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Physiological-based cord clamping versus immediate cord clamping for infants born with a congenital diaphragmatic hernia (PinC): study protocol for a multicentre, randomised controlled trial

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Manuscripts

1 **Physiological-based cord clamping versus immediate cord clamping for**
2 **infants born with a congenital diaphragmatic hernia (PinC): study protocol**
3 **for a multicentre, randomised controlled trial**

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26 Word count: 2742 words

1
2
3 **27 ABSTRACT**
4

5 **28 Introduction:** Pulmonary hypertension is a major determinant of postnatal survival in infants
6
7
8 **29** with a congenital diaphragmatic hernia (CDH). The current care during the perinatal
9
10 **30** stabilisation period in these infants might contribute to the development of pulmonary
11
12 **31** hypertension after birth – in particular umbilical cord clamping before lung aeration. An ovine
13
14 **32** model of diaphragmatic hernia demonstrated that cord clamping after lung aeration, called
15
16 **33** physiological-based cord clamping (PBCC), avoided the initial high pressures in the lung
17
18 **34** vasculature while maintaining adequate blood flow, thereby avoiding vascular remodelling and
19
20 **35** aggravation of pulmonary hypertension. We aim to investigate if the implementation of PBCC
21
22 **36** in the perinatal stabilisation period of infants born with a CDH could reduce the incidence of
23
24 **37** pulmonary hypertension in the first 24 hours after birth.

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27
28 **38 Methods and analysis:** We will perform a multicentre, randomised controlled trial in infants
29
30 **39** with an isolated left-sided CDH, born at ≥ 35.0 weeks. Before birth, infants will be randomised
31
32 **40** to either PBCC or immediate cord clamping, stratified by treatment centre and severity of
33
34 **41** pulmonary hypoplasia on antenatal ultrasound. PBCC will be performed using a purpose-built
35
36 **42** resuscitation trolley. Cord clamping will be performed when the infant is considered respiratory
37
38 **43** stable, defined as a heart rate >100 bpm, preductal oxygen saturation $>85\%$, while using a
39
40 **44** fraction of inspired oxygen of <0.5 . The primary outcome is pulmonary hypertension diagnosed
41
42 **45** in the first 24 hours after birth, based on clinical and echocardiographic parameters. Secondary
43
44 **46** outcomes include neonatal as well as maternal outcomes.

45
46
47 **47 Ethics and dissemination:** Central ethical approval was obtained from the Medical Ethical
48
49 **48** Committee of the Erasmus MC, Rotterdam, The Netherlands (METC 2019-0414). Local ethical
50
51 **49** approval will be obtained by submitting the protocol to the regulatory bodies and local
52
53 **50** institutional review boards.
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3 51 **Trial registration:** Netherlands Trial Register, NL7853. Registered on 3rd of July, 2019.

4
5 52 ClinicalTrials.gov, NCT04373902. Registered on 14th of April, 2020.

6
7 53 **Keywords:** Physiological-based cord clamping, congenital diaphragmatic hernia, perinatal
8
9 54 stabilisation, pulmonary hypertension, birth defect, resuscitation.

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11 55 **Word count:** 300 words.
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17 57 **STRENGTHS AND LIMITATIONS OF THIS STUDY**

- 18
19 58 - This is the first trial evaluating the effect of physiological-based cord clamping (PBCC)
20
21 59 on the incidence of pulmonary hypertension in the first 24 hours, a clinically relevant
22
23 60 outcome in infants with a congenital diaphragmatic hernia (CDH).
24
25 61 - Real-time monitoring of physiological parameters will improve our understanding of
26
27 62 the physiological changes occurring during the perinatal stabilisation period in infants
28
29 63 born with a CDH.
30
31 64 - As demonstrated in two recent phase II studies, implementing PBCC in infants born
32
33 65 with a CDH is feasible and does not result in unexpected adverse outcomes or safety
34
35 66 concerns, although CDH is a condition inherently associated with a significant risk of
36
37 67 complications.
38
39 68 - To detect a clinically relevant difference in mortality a significantly larger sample size
40
41 69 would be required, thereby prolonging the length of the study. As pulmonary
42
43 70 hypertension is generally regarded as a major contributor to mortality, we consider this
44
45 71 as an adequate proxy for postnatal survival.
46
47 72 - We will collect umbilical cord blood samples from the infants, enabling the detection
48
49 73 of biomarkers that could eventually be used as early predictors of both short-term and
50
51 74 long-term outcomes, thereby allowing early interventions and individualised treatments
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53 75 and specialised packages of care.
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76 INTRODUCTION

