

# Pulmonary artery perfusion versus no pulmonary perfusion during cardiopulmonary bypass

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This review has been accepted as a thesis together with 5 original papers by University of Copenhagen 23th of August 2016 and defended on 28th of October 2016. Please contact the author for supplementary material including the appendix.

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Dan Med J 2018;65(3):B5473

## ORIGINAL PAPERS

### Paper I

**Buggeskov KB**, Wetterslev J, Secher NH, Andersen LW, Jonassen T, Steinbrüchel DA.

Pulmonary perfusion with oxygenated blood or Custodiol HTK solution during cardiac surgery for postoperative pulmonary function in COPD patients: a trial protocol for the randomized, clinical, parallel group, assessor and data analyst blinded pulmonary protection trial. *Trials*. 2013;14:30.

### Paper II

**Buggeskov KB**, Jakobsen JC, Secher NH, Jonassen T, Andersen LW, Steinbrüchel DA, Wetterslev J. Detailed statistical analysis plan for the pulmonary protection trial. *Trials*. 2014;15:510.

### Paper III

**Buggeskov KB**, Sundskard MM, Jonassen T, Andersen LW, Secher NH, Ravn HB, Steinbrüchel DA, Jakobsen JC, Wetterslev J. Pulmonary artery perfusion versus no pulmonary perfusion during cardiopulmonary bypass in patients with chronic obstructive pulmonary disease: a randomised clinical trial. *BMJ Open Respir Res*. 2016 Jan 9;3(1):e000146.

### Paper IV

**Buggeskov KB**, Nielsen JB, Wetterslev J. Pulmonary perfusion versus no pulmonary perfusion during cardiopulmonary bypass

for cardiac surgery. Protocol. *Cochrane Database of Systematic Reviews* 2014, Issue 5. Art. No.: CD011098. DOI: 10.1002/14651858.CD011098.

### Paper V

**Buggeskov KB**, Grønlykke L, Risom EC, Wei M, Wetterslev J. Pulmonary artery perfusion versus no pulmonary perfusion during cardiopulmonary bypass. Submitted to *Cochrane Database of Systematic Reviews* 2016 Aug.

## ABBREVIATIONS

AVR	Aortic valve replacement
CABG	Coronary artery bypass graft
CCT	Controlled clinical trial
CO <sub>2</sub>	Carbon dioxide
CI	Confidence interval
CPB	Cardiopulmonary bypass
COPD	Chronic obstructive pulmonary disease
FEV <sub>1</sub>	Forced expiratory volume in 1 sec
FiO <sub>2</sub>	Inspired fraction of oxygen
FVC	Forced vital capacity
HTK	Histidine-tryptophan-ketoglutarate
MD	Mean difference
MEM	Linear mixed-effects model
O <sub>2</sub>	Oxygen
PaO <sub>2</sub>	Partial arterial oxygen pressure
PF	PaO <sub>2</sub> /FiO <sub>2</sub>
RCT	Randomised clinical trial
SAE	Serious adverse event
TSA	Trial sequential analysis

## INTRODUCTION

Pulmonary dysfunction following cardiac surgery with cardiopulmonary bypass (cpb) is associated with prolonged hospitalization and increased incidence of morbidity and mortality. It varies from only a short need of mechanical ventilation to fever, productive cough, pulmonary oedema, respiratory failure, and in the most severe cases adult respiratory distress syndrome (1–5). A recent retrospective observational study found that more than 30% of the patients required an extended duration of pulmonary support, and that was associated with increased risk of postoperative mortality (6).

The frequency of CPB-dependent cardiac surgery has declined in Denmark during the last 15 years as minimal invasive and percutaneous cardiac surgery has evolved. In 2001, 2.760 patients underwent primary isolated coronary artery bypass grafting (CABG) compared with 1.751 patients in 2015. On the other hand, the patient population has become older with multiple co-morbidities making it increasingly important to protect and preserve organ function during major surgery as open cardiac surgery with CPB (7).

The severity of pulmonary dysfunction is related to the patients preoperative pulmonary function (8–10) and therefore are patients with reduced lung function facing a multitude of problems during and after cardiac surgery (11–13). Increasing evidence indicates that pulmonary protection by pulmonary artery perfusion with blood (14–20) or a preservation solution (21–24) during CPB may attenuate postoperative hypoxaemia, by supplying metabolic substrates to the lung tissue and diminishing the lungs exposure to warm ischemia (25).

This thesis is based on a randomised clinical trial (RCT) assessing effects of two pulmonary artery perfusion techniques in patients with chronic obstructive pulmonary disease (COPD), and a systematic review assessing benefits and harms of pulmonary artery perfusion during CPB. The thesis contains a description of the undertaken trial and systematic review, and a discussion of the applied methods and results. Finally, the evidence for pulmonary artery perfusion, and a possible future trial, will be discussed.

## BACKGROUND

The aetiology of pulmonary dysfunction is multifactorial and can roughly be separated into poor postoperative lung mechanics and abnormal gas exchange. Part of the alterations in lung function may be attributed to surgical factors as sternotomy, hemidiaphragm paresis with concomitant atelectasis, wound pain, drain discomfort, and pleural effusion (26). However, cpb is considered to be a main contributor to postoperative pulmonary dysfunction due to 1) the contact between blood and the heart and lung machines artificial surfaces, 2) non-physiologic laminar perfusion of the systemic circulation, 3) no active perfusion of the pulmonary circulation, resulting in 4) warm ischemic reperfusion injuries of especially the lungs, and 5) release of endotoxins from the splanchnic area, all leading to 6) activation of the inflammatory response and consequently for the lungs, 7) interstitial pulmonary oedema (27–29).

## PULMONARY DYSFUNCTION AFTER MAJOR SURGERY AND CPB

One question is whether the lung injury purely is related to the use of CPB, or if pulmonary dysfunction in some degree develops after any major surgery with general anaesthesia. It has been shown by CT scanning that general anaesthesia induces atelectasis in almost all patients (30), however CPB appears to cause an additional lung injury and to delay patients pulmonary recovery compared with other types of major surgery (31). This is probably due to damaging effects of the systemic inflammatory response associated with CPB (32). Continuing refinement of CPB materials and improved anaesthetic management (ex. early extubation leading to fast-track recovery) have in some degree reduced the additional lung injury.

## ON-PUMP VERSUS OFF-PUMP CARDIAC SURGERY

Off-pump CABG has been associated with a reduced cytokine response compared with on-pump CABG (33–35) hypothesising

that an attenuated inflammatory response could diminish postoperative pulmonary dysfunction.

Circulating neutrophils and monocytes, as well as the level of neutrophil elastase, procalcitonin, and oxidative stress, reflects the degree of lung injury and was significantly reduced following off-pump CABG compared to on-pump CABG (35–37). Still, both on-pump and off-pump CABG patients experience similar degrees of decreased partial arterial oxygen pressure (PaO<sub>2</sub>), increased Alveolar-arterial O<sub>2</sub> gradient, and a higher percentage of pulmonary shunt fractions following CABG (38–40). In a systematic review off-pump CABG increased long-term follow-up mortality compared to on-pump CABG with no differences in myocardial infarction, stroke, renal insufficiency, or coronary re-intervention between the two groups (41). For the outcome intubation time off-pump repeat CABG in high-risk patients may be beneficial (42), but the positive effect was not found for patients undergoing primary CABG (38,40,43). The disturbances in lung mechanisms following on-pump cardiac surgery (39) can not only be explained by the CPB itself, although it seems to be a major contributor to the postoperative pulmonary dysfunction (38).

## HYPOTHERMIC VERSUS NORMOTHERMIC CPB

Effects of hypothermic versus normothermic CPB are still debated and different regimes are used for different types of cardiac surgery. One study found that the perfusion temperature did not significantly affect gas exchange (44), another study reported reduced values for intrapulmonary shunt function and Alveolar-arterial O<sub>2</sub> and CO<sub>2</sub> gradients indicating that normothermia may better preserve lung function during CPB (45).

## PULMONARY PHYSIOLOGICAL AND HISTOLOGICAL CHANGES FOLLOWING CPB

The physiologic disturbances after CPB, seen by changes in lung compliance with increase in pulmonary vascular resistance and intrapulmonary shunting, are all leading to pulmonary oedema and consequently hypoxaemia in terms of a low PF ratio (PaO<sub>2</sub> divided by fraction of inspired oxygen (FiO<sub>2</sub>)). Besides a decreased PF ratio, changes in functional residual and vital capacity as well as reduced diffusion capacity for carbon monoxide has also been observed in patients after CPB (31,39,46,47).

The histological changes following CPB are due to endothelial damage resulting in increased lung permeability (48–52). The permeability of the alveolar endothelia is closely related to the formation of pulmonary oedema, alveolar protein accumulation, and the facilitation of inflammatory cell sequestration, confirmed by intraoperative lung biopsy following CPB (53). On electron microscopy, pneumocytes and endothelial cells appear swollen and necrotic with consequently increased alveolar septal thickness, hence a smaller alveolar surface area, all compromising oxygenation (54).

Increase in alveolar endothelial permeability following CPB was demonstrated by increased transfer rate of technetium 99m-labeled diethylenetriamine pentaacetate (51), protein accumulation of radiolabelled transferrin (50,52), the systemic/bronchoalveolar urea ratio (52), and other similar permeability indicators (49,55). As a result, protein content in the bronchoalveolar lavage sample increased by more than three- to fourfold in patients following CPB (48). CPB may also affect the pulmonary surfactant activity, particularly in infants and neonates (47,48), but without major contribution to the initial stages of lung injury following CPB (50,56).

