Lactate clearance. During NRP, lactate should decrease significantly, but in case of open vena cava clamp at least 1 mmol/l per hour.

▪ Glucose levels. A high glucose level indicates adequate liver stress response. During NRP, glucose should be decreasing as indicator of normalization of physiology.

Pancreas

▪ Macroscopic aspect, i.e. evaluation of edema formation

Guidelines for determining acceptance of the liver for transplantation:

Livers from non-extended donors are: donor age between 50 and 60, donor BMI <30 and pre-op transaminases <3x ULN (~100 U/ml). In the Netherlands, these non-extended donors are allocated according to standard ET patient allocation rules to a specific patient on the national waiting list. These livers additionally receive DHOPE in the recipient center. These livers can be transplanted, regardless of the lab values during the NRP, because this category is now transplanted as-is, even without NRP assessment. Only in case of difficult NRP procedure, additional injury to the graft should be ruled out.

Extended donors are donors over the age of 60, or transaminases > 3x ULN or a BMI > 30. These livers are offered as center allocation outside national protocol, in which the liver is transplanted by the same team that does the retrieval for close communication and short ischemia times. These livers additionally receive DHOPE in the recipient center. The acceptance criteria during NRP for these livers may differ from center to center, but usually incorporate:

- Transaminases stabilized during perfusion and < 500 U/ml

- Bile production

- pH bile > 7.40 at any time during the procedure

- additional bile quality requirements up to center preference

6.5. Post NRP

After 2 hours, the NRP is discontinued and the perfusionist will open the circuit waste-bag allowing the perfusate to drain. Cold regular preservation fluid (UW) is connected to the arterial cannula and the cold perfusion can be started under pressure by the transplant coordinator. The abdomen is cooled with ice and cold saline, according to standard retrieval procedure.

The preparation of the back-table proceeds according to the Model Protocol for Organ Retrieval.

6.6 Sampling and determination

Blood gas and biochemistry

The iSTAT/EPOC and the Skyla HB1 are used for arterial blood gas analysis and biochemistry determinations, respectively. The arterial blood gas analysis and biochemistry determinations will be used to map organ function and viability. During NRP, the following determinations are made at time 5, 30, 60, 90 and 120 minutes:

Arterial blood gas: pH, pCO2, pO2, HCO3-, BE, Na+, K+, Glucose, Lactate, Ht, Hb, Sat.

Biochemistry: Bilirubin, AF, ALT, AST, GGT, Albumin, Urea, Creatinine

blood samples

During NRP, blood samples will be taken for quality control. Blood samples will be collected at the following times: Before switch-off, after 5, 30, 60, 90 and 120 minutes of NRP. The samples before switch-off will be collected by the transplant coordinator. Three blood tubes are used as standard: EDTA, SST and Citrate.

The blood samples are processed in the usual way. After 5, 60 and 120 minutes of NRP, the surgeon will also take blood samples from the v. porta and v. hepatica, which are then handed over to the perfusionist.

Bile and urine samples

A urine sample should be taken by the transplant coordinator before switch off. After 5, 60 and 120 minutes of NRP, urine will be collected if there is production. After 30, 60, 90 and 120 minutes of NRP, bile will be collected if there is production. Bile will be assessed by the iSTAT/EPOC for pH.

Tissue samples

Tissue samples will be collected during NRP for quality control. After 5 and 120 minutes of NRP, the surgeon will take biopsies of the liver, common bile duct and pancreas which are then presented to the perfusionist. After 120 minutes of NRP, the surgeon will also take a kidney biopsy if the kidney has been allocated to Erasmus MC or LUMC. All biopsies are stored in formalin, in RNA later or snap-frozen in isopentane on dry ice.

Processing Samples

See the SOP sampling (Appendix 2) for the sample collection and processing protocol.

Appendix 3: concomitant hypothermic lung retrieval / NRP protocol

Technique

Rapid sternotomy and laparotomy are undertaken, aortic and IVC are cannulated in the abdomen. Meanwhile, the descending thoracic aorta is clamped by the thoracic surgeon through the posterior pericardium or through the left pleural cavity, as desired, just above the diaphragm. The tip of the NRP venous cannula is withdrawn below the level of the diaphragm and the supra-hepatic IVC is stapled (TA) or clamped at the cavo-atrial junction. This maintains a good venous return into the NRP circuit. Take care to leave enough supra-hepatic IVC cuff for LTx.

Based on the first experience with staple insufficiency at the suprahepatic IVC, placement of an extra vascular clamp is advised.

The ascending aorta is stapled (TA) or clamped, the main pulmonary artery (PA) is cannulated with a purse-string suture secured cannula for cold flush-perfusion. The left atrial appendage is vented widely. Alternatively, the main pulmonary artery (PA) may be flushed through the valve (be careful not to damage the heart valve in case of tissue donation), following an incision in the RVOT (not in case of DCD heart donation).

Whilst abdominal NRP circuit is established, the donor is reintubated and a bronchoscopy is performed. The lungs are inflated with a single recruitment manoeuvre and kept inflated with 100% oxygen.

While waiting for the pulmonary flush to be delivered, the superior vena cava (SVC) is stapled (TA) and divided just below the azygos take off and the systemic connections of the heart are disconnected, leaving the IVC clamped within the pericardium. The division of the main vessels proximal to the clamps ensures that there is no blood loss, to avoid compromising the NRP flow. After pulmonary flush with cold Perfadex solution is commenced, ventilation is started at half tidal volume with 5cmH_20 PEEP and 40% oxygen. The pleurae are opened widely and lungs inspected and palpated, ensuring adequate delivery of flush and topical cooling with copious volumes of 4°C saline. The diaphragmatic surface of the liver can be protected from trans-diaphragmatic cooling using a folded gauze pack soaked in warm saline, topped up throughout the duration of the lung procurement.

Once the cold pulmonary flush is completed, the main pulmonary artery is divided just proximal to its bifurcation. The lungs are allowed to deflate at this stage. The left atrium is divided, leaving behind

an adequate cuff for the lungs and the excised heart is removed for later recovery of heart valves. The pericardium above the diaphragm is further incised, the inferior pulmonary ligaments are divided and the plane up to and behind the trachea is developed. The trachea is dissected bluntly circumferentially in the space between the SVC and aorta and pulled down to gain as much length as possible. Direct bronchial artery branches from the aorta are stapled or cross clamped. Special care is given to tying or clamping the (hemi)azygos vein, as this is a major source of back bleeding to the heart and instability during NRP.

The ETT tube is withdrawn, a breath with 50% tidal volume is delivered and the trachea is stapled with a bronchial stapler and divided above the staple line. The lung block is removed and complete haemostasis of the mediastinum should be ensured. On the back-table retrograde pulmonary venous flush of the lungs is performed with 1000 ml of Perfadex before packaging of the lungs.

Normothermic regional perfusion should continue as per protocol.