77 A congenital diaphragmatic hernia (CDH) is a birth defect characterised by incomplete closure
78 of the diaphragm. Abdominal organs herniate into the fetal thorax and interfere with lung
79 development, thereby contributing to the development of pulmonary hypoplasia.¹⁻⁸ Pulmonary
80 hypoplasia translates in delayed lung aeration after birth, thereby requiring prompt resuscitation
81 and respiratory support. In anticipation of this requirement, the umbilical cord is usually
82 clamped immediately after birth so that the infant can be transferred to a resuscitation table.

83 Despite extensive respiratory support, infants with a CDH face significant mortality (around
84 30% in most series) and long-term morbidity, with many survivors suffering from chronic
85 respiratory problems and pulmonary hypertension.⁹⁻¹² The aetiology of pulmonary hypertension
86 in infants with a CDH is multifactorial. Abnormal structural development of the vasculature,
87 altered vasoreactivity, and progressive vascular remodelling are considered important factors
88 in developing and maintaining high perfusion pressures in the lungs.¹³⁻¹⁵ Postnatal left
89 ventricular systolic dysfunction correlates with outcomes in infants with a CDH and also
90 contributes to the development of pulmonary hypertension.^{16 17} Pulmonary hypertension can
91 develop in the first hours after birth and can persist for weeks to even months. The presence of
92 severe pulmonary hypertension at 1 month of life is associated with a 56% mortality rate prior
93 to discharge.¹⁸ Current treatment options for pulmonary hypertension are limited and mainly
94 consist of pulmonary vasodilator drugs with varying responses and the use of extracorporeal
95 membrane oxygenation.¹⁹

96 Currently, immediate cord clamping is performed in almost all infants born with a CDH.
97 Before cord clamping, oxygenated blood in the umbilical veins shunts to the left atrium via the
98 ductus venosus and foramen ovale, thereby guaranteeing venous return to the left ventricle of
99 the heart.^{20 21} Thus, clamping the cord separates the infant from both its oxygen source as well
100 as the blood flow required to maintain left ventricular preload.^{20 21} In addition, left ventricular

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3 101 afterload increases when the low-resistance circulation of the placenta is removed.^{20 21} As a
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5 102 result, cardiac output decreases. In term neonates with normal lung development, lung aeration
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7 103 causes the pulmonary vascular resistance to decrease and the pulmonary blood flow to increase,
8
9 104 allowing the lungs to take over from the placenta in providing gas exchange (oxygenation) and
10
11 105 maintaining cardiac output.^{20 21}

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13
14 106 In contrast, most infants born with a CDH are faced with a complicated transition from the
15
16 107 fetal to the neonatal phenotype. Due to the abnormally developed pulmonary vasculature and
17
18 108 hypoplastic lungs, lung aeration is delayed and, thus, the pulmonary vascular resistance does
19
20 109 not decrease sufficiently to accommodate the entire output of the right ventricle.²¹⁻²³ Pulmonary
21
22 110 vascular pressures then increase and potentially result in a reactive vasospasm triggering
23
24 111 vascular remodelling, perpetuated by ongoing hypoxic pulmonary vasoconstriction.²² When
25
26 112 lung aeration is established prior to clamping the cord, called physiological-based cord
27
28 113 clamping (PBCC), the lungs will already have taken over the placental function before the cord
29
30 114 is clamped, thereby avoiding the hypoxia and high pulmonary arterial pressures that can occur
31
32 115 after immediate cord clamping (Figure 1). Recently, in an ovine model of a diaphragmatic
33
34 116 hernia, we have confirmed that PBCC resulted in significantly lower pulmonary arterial
35
36 117 pressures while maintaining higher pulmonary blood flows up to 20 and 120 minutes after birth
37
38 118 respectively.²² PBCC thus has the potential to influence the functionality of the pulmonary
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40 119 vessels.