## PULMONARY BLOOD SUPPLY DURING CPB

The lungs have a bimodal blood supply from the pulmonary and bronchial arteries with extensive collateral connections. Blood supply to the lungs during total CPB is limited to flow through the bronchial arteries and an ischemic injury will occur, if bronchial blood flow is insufficient to meet the lungs metabolic demands. Virchow demonstrated in 1847 that ligation of the pulmonary artery in dogs resulted in increased bronchial artery blood flow (57). This led to the hypothesis that ceasing pulmonary artery blood flow during CPB would result in a compensatory increase in bronchial arterial blood flow. But it has now been confirmed in several animal models that bronchial artery blood flow is decreased substantially during CPB, and that although exposure to CPB alone is enough to cause pulmonary injury, concomitant cessation of pulmonary artery blood flow contributes significantly to the pulmonary insult (54,58–60).

## PULMONARY HYPOTHERMIC PRESERVATION

Considering experiences from lung transplantation it is hypothesised, that cooling of the lungs by pulmonary artery perfusion with a hypothermic preservation solution protects the lungs from warm ischemic injury (61). Hypothermia is also used as a part of the treatment for post cardiac arrest patients to limit ischemic reperfusion injuries, although the benefits and potentially harms of hypothermic treatments remains debated (62–64). A similar cascade of events occurs when interrupting the pulmonary artery blood flow during CPB, leaving the lungs relatively warm compared with the hearts decreased temperature by local and systemic cooling.

Histidine-tryptophan-ketoglutarate (HTK) solution (Dr. Franz Köhler Chemie GmbH, Bensheim, Germany) is one of the preservation solutions used worldwide for multi-organ preservation (65). Its buffer system is thought to withdraw extracellular sodium and calcium, together with buffering of the extracellular space by means of histidine and histidine-hydrochloride, prolonging the period during which organs tolerates interruption in blood supply.

## EVIDENCE FOR PULMONARY ARTERY PERFUSION PRIOR TO THE PP-TRIAL

When planning the Pulmonary Protection Trial (PP-Trial) in the early 2011 we reviewed the literature and only found a handful small scaled RCTs and non-randomised controlled clinical trials (CCT) with high risk of bias including adults and children with few or no comorbidities. The majority of studies reported intubation time, length of stay at the intensive care unit, and surrogate outcomes as PF ratio and inflammatory biomarkers. Conflicting results for different pro- and anti-inflammatory biomarkers measured in various tissue types at different time points were reported making it difficult to compare the results across studies. Therefore, only clinical and surrogate outcomes, as well as histological changes of the lung tissue, are described in the literature review below (Table S1; Supplementary appendix I). Moreover, studies with co-interventions (i.e. perfusion and ventilation of the lungs during CPB) are excluded for the sake of simplicity. A systematic review including all RCTs in adult (Paper V) is a part of this PhD thesis (Study II). The results of these RCTs and meta-analyses will therefore not be presented in the literature review below which includes RCTs in children, CCTs in adults, and animal studies. Results from five animal studies are not described due to three of the studies only provided an abstract in English (66), or a conference abstract (67,68), and further two studies did not have

a control group (69,70). For search strategy see Study II and Supplementary appendix III.

Immature lungs are more vulnerable to the effects of CPB compared to mature lungs and therefore, postoperative lung injury is more common and serious in infants, especially if concomitant pulmonary hypertension is present (71). In infants, pulmonary perfusion with oxygenated blood significantly increased PF ratio and shortened intubation time (15), but not consistently (16). In two Chinese studies, the effect of pulmonary perfusion with a hypothermic preservation solution demonstrated significantly increased PF ratio in the perioperative period and a shortened intubation time (23,24). In adults, pulmonary artery perfusion with a preservation solution also significantly increased PF ratio and fewer patients needed mechanical ventilation >72 hours (22), although not consistently, as some studies showed no significant differences in PF ratio, intubation time, serious adverse events (SAEs), or mortality for pulmonary artery perfusion with a preservation solution (21) or blood (14). In animals, pulmonary artery perfusion with blood or a preservation solution demonstrated less increase in lung tissue lactate following CPB (25), improved or with no difference in oxygenation, and less pronounced post-CPB histological changes consistent with lung injury (72–75). However, it has to be emphasised that these encouraging results have all been obtained in rather small studies comprising between 12-64 patients or animals (for details see Table S1; Supplementary appendix I).

Due to lack of evidence from a trial with low risk of bias including contemporary relevant patients the PP-Trial was planned, randomizing high-risk elderly patient with COPD to pulmonary artery perfusion with oxygenated blood or a preservation solution compared with no pulmonary perfusion during CPB.

## AIMS OF STUDIES

Our aims were to conduct an explanatory trial (study i) assessing the effects of pulmonary artery perfusion during cpb on postoperative oxygenation (primary outcome measure), intubation time, saes, and mortality. Secondly (study ii), we aimed at comparing our results with those of other rcts by conducting a systematic review with meta-analyses of evidence for benefits and harms of pulmonary artery perfusion during cpb.

## MATERIALS AND METHODS

Detailed description of the two studies design and methodology can be found in the published protocols (paper i and vi). In the following section, a brief outline of the two patient populations, anaesthetic, and cpb procedures used in the pp-trial (study i) will be presented with minor deviations in regards to methods used in the rcts included in the systematic review (study ii).

## POPULATION

For both Study I and II the included patients had one (or two in combination) of the following operations:

- Coronary artery bypass graft (CABG)
- Aorta valve replacement (AVR)
- Mitral or Tricuspid valve reconstruction
- Concomitant surgery on the ascending aorta

## ANAESTHESIA AND CARDIOPULMONARY BYPASS

For Study I anaesthesia was induced with fentanyl (10 µg/kg), propofol (1-2mg/kg) and cisatracurium (0.1 mg/kg) and main-

tained with sevoflurane 0.5% - 3% and continuous infusion of remifentanyl (15-30 µg/kg/hour). Venous access was obtained using two peripheral veins. A sheath for the pulmonary artery catheter and central line were inserted in one of the internal jugular veins. The ventilation mode was preferentially volume controlled and after CPB positive end expiratory pressure was set to 5 mmHg and lung recruitment performed. Ventilation was ceased during sternotomy and the CPB period.

After heparinization (350 IU/kg, ACT >480 sec), normothermic CPB with passive cooling was initiated. In the ascending aorta an angled arterial cannula was placed (DLP 24 FR, Medtronic, MM, USA), and the right atrial appendage was provided with a two-stage venous cannula (36/46 FR, Medtronic). A membrane oxygenator (Capiiox RX25, Terumo, Tokyo, Japan) and a roller pump (Stockert S5, Sorin Group, Milano, Italy) were used for perfusion of the systemic circulation with laminar flow without active perfusion of the pulmonary artery. The arterial line included a 40-µm filter (AL06, Pall, NY, USA). Pump flow was 2.4 l/min/m<sup>2</sup> body surface area and mean arterial pressure was kept between 40 and 70 mmHg by administration of a vasoconstrictor or dilator as appropriate.

### DESCRIPTION OF THE INTERVENTIONS

Pulmonary artery perfusion was administered during aortic cross-clamping, where the natural lung perfusion pathways were clamped and mechanical ventilation paused. Perfusion was administered until release of the aortic cross-clamp, re-establishing the natural perfusion of the pulmonary circulation. The blood or preservation solution was administered continuously, as a single shot or repetitively during the aortic cross-clamp period. For the control group standard CPB was performed without any active pulmonary artery perfusion.

The interventions included in Study I and II can depending on the product perfused be divided into two groups: 1) pulmonary artery perfusion with oxygenated or deoxygenated blood, and 2) pulmonary artery perfusion with a preservation solution. Secondly subdivide into: 1) single shot pulmonary artery perfusion performed ones or repetitively, and 2) continuous pulmonary artery perfusion during the whole aortic cross-clamp period.

#### Pulmonary artery perfusion with blood

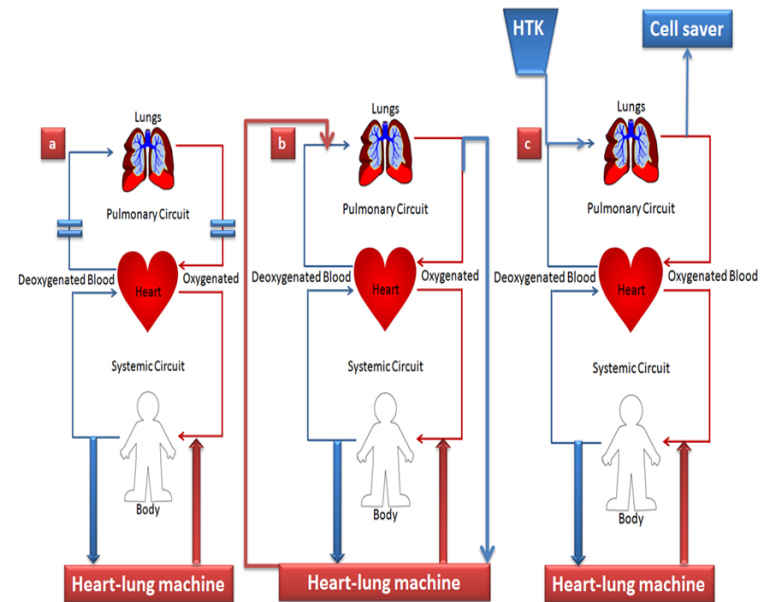
For Study I the pulmonary artery was provided with a straight arterial cannula (14 FR DLP, Medtronic), the aorta cross-clamped, cardioplegia administered, and normothermic pulmonary artery perfusion with oxygenated blood was initiated when CPB reached full flow. We aimed at a pulmonary artery flowrate of 300-400 ml/min equalling approximately 10% of the patient's total flow with an overall CPB flow of 110%. The blood returning from the pulmonary circuit was drained to the venous reservoir via a left atrial vent (VT-53218, CalMed Laboratories, CA, USA) inserted through one of the pulmonary veins (Figure 1).

The pulmonary artery perfusion pressure was online monitored by a pressure gauge on a side branch of the pulmonary artery cannula, aiming for a mean pressure not exceeding 20 mmHg. Before aortic cross-clamp release the perfusion was terminated, the pulmonary artery cannula removed, and the purse-string suture tied to seal the insertion puncture.

#### Pulmonary artery perfusion with HTK solution

For Study I the pulmonary artery was cannulated as described above and 2 litres of 4°C Custodiol HTK solution was administered within 8-10 min without pausing the surgical procedure concomi-

tantly with primary cardioplegia. The pulmonary artery perfusion pressure was monitored and used to guide the infusion rate. The blood and HTK solution returning from the pulmonary circulation to the left atrium was filtered by a cell saver. The HTK solution was discarded and the remaining red blood cells returned to the patient via the venous reservoir (Figure 1).



**Figure 1** a) standard treatment, b) pulmonary perfusion with oxygenated blood, c) pulmonary perfusion with histidine-tryptophan-ketoglutarate solution

### STUDY I

#### Pulmonary artery perfusion versus no pulmonary perfusion during cardiopulmonary bypass in patients with chronic obstructive pulmonary disease: a randomised clinical trial

##### TRIAL DESIGN AND OVERVIEW

The PP-Trial was a randomised, parallel group, participants, statistician and conclusion drawer's blinded clinical trial randomising patients admitted to the Department of Cardiothoracic Surgery, Rigshospitalet, Denmark for AVR, CABG or the two procedures combined from July 2012 to November 2013.

Patients were assigned in a 1:1:1 ratio to one of three interventions 1) pulmonary artery perfusion with normothermic oxygenated blood, 2) pulmonary artery perfusion with hypothermic HTK solution, or 3) no pulmonary perfusion during CPB. Randomisation was performed centrally, on a website hosted by The Copenhagen Trial Unit, with a computer-generated allocation sequence in permuted blocks of varying size, stratified according to the preoperative lung function into two groups 1) mild COPD, and 2) moderate to very severe COPD.

##### PATIENTS

Patients were 18 years of age or older and diagnosed with an irreversible airway obstruction (forced expiratory volume in 1 sec (FEV1)/forced vital capacity (FVC) < 0.70). Classified as mild, moderate, severe, or very severe COPD in accordance with the global initiative for chronic obstructive lung disease (GOLD) classification

(76). The main exclusion criteria were previous heart or lung surgery, previous thoracic radiation, left ventricular ejection fraction < 20%, tracheal intubation or medically treated pneumonia prior to surgery. Written informed consent was obtained from each patient and the trial was conducted in accordance with the ethical standards of the Helsinki Declarations of 1975 (77).

### ETHICS

The trial was approved by the ethics committee, medicines agency, data protection agency, and registered at the website ClinicalTrials.gov (identifier: NCT01614951) prior to enrolment of the first patient. The good clinical practice unit at Copenhagen University Hospital monitored adherence to the protocol and data registration.

### OUTCOMES

The co-primary outcome was the inverse oxygenation index (PaO<sub>2</sub> divided by FiO<sub>2</sub> times mean airway pressure) measured at 21 hours and longitudinally at 1, 3, 5, 7, and 21 hours after CPB start. The secondary outcomes were: 1) intubation time, 2) days alive outside the intensive care unit, 3) days alive outside the hospital, 4) 30- and 90-day mortality, and 5) patients with one or more of the selected SAEs: pneumothorax or pleural effusion, major bleeding, reoperation, severe infection, cerebral event, hyperkalaemia, acute myocardial infarction, cardiac arrhythmia, renal replacement therapy, and readmission to the hospital with respiratory-related symptoms.

### STATISTICAL ANALYSES

The primary analysis included a modified intention-to-treat population defined as all randomised patients excluding patients who did not undergo CPB dependent cardiac surgery, and a per-protocol population excluding patients with major protocol violations. All analyses on the co-primary outcome were adjusted for the stratification variable (mild COPD or moderate to very severe COPD) and the patients' baseline oxygenation index. We secondly adjusted for both the stratification and design variables (age, FEV<sub>1</sub>, left ventricular ejection fraction and the patients' baseline oxygenation index) (78).

Linear regression was used to compare the three groups' oxygenation indices measured 21 hours after CPB start. To analyse differences in the longitudinal measurements of the oxygenation indices (1, 3, 5, 7, and 21 hours after CPB start) a linear mixed-effects model (MEM) was used.

SAEs and 30- and 90 days mortality were analysed as dichotomous variables using logistic regression adjusted for the stratification variable. The remaining secondary outcomes were count data analysed with the van Elteren test (79) adjusted for the stratification variable. We pre-published a detailed statistical analysis plan (Paper II) prior to data analysis (78).

### RESULTS

Of 3688 patients evaluated for eligibility, 387 meet the inclusion criteria, but only 90 patients were randomised due to staff shortage. Due to one post-randomisation exclusion 89 patients were included in the intention to treat analysis and 86 patients in the per-protocol analysis (Figure 2 and Table S1; Supplementary appendix II).

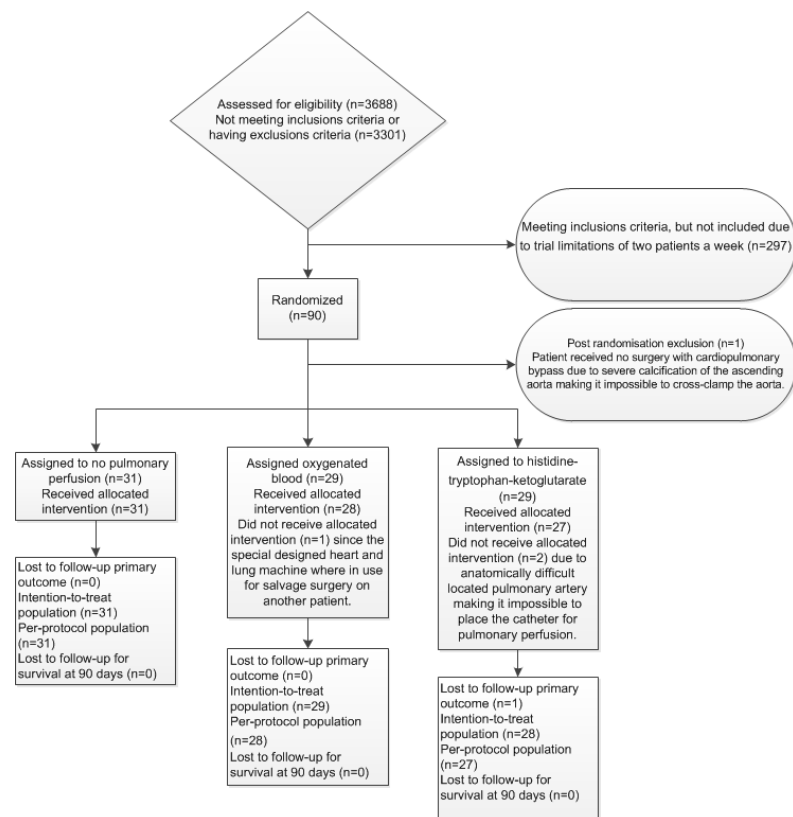


Figure 2 Flow of patients in The Pulmonary Protection Trial.

The baseline characteristics of the patients, comorbidities, pre-operative pulmonary status, and surgical data are summarized in Table 1. Mean age was 70 years, and 75% were men. We found for the baseline characteristics no statistical significant differences between the three groups.