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43 120 Two recent feasibility studies described the concept of initiating respiratory support prior to
44
45 121 cord clamping in infants with a CDH.^{24 25} Both studies confirmed that this approach was feasible
46
47 122 and had promising effects on the cardiovascular adaptation in the first hours after birth, although
48
49 123 neither studies were powered to detect differences in outcomes.^{24 25} Hence, the logical next step
50
51 124 is a randomised clinical trial to determine the true benefit of PBCC for infants with a CDH.²⁶
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53 125 We hypothesise that implementing a non-invasive intervention (such as PBCC) during the
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3 126 perinatal stabilisation period avoids initial high pulmonary perfusion pressures that initiate a
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5 127 vasoreactive response, thereby reducing the risk of pulmonary hypertension. The primary aim
6
7 128 of this study is to investigate if the implementation of PBCC in the stabilisation period of infants
8
9 129 born with a CDH reduces the incidence of pulmonary hypertension in the first 24 hours after
10
11 130 birth, a clinically relevant outcome in these infants. The secondary aim of this study is to
12
13 131 perform real-time monitoring of physiological parameters, which will improve our
14
15 132 understanding of the physiological changes occurring during the perinatal stabilisation period
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17 133 in this population of neonates.
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135 **METHODS AND ANALYSIS**

136 **Study design**

137 The PBCC in CDH (PinC) trial is an international randomised controlled trial, that will be
138 conducted in multiple academical centres in Europe and Australia. Infants will be randomised
139 to either PBCC or immediate cord clamping (Figure 2), whereas ongoing management will be
140 according to a consensus-based postnatal management protocol.¹⁹
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141

142 **Patient and public involvement**

143 Patients were not involved in the design of this study.
144

144

145 **Patient population**

146 We will include infants diagnosed with an isolated left-sided CDH on prenatal ultrasound with
147 gestational age at delivery ≥ 35.0 weeks. Exclusion criteria are right-sided and bilateral CDH,
148 antenatal diagnosed major associated structural or genetic abnormalities, high urgency
149 caesarean section (intended interval to delivery < 15 min), cases that have been treated during
150 pregnancy with experimental drug therapy aiming to decrease the occurrence of pulmonary
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3 151 hypertension, twin pregnancies in which the infant diagnosed with a CDH is born first, multiple
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5 152 birth >2, and placental abnormalities (anterior placenta praevia, placental abruption).
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10 154 **Randomisation**

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12 155 Participants will be randomised using Castor EDC, an electronic data capture system that uses
13
14 156 a computer-generated randomisation list. Infants will be randomised 1:1 to either PBCC or the
15
16
17 157 current standard approach of immediate cord clamping. Allocation will be stratified by
18
19 158 predicted lung size (determined by observed/expected lung-to-head ratio and liver position,
20
21 159 graded as mild/moderate/severe lung hypoplasia, measured between 20-26 weeks or at the
22
23 160 initial visit) and by treatment centre, using variable random permuted block sizes (4-8).²⁷
24
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28 162 **Study procedures**

29
30 163 Providing adequate respiratory support immediately after birth while performing PBCC
31
32 164 requires a resuscitation table near the mother. To facilitate this approach, several trolleys have
33
34 165 been developed and we will preferably use the Concord Birth Trolley (Concord Neonatal B.V.,
35
36 166 Leiden, The Netherlands). This trolley was purpose-built for PBCC and has shown excellent
37
38 167 feasibility in preterm infants.²⁸ The trolley is fully equipped for stabilisation of infants with a
39
40 168 CDH. In all infants, we will use a monitor that records vital parameters during stabilisation.
41
42 169 Prior to the start of the study, all caregivers involved in delivery room care will be trained using
43
44 170 the Concord.