Table 1 Characteristics of the Patients at Baseline and Surgical Data <sup>a</sup>

Characteristics	Oxygenated blood (N = 29)	HTK solution (N = 29)	No Pulmonary perfusion (N = 31)
<b>Demographic characteristics</b>			
Age – yr.	69.9 ± 10.8	69.6 ± 8.0	70.5 ± 8.9
Male sex – no. (%)	22 (75.9)	20 (69.0)	25 (80.6)
<b>Comorbidities – no./total no. (%) [no. of patients with missing values]</b>			
Self-reported COPD	15/29 (51.7)	18/29 (62.1)	19/31 (61.3)
Pulmonary hypertension determined by preoperative echocardiographic	3/29 (10.3) [5]	7/29 (24.1) [3]	6/31 (19.4) [8]
Arterial hypertension	23/29 (79.3)	24/29 (82.8)	22/31 (71.0)
Chronic atrial fibrillation or flutter	4/29 (13.8)	3/29 (10.3) [1]	5/31 (16.1)
Chronic heart failure <sup>b</sup>	13/29 (44.8)	6/29 (20.7) [2]	9/31 (29.0)
ASA class IV or worse	1/29 (3.4)	4/29 (13.8) [2]	4/31 (12.9) [2]
Median left ventricular ejection fraction (IQR)	52.5 (41.2-60.0) [1]	50.0 (37.5-60.0)	55.0 (45.0-60.0)
Recent AMI <sup>c</sup>	7/29 (24.1)	11/29 (37.9)	7/31 (22.6)
Insulin dependent diabetes mellitus	3/29 (10.3)	3/29 (10.3)	2/31 (6.5)
Median estimated creatinine clearance (IQR) <sup>d</sup>	82.5 (64.2-121.9)	82.7 (62.8-	86.1 (65.2-106.8)

		107.3)	
Previous transit ischemic attack or stroke	1/29 (3.4)	7/29 (24.1)	7/31 (22.6)
Previous percutaneous coronary intervention	11/29 (37.9)	7/29 (24.1)	7/31 (22.6)
Implantable cardioverter defibrillator or pacemaker	2/29 (6.9) [2]	1/29 (3.4) [1]	1/31 (3.2) [1]
Greater alcohol consumption <sup>e</sup>	11/29 (37.9)	7/29 (24.1)	7/31 (22.6)
Median tobacco pack years (IQR)	40.0 (18.5-51.0)	45.0 (32.5-50.0)	35.0 (20.0-45.0)
<b>Pulmonary function</b>			
Median percent predicted FEV1 (IQR)	75.0 (56.5-87.5)	79.0 (58.5-88.0)	74.0 (58.0-88.0)
GOLD classification Mild	14/29 (48.3)	14/29 (48.3)	12/31 (38.7)
GOLD classification Moderate	11/29 (37.9)	14/29 (48.3)	17/29 (54.8)
GOLD classification Severe	3/29 (10.3)	1/29 (3.4)	2/31 (6.5)
GOLD classification Very severe	1/29 (3.5)	0/29 (0.0)	0/29 (0.0)
<b>Surgical data</b>			
Elective surgery	18/29 (62.1)	16/29 (55.2)	23/31 (74.2)
Urgent surgery	11/29 (37.9)	13/29 (44.8)	8/31 (25.8)
<b>Type of surgery</b>			
AVR	7/29 (24.1)	7/29 (24.1)	12/31 (38.7)
CABG	15/29 (51.7)	17/29 (58.6)	17/31 (54.8)
CABG and AVR	7/29 (24.1)	5/29 (17.2)	2/31 (6.5)
Cardiopulmonary bypass time (min)	102.2 ± 53.7	100.9 ± 26.5	98.45 ± 44.6
Aortic cross-clamp time (min)	58.8 ± 37.9	64.3 ± 18.3	61.9 ± 34.7

<sup>a</sup> Plus-minus values are means ±SD. There were no statistically significant differences between the three groups for any of the variables. AMI denotes acute myocardial infarction; ASA denotes American society of anaesthesiologists; AVR denotes aortic valve replacement; CABG denotes coronary artery bypass graft; COPD denotes chronic obstructive pulmonary disease; FEV1 denotes forced expiratory volume in 1 sec; GOLD denotes global initiative for chronic obstructive lung disease; HTK denotes histidine-tryptophan-ketoglutarate; IQR denotes interquartile range

<sup>b</sup> Chronic heart failure was defined as New York heart association Class III or worse.

<sup>c</sup> Recent AMI is within 3 months prior to surgery.

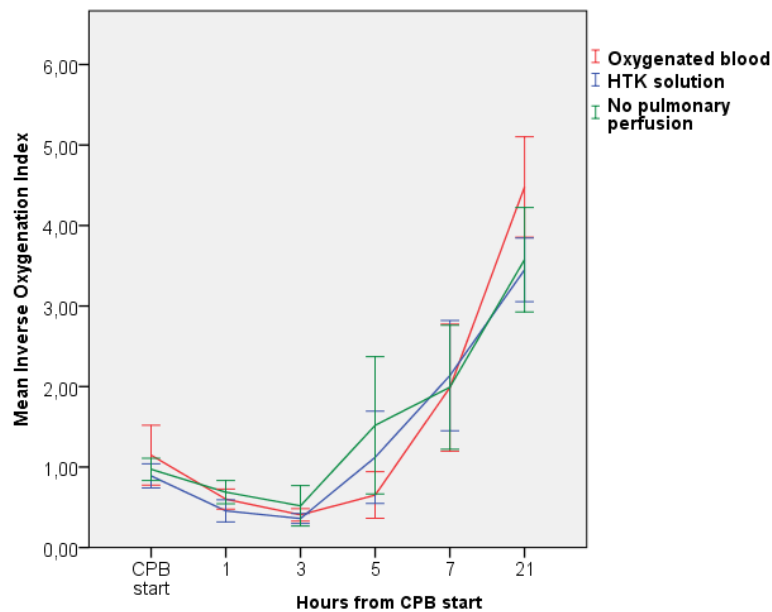
<sup>d</sup> Estimated creatinine clearance by Cockcroft-Gault equation in mL/min/1.73m<sup>2</sup>.

<sup>e</sup> Greater alcohol consumption was defined as > 14 for women and > 21 for men units' alcohol per week.

## PREDEFINED OUTCOME MEASURES

### Primary outcome – Oxygenation index

Results for the inverse oxygenation index measured at 21 hours and longitudinal 1, 3, 5, 7, and 21 hours after CPB start are illustrated in Figure 3 and listed in Table 2 also including mean PF ratios (not inversed oxygenation indices see Table S2; Supplementary appendix II).



**Figure 3** The figure shows unadjusted means of the inverse oxygenation indices for the three intervention groups from CPB start and until 21 hours after.

For the PF ratio all three groups demonstrated their lowest value 1 hour after CPB start with the HTK group showing the most pronounced deterioration of all three groups. Point estimates for the group receiving no pulmonary perfusion demonstrated the greatest change in PF ratio of 122 mmHg from baseline to 21 hours, compared to only 61 mmHg and 84 mmHg for the groups receiving oxygenated blood and HTK solution, respectively.

At 21 hours after CPB start, patients who received pulmonary artery perfusion with oxygenated blood had a higher inverse oxygenation index compared to patients receiving no pulmonary perfusion during CPB (mean difference (MD) 0.94; 95% confidence interval (CI), 0.05 to 1.83; P=0.04). Although with Bonferroni correction for multiple comparisons the result becomes insignificant.

<b>Table 2. Unadjusted Inverse Oxygenation Indices and PF ratio<sup>a</sup></b>		
	<b>OI mean [95% CI]</b>	<b>PF mean [95% CI]</b>
<b>Baseline</b>		
PP with blood	1.15 [0.78 - 1.52]	392.35 [348.06 - 436.64]
PP with HTK	0.89 [0.74 - 1.04]	350.14 [314.80 - 385.48]
No PP	0.97 [0.83 - 1.11]	413.43 [371.37 - 455.49]
<b>1 hour<sup>b</sup></b>		
PP with blood	0.60 [0.48 - 0.73]	272.00 [225.90 - 318.09]
PP with HTK	0.46 [0.32 - 0.59]	199.96 [160.15 - 239.77]
No PP	0.69 [0.55 - 0.83]	293.02 [245.93 - 340.12]
<b>3 hours</b>		
PP with blood	0.41 [0.33 - 0.48]	282.11 [237.46 - 326.76]
PP with HTK	0.36 [0.30 - 0.42]	249.64 [214.58 - 284.70]
No PP	0.52 [0.27 - 0.77]	267.84 [216.66 - 319.03]
<b>5 hours</b>		
PP with blood	0.65 [0.37 - 0.94]	337.15 [260.56 - 413.74]
PP with HTK	1.12 [0.55 - 1.69]	262.86 [235.55 - 290.18]
No PP	1.52 [0.67 - 2.37]	285.84 [238.32 - 333.36]
<b>7 hours</b>		
PP with blood	1.99 [1.20 - 2.77]	321.00 [286.47 - 355.54]
PP with HTK	2.14 [1.46 - 2.82]	274.82 [250.64 - 299.11]
No PP	1.99 [1.23 - 2.76]	277.43 [239.55 - 315.32]
<b>21 hours</b>		
PP with blood	4.48 [3.86 - 5.10]	331.04 [285.54 - 376.54]

PP with HTK	3.45 [3.06 – 3.84]	266.03 [238.91 – 293.15]
No PP	3.58 [2.93 – 4.22]	291.37 [254.58 – 328.16]

*a* Intention-to-treat population. *b* after CPB start. CI denotes confidence interval; CPB denotes cardiopulmonary bypass; HTK denotes histidine-tryptophan-ketoglutarate; OI denotes oxygenation index; PaO<sub>2</sub> divided by FiO<sub>2</sub> measured in mmHg; PF denotes; PP denotes Pulmonary Perfusion.

The inverse oxygenation index was significantly higher at 21 hours after CPB start (MD 0.99; CI, 0.29 to 1.69; P=0.007), and longitudinally ((MEM, P=0.009), for patients receiving pulmonary artery perfusion with oxygenated blood compared to pulmonary artery perfusion with hypothermic HTK solution (Table 3). This corresponds to a PaO<sub>2</sub> difference of 23 mmHg with a median FiO<sub>2</sub> of 0.32. We found no additional significant differences for the remaining comparisons of the inverse oxygenation indices. The per-protocol and secondary analyses did not noticeably change the results of the primary outcome (Table S3; Supplementary appendix II).

Table 3. Effects of Pulmonary Artery Perfusion versus No Pulmonary Perfusion on the Oxygenation Indices <i>a</i>		
Linear Regression at 21 hours after CPB start	Mean difference [95% CI]	P value
Oxygenated blood vs. no pulmonary perfusion	0.94 [0.05 – 1.83]	0.04
HTK solution vs. no pulmonary perfusion	0.06 [-0.73 – 0.86]	0.87
Oxygenated blood vs. HTK solution	0.99 [0.29 – 1.69]	0.007
Linear Mixed-Effects Model (longitudinally)		
Oxygenated blood vs. no pulmonary perfusion		0.57
HTK solution vs. no pulmonary perfusion		0.17
Oxygenated blood vs. HTK solution		0.009

*a* Analysis of the intention-to treat-population adjusted for stratification variable and baseline oxygenation index. CI denotes confidence interval; CPB denotes cardiopulmonary bypass; HTK denotes histidine-tryptophan-ketoglutarate; vs. denotes versus.

### Secondary outcomes

We found no statistically significant differences for any of the secondary outcomes (Table 4; Table S4; Supplementary appendix II) although point estimates, except for SAEs, favoured pulmonary artery perfusion with oxygenated blood compared with no pulmonary perfusion during CPB.

The proportions of patients with one or more SAEs did not differ between the three groups and there were no serious adverse reactions or suspected unexpected serious adverse reactions for any of the trial patients. The number of patients with 0-6 SAEs are listed in Table S5 and described in details in Table S6 both in the Supplementary appendix II.

Table 4. Effects of Pulmonary Artery Perfusion versus No Pulmonary Perfusion on the Secondary Outcomes <i>a</i>		
Intubation time <i>b</i>	P value	
PP blood vs. no PP		0.47
PP HTK vs. no PP		0.10
PP blood vs. PP HTK		0.23
Days alive outside ICU <i>b</i>		
PP blood vs. no PP		0.42
PP HTK vs. no PP		0.61
PP blood vs. PP HTK		0.92
Days alive outside the hospital <i>b</i>		
PP blood vs. no PP		0.86
PP HTK vs. no PP		0.99
PP blood vs. PP HTK		0.97
Death at 90 days <i>c</i>	Odds ratio [95% CI]	P value

PP blood vs. no PP	0.69 [0.09 – 3.10]	0.98
PP HTK vs. no PP	0.78 [0.01 – 65.37]	1.00
PP blood vs. PP HTK	0.47 [0.01 – 9.33]	0.96
Patients with one or more Serious Adverse Events <i>c</i>		
PP blood vs. no PP	0.79 [0.19 – 3.30]	0.75
PP HTK vs. no PP	0.61 [0.13 – 2.85]	0.53
PP blood vs. PP HTK	1.33 [0.27 – 6.53]	0.73

*a* Analysis of the intention-to-treat population CI denotes confidence interval; HTK denotes histidine-tryptophan-ketoglutarate; ICU denotes intensive care unit; ITT denotes intention to treat; PP denotes pulmonary perfusion; vs. denotes versus.

*b* Van Elteren test

*c* Exact logistic regression adjusted for stratification variable

### CONCLUSION

We found that pulmonary artery perfusion with normothermic oxygenated blood during CPB resulted in a higher postoperative inverse oxygenation index for cardiac surgery patients with COPD compared with both no pulmonary perfusion and pulmonary artery perfusion with hypothermic HTK solution. We found no statistically significant differences between the groups for any of the secondary outcomes.

### STUDY II

### Pulmonary artery perfusion versus no pulmonary perfusion during cardiopulmonary bypass: a systematic review of randomised clinical trials with meta-analyses and trial sequential analyses

### METHODS

#### Overview and design

The systematic review was conducted in accordance with recommendations from the Cochrane collaboration (80). A peer-reviewed protocol (Paper IV) was published before literature search was performed (81).

#### ELIGIBILITY CRITERIA

RCTs with adults were included when at least one intervention group was allocated to receive pulmonary artery perfusion (irrespective of pharmacological class of the administered fluid or drug(s), quantity and frequency) compared to no pulmonary perfusion during CPB. Trials on children, Quasi-randomised and non-randomised CCTs in adults as well as RCTs in children obtained through the search were included for assessment of harm.

#### Search strategy

Relevant RCTs were identified without language restrictions by searching the electronic databases: Cochrane Central Register of Controlled Trial, MEDLINE, EMBASE and World Health Organisations International Clinical Trials Registry Platform (82) for ongoing trials until June 1<sup>st</sup> 2016. References of published literature, hand searched study reports, and review articles were reviewed to identify additional relevant trials.

#### Data extraction and outcomes

Two authors independently identified trials and extracted data using a pre-planned data extraction form. Predefined primary outcomes were all-cause mortality and pulmonary related events (increase in PF ratio, prolonged mechanical ventilation, pneumo-

nia, adult respiratory distress syndrome). Secondary outcomes were number of patients with one or SAEs and changes in inflammatory markers.

### Bias assessment and GRADE

All RCTs were reviewed for risk of bias in major domains as recommended by the Cochrane Collaboration (80). Low risk of bias were granted to RCTs with low risk of bias in all domains except for blinding of surgical personnel, as blinding of the surgical intervention was not feasible. The quality of evidence for all-cause mortality, pulmonary related events, SAEs and changes in inflammatory markers were assessed using the grading of recommendations assessment, development and evaluation (GRADE) methodology (83).

### Statistical analyses

Pooled estimates of intervention effects in primary and secondary outcomes were calculated with the software package Review Manager version 5.3.5 (The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) using conventional meta-analysis. Trial sequential analysis (TSA) were performed as a supplemental analysis adjusting for repetitive significance testing whenever new trials were added and sparse data using TSA program version 0.9 beta (www.ctu.dk/tsa).

## RESULTS

### Characteristics of included trials

Four RCTs enrolling 210 patients were included. Trial proportion size ranged from 15 to 90 patients. The included trials were heterogeneous regarding type of patients, surgery, and intervention methods. In two trials the patients received continuous pulmonary artery perfusion with oxygenated blood during CPB (18–20), in one trial repetitive pulmonary artery perfusion with venous blood (17), and in a trial one group received continuously pulmonary artery perfusion with oxygenated blood and a second group received single-shot pulmonary artery perfusion with a hypothermic preservation solution (84). In all four trials the control group received no pulmonary perfusion during CPB. For the current version of the systematic review the comparison pulmonary artery perfusion with blood versus no pulmonary perfusion was only assessed. One trial had low risk of bias in all domains, except for blinding of personnel during the surgical procedure, while the remaining three trials had high risk of bias. The length of follow-up varied from index admission to 90 days post randomisation.

Only one trial (84) reported on pulmonary related events. Intubation time and last measured PF ratio were therefore added as two new explorative secondary outcomes. There were for the current available data on inflammatory markers a high degree of inconsistency and therefore could a meta-analysis not be performed.

### Mortality

In the analysis of all trials, there were no statistically significant differences in all-cause mortality between patients receiving pulmonary artery perfusion with blood compared with no pulmonary perfusion during CPB (Table 5). Subgroup analyses of trials with low risk of bias (except for blinding of personnel during the surgical procedure), preoperative lung function, and length of follow-up did not alter the result. TSA including all trials showed

that no boundaries were crossed (Figure S1, Supplementary appendix III). The quality of evidence was judged to be low.

**Table 5. Results of Conventional Meta- and Trial Sequential Analysis.**

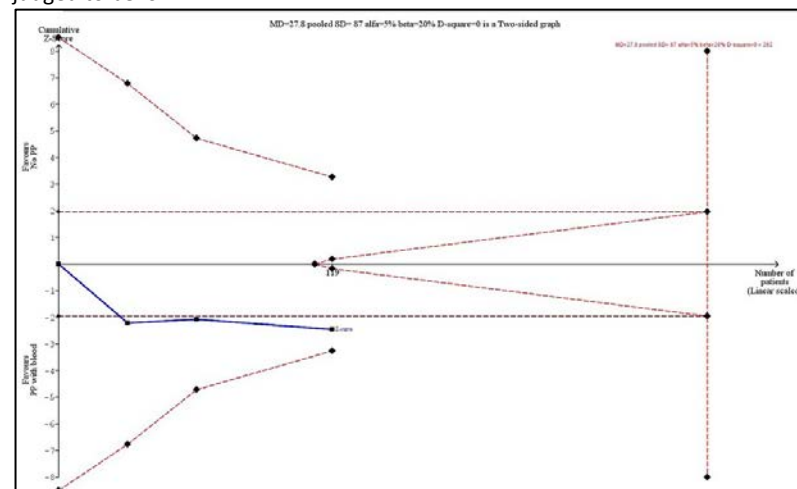
Outcomes	No. of trials	PP	No PP	RR/MD [95% CI]	TSA adjusted 95% CI
<b>Mortality <i>a</i></b>					
Low risk of bias	1	3/29	2/31	1.6 [0.3 - 8.9]	
All trials	4	5/104	3/106	1.7 [0.4 - 6.9]	0.01 - 493
<b>Serious adverse events <i>a</i></b>					
Low risk of bias	1	29/29	28/31	1.1 [1.0 - 1.3]	
All trials	3	45/89	39/91	1.2 [0.8 - 1.8]	0.9 - 1.4
<b>PF ratio (mmHg) <i>b</i></b>					
Low risk of bias	1	331.0 ± 117.3	291.4 ± 100.3	39.7 [-16.3 - 95.7]	
All trials	3	328.7 ± 72.9	301.9 ± 68.8	27.8 [5.7 - 50.0]	10.7 - 66.4
<b>Intubation time (hours) <i>b</i></b>					
Low risk of bias	1	5 ± 1.9	6 ± 3.0	-1.0 [-2.24 - 0.24]	
All trials	4	9.6 ± 13.19	10.3 ± 125.3	-0.4 [-1.1 - 0.4]	-1.3 - 0.5

*a* Events/total; CI denotes confidence interval; MD denotes mean difference; PF denotes PaO<sub>2</sub> divided by FiO<sub>2</sub> measured in mmHg; RR denotes relative risk; TSA denotes trial sequential analysis

*b* Mean ± standard deviation

### Serious adverse events

In the analysis of all trials contributing with SAE data there were no statistically significant differences in the number of SAEs between patients receiving pulmonary artery perfusion with blood compared with no pulmonary perfusion during CPB (Table 5). Subgroup analyses of trials with low risk of bias (except for blinding of personnel during the surgical procedure), preoperative lung function, and length of follow-up did not alter the result. TSA including all trials showed that no boundaries were crossed (Figure S2, Supplementary appendix III). The quality of evidence was judged to be low.



**Figure 4** Trial Sequential Analysis of the outcome PaO<sub>2</sub> divided by FiO<sub>2</sub> (PF ratio) at least 6 hours after surgery and anaesthesia. The blue line is the cumulative Z-curve and the boundaries for benefit and harm are shown in the upper and lower panel as a line between two points. The utility boundaries are to the right side shown as an inner triangular wedge with



its base adhering to the vertical line demonstrating the required information size. The required information size of 282 participants was not reached and none of the TSA boundaries for benefit, harm, or futility of a 10% improvement with pulmonary perfusion with blood was crossed. The TSA adjusted confidence interval being: -10.7 to 66.4.

#### **Last measured PaO<sub>2</sub> to FiO<sub>2</sub> ratio**

In the analysis of all trials contributing with data on last measured PF ratio, pulmonary artery perfusion with blood compared with no pulmonary perfusion during CPB was significantly associated with a higher PF ratio (Table 5). Subgroup analyses on risk of bias and preoperative lung function divided the trials in to two groups. For one trial (84) with overall low risk of bias (except for blinding of personnel during the surgical procedure) including COPD patients the significant association was not rediscovered, most likely due to the very wide CI (Table 5). In trials with overall uncertain or high risk of bias including patients with a normal lung function (19,20) the significant association between pulmonary artery perfusion with blood and a higher PF ratio remained (Table 5). Despite the overall significant result, TSA showed that only 39% of the required information to detect or reject a 10% increase in the PF ratio was reached and none of the TSA boundaries were crossed (Figure 4). The quality of evidence was judged to be very low.

#### **Intubation time**

In the analysis of all trials contributing with data on intubation time there was no statistically significant differences between patients receiving pulmonary artery perfusion with blood compared with no pulmonary perfusion during CPB (Table 5). Subgroup analyses of trials with low risk of bias or stratified by preoperative lung function did not alter the result. TSA including all trials showed that the boundary for lack of superiority (futility) was crossed refuting a shorten intubation time of 1.5 hours or more (Figure S3, Supplementary appendix III).

#### **CONCLUSION**

Conventional meta-analysis did not demonstrate any strong association between mortality, or any of the secondary outcomes, with the use of pulmonary artery perfusion with blood during CPB. However, we found a possible association between pulmonary artery perfusion with blood and a higher PF ratio. The overall quality of evidence was judged to be low. The TSAs showed that the required information size had not been reached for any of the outcomes nor had the boundaries for benefit, harm, or futility been surpassed for the outcomes of mortality, SAE, and PF ratio. However, a clinical relevant reduction in intubation time seems to be absent as a reduction of 1.5 hours could be refuted.

#### **DISCUSSION**

##### **PRINCIPAL FINDINGS**

In the randomised, participants, statistician, and conclusion drawers blinded PP-Trial including cardiac surgery patients with COPD we found, that pulmonary artery perfusion with normothermic oxygenated blood during CPB resulted in a higher inverse oxygenation index compared with both no pulmonary perfusion and pulmonary artery perfusion with hypothermic HTK solution. We found no statistically significant differences between the groups for intubation time, days alive outside the intensive care

unit, days alive outside the hospital, SAEs, 30- and 90-days mortality.

In the systematic review, the conventional meta-analysis did not demonstrate an association between pulmonary artery perfusion with blood and mortality, SAEs, PF ratio and intubation time compared with no pulmonary perfusion. However, we found a possible association between pulmonary artery perfusion with blood and a higher PF ratio. The overall quality of evidence was judged to be low and the TSAs showed that the required information size had not been reached for the majority of outcomes.

#### **LIMITATIONS AND STRENGTHS – STUDY I (THE PP-TRIAL)**

##### **Design**

The PP-Trial was designed as a RCT with overall low risk of bias (except for blinding of operative personnel) by a pre-published protocol, centralised computer based randomisation, and concealed allocation. The interventions were masked for physicians, personnel at the intensive care unit and ward, personnel performing the 90 days follow-up, statisticians and the conclusion drawers. The possibility of introduction of bias by the lack of blinding may still be present as the surgical team was aware of the intervention assignment due to the inherent problem with blinding of a surgical intervention.

The trial was designed as an explanatory trial accessing effects of pulmonary artery perfusion with oxygenated blood or HTK solution on a surrogate outcome reflecting oxygenation in cardiac surgery patients with COPD. This was the first well conducted RCT with lowest possible risk of bias evaluated specifically in high-risk elderly patients with COPD. The goal was to investigate if pulmonary artery perfusion during CPB could improve oxygenation and secondly to report potential SAEs. The trial was not intended to, and therefore not powered to, demonstrate an association between pulmonary artery perfusion and clinical outcomes such as mortality, but meant as a stepping stone to a larger pragmatic multicentre RCT. Thus, the explanatory trial design supported the aim of PP-Trial but on the other hand did not provide, due to lack of power, any definite results on mortality and SAEs which would be the aim of a future pragmatic trial.

##### **Patient selection**

Pulmonary artery perfusion with oxygenated blood or HTK solution has not previously been tested in cardiac surgery patients with COPD and this patient group was chosen as they demonstrate serious comorbidities and more severe postoperative pulmonary dysfunction. Pulmonary artery perfusion during CPB could potentially be beneficial for this high-risk patient group's postoperative oxygenation, but could also worsen outcome.

A limitation for the generalizability of our results was that patients in the PP-Trial were much older and sicker than patients included in similar trials (18–20) making our cohort less comparable to other RCTs. Furthermore, in comparable studies, unstable patients and patients having complications following surgery were excluded post-randomisation risking results to be selection biased (18,19). To avoid patient selection bias and to ensure external validity we had broad inclusion criteria, few exclusion criteria, and only one of our patients was excluded post-randomisation. During the enrolment period all 3.688 referred patients were screened for eligibility with 387 meeting the inclusion criteria. Due to staff shortage only 90 of the 387 patients were randomised limiting the results external validity.

Another patient selection limitation was our diagnosis criteria for COPD. Diagnosis of reduced lung function in patients with concomitant acute or chronic heart failure is a complex problem that current research does not have the answer to (85,86). In the PP-Trial the COPD diagnosis was based on anamnestic exposure to risk factors, dyspnoea, and the Tiffeneau-Pinelli index (post-bronchodilator irreversible FEV1/FVC < 0.7) (87), but not chronic cough and chronic sputum production in accordance with the strict GOLD criteria (76). In fact, only 58% of the patients self-reported COPD with the remaining patients not prior diagnosed with a pulmonary disease.

Cardiac diseases share predisposing factors with pulmonary diseases raising the question if the trial patients preoperative COPD diagnosis was due to a reduced lung or heart function, or maybe both. In addition, dyspnoea and reduced lung function is a symptom of both COPD and heart failure. In a German population-based, prospective cohort study with 15.010 individuals mild alterations in lung function were related to measurable echocardiographic changes and manifest heart failure (85). FEV1/FVC ratio, percent predicted FEV1 and FVC were all negatively associated with prevalent heart failure, both with preserved and reduced ejection fraction. One standard deviation increase in FEV1 was related to a 0.52-fold decrease in the odds for heart failure, with a similar strong association for FVC. The cohort focused on pulmonary function variables as predictors of cardiac function, but it could also be the other way around, a primary impairment of cardiac function in relation to pulmonary function. Subclinical and transitory elevation of left ventricular filling pressures may increase left atrial and pulmonary venous pressure leading to consecutive capillary fluid extravasation with mechanical obstruction of bronchiolae and thereby a reduction in FEV1 and FEV1/FVC ratio (85). In The British Regional Heart Study reduced FEV1, even within the low normal range, was found associated with increased risk of heart failure after adjustment for established heart failure risk factors known to be associated with lung function (86). A possible theory could be that poor lung function is reflecting preclinical heart failure, although the significant association between reduced FEV1 and incident heart failure was partially attenuated by adjustment for baseline NT-proBNP (N-terminal pro-brain natriuretic peptide) and cardiac troponin T.

With the PP-Trials median FEV1 of 76% classifying almost half of the patients with mild COPD it could be speculated whether the reduced lung function was still present postoperatively when the heart function may have improved. However, we did not perform a subgroup analysis of patients with persistent postoperative reduced lung function although lung function test data from the 90 days follow-up is still to be investigated. Further, the patients in the PP-Trial had a near normal median left ventricular ejection fraction of 53% questioning the general presence of preoperative clinical significant heart failure.

### Intervention

The primary strength of the PP-Trial was that the pre-published protocol in details described, and clearly defined, the intervention groups in terms of the surgical, anaesthetic, and CPB procedures leading to very few protocol violations. The number of protocol violations was identical in the three groups and all due to technical or anatomical problems, and not un-awareness of the trial protocol or a result of deliberate action from the attending clinicians. Therefore, results from the per protocol analyses, excluding patients with protocol violations, were not noticeable different from the primary analysis.

The PP-Trial was neither designed nor powered to report differences in oxygenation between patients receiving pulmonary artery perfusion with oxygenated blood compared with pulmonary artery perfusion with HTK solution, although this comparison demonstrated the greatest difference. There are inherent difficulties in comparing these two interventions. The substrate is different, the perfusion techniques identical in regards to the pulmonary artery cannula and perfusion pressure, but different in relation to perfusion time as the oxygenated blood was administered continuously during the whole aortic-cross clamp period compared to the HTK solution, which was administered as a single-shot together with the primary cardioplegia. It was reasonable to administer the HTK solution as a single dose as the preservation solution is used similarly in the clinical setting when administered as a cardioplegic solution or as a preservation solution for perfusion of organs prior to harvesting (65). Finally, the oxygenated blood was normothermic and the HTK solution hypothermic. To increase comparability of the two interventions and reduce confounding we could have designed the PP-Trial with both infusion substrates having the same temperatures.

Clinical equipoise when allocating patients to different intervention groups is an ethical imperative for medical research (88). For the investigators of a RCT there should be no ethical imperative to support any of the chosen treatment arms. When planning the PP-Trial there was only a few small scale high-risk RCTs in adults and children reporting conflicting results but overall a positive or non-significant effect of both pulmonary artery perfusion with blood or HTK solution compared with no pulmonary perfusion during CPB (15,16,18,19,23,24). Therefore, both individual and collective equipoise ensuring non-biased enrolment of patients was present, as the trial design was consistent with current evidence of the intervention effects.

### Outcomes

The strength of our trial was that we reached the pre-planned number of included patients powered to detect a relevant difference in the OI. The OI is associated with the degree of acute lungs injury (89), but still a surrogate outcome that may lead to overestimation of intervention effects (90). The validity of this surrogate outcome can be questioned in terms of its short and long term clinical relevance as well as benefit or eventual harm of a high PaO<sub>2</sub>. Results of the primary analysis seem reliable as the same results were found in the fully adjusted, unadjusted, and per-protocol analyses. We achieved 98% follow-up for the primary outcome and 96% follow-up for the secondary outcomes eliminating bias due to drop-outs with no need for multiple-imputation due to missingness.

With respect to the sample size, the assumption was a PF ratio mean difference for the comparisons of pulmonary artery perfusion with oxygenated blood or HTK solution compared with no pulmonary perfusion of 55 mmHg and 110 mmHg, respectively (81). It is a fairly large difference considering that the only available studies for our sample size calculations were small, had high risk of bias, and included no more than 12-15 patients in each intervention group (19,24). We found for the comparison oxygenated blood versus no pulmonary perfusion a statistically significant mean difference in the OI of 0.94 corresponding to a difference in the PaO<sub>2</sub> of 22 mmHg at a FiO<sub>2</sub> of 0.32, and for the comparison HTK solution versus no pulmonary perfusion a non-significant mean difference in the OI of 0.06. As the PF ratio and OI are not identical measures of oxygenation the results cannot be directly compared, but overall we found a relatively smaller

but still statistically significant effect of pulmonary artery perfusion with oxygenated blood, but, no statistically significant effect of pulmonary artery perfusion with HTK solution compared with no pulmonary perfusion. The significant change in PaO<sub>2</sub> of 22 mmHg is, however, still an important and clinically relevant difference.

Our result differs from the results by Kiessling et. al (17) showing a non-significant difference in the PF ratio for repetitive pulmonary artery perfusion with venous blood compared with no pulmonary perfusion in 59 COPD patients. The different results could be due to the fact that Kiessling et al. (17) administered venous blood repetitively compared with our continuous administration with oxygenated blood during the whole aortic-cross clamp period. In the RCTs by Santini et al. (18,19) and Liu et al. (20) the patients received continuous pulmonary artery perfusion with oxygenated blood and found, similarly to our observations, a statistically significant difference in PF ratio favouring the perfusion group. To this date there has not been published other RCTs in adults comparing pulmonary artery perfusion with a preservation solution with no pulmonary perfusion or pulmonary artery perfusion with blood.

For the secondary outcomes mortality, SAEs, intubation time, days alive outside the intensive care unit and days alive outside the hospital we found no statistically significant differences. As mentioned, the PP-Trial was not powered to show any significant differences in clinical outcomes. In a similarly powered trial Kiessling et al. (17) were unable to demonstrate any significant differences. This was also the case for the findings of Santini et al. (18,19), however, it is noteworthy that Liu et al. (20) in a trial with half as many participants found a statistically significant difference, favouring pulmonary artery perfusion with oxygenated blood, for the clinical outcomes intubation time and length of stay at the intensive care unit.

### Statistics

Our trials results are strengthened by the pre-published statistical analysis plan (Paper II) and by the fact, that primary analysis was adjusted for the stratification variables (mild versus moderate to severe COPD; baseline OI) taking correlation between patients within each stratum into account (91,92). The primary analysis was performed on the modified intention-to-treat population (93), as one patient was excluded post-randomisation (94). The change in the surgical plan from on-pump to off-pump was first made during the operation and we could therefore not have avoided this post-randomisation exclusion.

The statistical analysis plan (Paper II) included an instruction for handling of missing data by multiple imputation, and if used a best-worst worst-best case scenario analysis was performed as a supplement, predicting the limits for the true intervention effects (95). For the primary outcome we had 2% missingness and for the secondary outcome no more than 4% missingness, therefore a complete case analysis without imputation of missing values was performed.

A difficulty to the statistical interpretation of our trial was that the OI (FiO<sub>2</sub> x mean airway pressure/PaO<sub>2</sub>) data were not normally distributed. However, the inverse OI<sup>-1</sup> (PaO<sub>2</sub>/FiO<sub>2</sub> x mean airway pressure) was normally distributed and this transformation was therefore used in the statistical analyses as the use of parametric methods made sense. Although this strengthened the results, it made the interpretation of the results slightly more complex. The same applied for the statistical challenges in analysing and reporting results from a three-armed versus a two-armed

RCT. To simplify and strengthen the statistical analyses we defined our a priori comparison of interest in our statistical analysis plan (Paper II). In relation to other known statistical concerns in multiple-armed RCTs (96) we waived from performing global comparison tests (assessing global differences between all arms), but only performed pair-wise comparisons, did not pool data from two arms, or report selectively (ex. only statistically significant comparisons), which all diminished the risk of a type 1 error.

In order to restrict the family wise error rate to less than the traditionally 0.05 we used Bonferroni adjustment of the P values (97) as well as an a priori defined strict significance level. As outlined in the statistical analysis plan we planned to assess the validity of the trial results according to the five-point procedure as suggested by Jakobsen et al. (98) with a P value threshold for significance of 0.025 (0.05/2) for the two comparisons of the primary outcome. For the secondary outcomes we planned to evaluate the P value according to the following scheme: P≥0.05 not statistically significant; P=0.01 to 0.05: dubious statistical significance; and P<0.01: statistically significant. Nevertheless, our RCT had three treatment arms and two co-primary outcomes leaving us with 6 possible comparisons. Moreover, the three pair-wise comparisons of the intervention groups and the a priori decided primary comparison hampered the presentation of the results with risk of important information getting lost in the large amount of data.

The Bonferroni adjustment of the significance level, considering 6 independent comparisons renders a statistical significance level of 0.05/6=0.008. Thereby, the statistically significant difference (at the 0.05 level) in OI at 21 hours for the comparison pulmonary artery perfusion with blood versus no pulmonary perfusion becomes insignificant indicating that it could be a chance finding. However, this adjustment of the significance level is done under the assumption that the co-primary outcomes are completely independent, which they are obviously not, and the three comparisons due to three intervention groups may also contain some interdependency due to common groups in the comparisons. On the other hand, using no adjustment at all assumes that the type 1 error risk is not increased when several comparisons are done this is also obviously wrong. A P value threshold halfway between 0.05 and 0.008 (0.05/((6+1)/2)=0.014) might be a more realistic significance level (99,100), that would still render the comparison of the OI difference at 21 hours statistically significant.

## LIMITATIONS AND STRENGTHS – STUDY II (THE SYSTEMATIC REVIEW)

### Design

The main strengths of our systematic review was adherence to Cochrane methodology including GRADE (83) and reporting according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) guidelines (101). This included a pre-published, peer-reviewed protocol, a structured and up to date extensive literature search in relevant databases with no language restriction, independent screening and data extraction of all references by two authors, contact with corresponding authors for additional information and data, and inclusion of trials irrespective of publication status and reported outcomes, and bias risk assessment. Despite a thorough pre-planned search strategy in relevant databases supported by hand search we cannot completely rule out the possibility of reporting and publication bias.

Although statistical heterogeneity was low among trials, it is obvious that we have pooled data from clinically heterogeneous trials in regards to clinical setting, type of operation, patient age, comorbidities etc. In addition, a different variety of pulmonary artery perfusion strategies were used in the intervention arms all increasing the risk of type-II error. We excluded RCTs with infants and children as well as trials with co-interventions, to increase clinical comparability. We have on the other hand conducted a broad meta-analysis resulting in increased power and precision of pooled analyses compared to a single trial results. Furthermore, we explored the hypothesis that effects of pulmonary artery perfusion with blood vary by the patients' preoperative lung function, and found no significant differences between subgroups.

### **Trial sequential analysis**

Meta-analyses increases power and precision of pooled estimates, however, the analyses may produce random errors due to sparse data and repetitive testing of accumulated data (102,103). In a TSA, the number of patients needed to show or reject a specific intervention effect (the required information size) was calculated and used to evaluate the strength of a meta-analysis's P value and to adjust confidence intervals for sparse data and repetitive testing in cumulative meta-analysis. This method is similar to an interim analysis of a single trial and it is argued, that TSAs should be applied to conventional meta-analyses with the same methodological effort (104,105). All meta-analyses can therefore be considered as interim analyses on the way to achieving the required information size of plausible effects (106) and the TSA is therefore needed for interpretation of traditional meta-analyses likewise for interpretation of single trials interim analyses. In a recent empirical review exploring the role of TSA in assessing the reliability of conclusions in underpowered meta-analyses Imberger et al. (107) found that the proportion of false positives is likely higher than 7% of the included meta-analyses.

The required information size is calculated from 1) the risk of type I and II errors (usually 5% and 20%, respectively), 2) the anticipated size of the intervention effect, and 3) event proportion. The size of the intervention effect can be selected among 1) the a priori anticipated effect, 2) the observed overall intervention effect in the meta-analysis, or 3) the observed intervention effect in trials with low risk of bias. A limitation to the TSA is that the listed parameter estimates can be based on prior knowledge or on the results of the meta-analyses. The results of the TSA therefore depend on the selected parameters. There are no rights or wrong for this dilemma as anticipated values from a single trial may be imprecise, and calculated values biased if only a few small trials are included or if the results of the meta-analysis primarily are based on data from one very large trial. To strengthen our results we pre-specified the choice of parameter values in our pre-published review protocol.

### **CONCLUSION AND FUTURE PERSPECTIVES**

The PP-Trial provided additional knowledge about the use of pulmonary artery perfusion with either oxygenated blood or HTK solution in cardiac surgery patients with COPD. The oxygenation was improved for patients receiving pulmonary artery perfusion with oxygenated blood compared with both no pulmonary perfusion and pulmonary artery perfusion with HTK solution. Pulmonary artery perfusion with HTK solution did not result in an improved oxygenation. In line with this, the systematic review

including data from additional RCTs showed a possible association between pulmonary artery perfusion with blood and improved postoperative oxygenation, but no significant associations with mortality, SAEs, or intubation time. However, all data are still too sparse to be conclusive.

With our trial we wanted to test if two of the suspected etiological causes to postoperative pulmonary dysfunction following cardiac surgery with CPB could be modelled with improvement of the patients' oxygenation. Our theory was that pulmonary protection during CPB by supplying oxygen to or cooling the lungs could diminish the ischemic insult, and thereby reperfusion injuries, leading to reduced endotoxin release hence attenuation of the systemic inflammatory response syndrome. On the histological and clinical level, less pronounced interstitial pulmonary oedema and alveolar clustering of white blood cells, preserved alveolar surface and blood-gas interphase area could lead to improved oxygenation of the lung tissue and other affected organs. However, given the fact that pulmonary artery perfusion with blood has not proven beneficial for clinical relevant outcomes as mortality its general use cannot yet be recommended. A pragmatic RCT with few in- and exclusion criteria, easy enrolment procedure, inclusion from multiple international centres, an optimized and simplified intervention procedure comparing pulmonary artery perfusion with oxygenated blood to no pulmonary perfusion, and clinical relevant outcomes as mortality, time outside the hospital, pulmonary related events, and SAEs is needed before any conclusive evidence can be made. Adult patients with both normal and reduced lung function should be included only excluding patients with severe heart failure (left ventricular ejection fraction  $\leq$  20%) and salvage surgery in unstable patients. To minimize bias the RCT the following steps should be taken, 1) blinding of all possible domains, 2) pre-randomisation publication of a protocol with defined outcomes and a sample size estimation based on mortality data from low risk RCTs and 3) a defined statistical analysis plan prior to data handling.

### **FUNDING**

This PhD project was funded by The Lundbeck Foundation; Pharmedica A/S; The Aase & Ejnar Danielsen Foundation; The Moeller Foundation; The Ehrenreich Foundation and by The Heart Centre Research Committee at Rigshospitalet, Copenhagen University Hospital, Denmark.

### **CONFLICTS OF INTERESTS**

The author (KBB) declares to have received an unrestricted grant from The Lundbeck Foundation which is not involved in producing or distributing Custodiol histidine-tryptophan-ketoglutarate solution. In addition, KBB has been supported by Pharmedica A/S distributing, but not producing, Custodiol histidine-tryptophan-ketoglutarate solution. The support was given as an unrestricted grant in January 2012 and used for 4 months' salary to write the protocol for The Pulmonary Protection Trial.

### **SUMMARY**

#### **BACKGROUND**

During conventional cardiopulmonary bypass (CPB) there is no active perfusion of the pulmonary circulation and the mechanical ventilation is ceased leaving the lungs exposed to warm ischemia.

Pulmonary dysfunction is seen in varying degrees after major surgery, but more severe in cardiac surgery patients probably due to the effects of CPB. The evidence for effect and safety are limited, but active pulmonary artery perfusion during CPB could be beneficial for the patients' postoperative oxygenation.

Our aim was in a randomised clinical trial to assess primarily the effect of pulmonary artery perfusion during CPB on postoperative oxygenation in patients diagnosed with chronic obstructive pulmonary disease (COPD), secondarily to assess other possible benefits and harms. Furthermore, we wanted in a systematic review with meta-analyses of all randomised clinical trials to investigate the pooled effects of pulmonary artery perfusion during CPB.

## METHODS

We planned and conducted a randomised, partly blinded, clinical trial assigning cardiac surgery patients diagnosed with COPD to receive pulmonary artery perfusion with oxygenated blood or histidine-tryptophan-ketoglutarate (HTK) solution compared to no pulmonary perfusion during CPB. The primary outcome was the oxygenation index measured during and after surgery. Secondary outcomes were intubation time, serious adverse events, days alive outside the intensive care unit and outside the hospital, 30- and 90-days mortality.

Secondly, we conducted a systematic review of randomised clinical trials comparing benefits and harms of using pulmonary artery perfusion versus no pulmonary perfusion during CPB pooling results in meta-analyses and trial sequential analyses (TSA).

## RESULTS

Of the 90 randomised patients 89 were included in analysis of the primary outcome, the inverse oxygenation index, measured at a single time point 21 hours after CPB start and longitudinally 1, 3, 5, 7, and 21 hours after CPB start. At 21 hours, patients randomised to pulmonary artery perfusion with oxygenated blood had a higher inverse oxygenation index compared to patients randomised to no pulmonary perfusion during CPB (mean difference (MD) 0.94; 95% confidence interval (CI), 0.05 to 1.83;  $P=0.04$ ). The inverse oxygenation index was also significantly higher at 21 hours after CPB start (MD 0.99; CI, 0.29 to 1.69;  $P=0.007$ ), and longitudinally ( $P=0.009$ ), for patients receiving pulmonary artery perfusion with oxygenated blood compared to pulmonary artery perfusion with HTK solution. This corresponds to a  $\text{PaO}_2$  difference of 23 mmHg with a median  $\text{FiO}_2$  of 0.32. We found no additional significant differences for the remaining comparisons of the inverse oxygenation index neither for any of the secondary outcomes.

The systematic review identified 4 trials with a total of 210 patients. In meta-analyses pulmonary artery perfusion with blood versus no pulmonary perfusion during CPB was not associated with relative risk of death (1.7; 95% CI, 0.4 to 6.9; 210 patients in three trials with high and one trial with low risk of bias), serious adverse events (1.2; 95% CI, 0.8 to 1.8; 180 patients in two trials with high and one trial with low risk of bias) or intubation time (-0.4 hours; 95% CI, -1.1 to 0.4; 176 patients in three trials with high and one trial with low risk of bias). TSA on mortality, serious adverse events, and  $\text{PaO}_2/\text{FiO}_2$  ratio showed that required information sizes have not been reached, but pulmonary artery perfusion with blood was associated with a higher  $\text{PaO}_2/\text{FiO}_2$  ratio (27.8 mmHg; 95% CI, 5.7 to 50.0 mmHg; 119 patients in two trials with high and one trial with low risk of bias). TSA on intubation time showed that the boundary for lack of

superiority (futility) was crossed refuting a shorten intubation time of 1.5 hours or more.

## CONCLUSION

Our trial provided additional knowledge about the use of pulmonary artery perfusion during CPB in cardiac surgery patients with COPD, and improved oxygenation for patients receiving pulmonary artery perfusion with oxygenated blood. Pulmonary artery perfusion with HTK solution did not result in an improved oxygenation. In line with this, the systematic review including data from additional trials showed a possible association between pulmonary artery perfusion with blood and improved oxygenation, but no significant associations with mortality, serious adverse events or intubation time. However, all data are too sparse to be conclusive.

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