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47 171 In PBCC, the Concord will be placed next to the bed of the mother and all equipment will
48
49 172 be checked before the second stage of labour has started (Figure 3). The infant will be placed
50
51 173 on the platform of the Concord immediately after birth, avoiding any traction or pressure on the
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53 174 cord and avoiding heat loss by radiation heating. The umbilical cord will not be clamped until
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55 175 the infant is considered respiratory stable, which is defined as the presence of a heart rate >100
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3 176 bpm and preductal oxygen saturation >85%, while using a fraction of inspired oxygen of <0.5.
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5 177 Oxytocin administration will be postponed until after cord clamping if there are no obstetric
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7 178 concerns. To both guarantee an optimal placental-to-fetal transfusion as well as avoid excessive
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9 179 maternal blood loss, the minimum and maximum times of cord clamping are three and ten
10
11 180 minutes after birth, respectively.²⁹ At any time, the attending neonatologist and obstetrician can
12
13 181 decide that PBCC should not be performed or be interrupted. In that case, the infant can be
14
15 182 placed on the standard resuscitation table for (further) stabilisation. In the immediate cord
16
17 183 clamping group, the cord will be clamped immediately after birth. The infant will then be
18
19 184 transferred to the standard neonatal resuscitation table. Thermomanagement during stabilisation
20
21 185 is an important focus in both groups since hypothermia is a known trigger for pulmonary
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23 186 hypertension. Normal precautions will be taken to prevent heat loss, such as dry towels, caps,
24
25 187 and a radiant warmer. After cord clamping, all infants will be managed according to the
26
27 188 standardised neonatal management protocol for infants with a CDH, which is a consensus of
28
29 189 current clinical guidelines by the CDH EURO consortium.¹⁹ A 2D echocardiography will be
30
31 190 performed within the first 24 hours of life to evaluate the presence or absence of pulmonary
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33 191 hypertension.

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39 192 This trial provides the unique possibility of collecting umbilical cord blood samples
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41 193 from a significant number of infants with a CDH that will have been randomised for two
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43 194 different stabilisation methods. We speculate that the physiological changes during the
44
45 195 stabilisation period could trigger the release of biomarkers, such as free oxygen radicals,
46
47 196 iron/hepcidin and metabolomics. Free oxygen radicals stimulate pulmonary vasoconstriction
48
49 197 and could thus contribute to the occurrence and therapy-resistance of pulmonary hypertension.³⁰
50
51 198 These biomolecules also induce lipid peroxidation modifying certain metabolic pathways, such
52
53 199 as the endocannabinoid metabolism. Endocannabinoids are an interesting target for further
54
55 200 analysis because of their involvement in supporting the fetal-to-neonatal transition.³¹ A second
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3 201 promising pathway is iron homeostasis, in particular the regulatory protein hepcidin. Iron-
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5 202 deficiency seems to alter smooth muscle cell activity, influence pulmonary vascular function,
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7 203 and, thus, contribute to the severity of pulmonary hypertension.³² Hepcidin treatment in rats
8
9 204 with pulmonary hypertension resulted in a decrease in right ventricular systolic pressure and
10
11 205 mean pulmonary arterial pressure, and with that in a decrease in pulmonary lesions induced by
12
13 206 pulmonary hypertension.³³ Samples will be collected and stored in a Biobank in the Erasmus
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15 207 MC. Cord blood will be analysed to detect relevant biomarkers in the prediction of postnatal
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17 208 outcomes. The above-mentioned biomarkers could eventually be used as early predictors of
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19 209 both short- and long-term outcomes, thereby allowing early interventions and individualised
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21 210 treatments and specialised package of care.
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28 212 **Primary and secondary outcomes**

30 213 The primary study outcome is pulmonary hypertension diagnosed in the first 24 hours after
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32 214 birth combining clinical and echocardiographic parameters (Table 1). To evaluate for the
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34 215 presence or absence of the echocardiographic criteria, we will collect the following
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36 216 echocardiographic parameters: right ventricular systolic pressure, right ventricular size,
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38 217 pulmonary artery acceleration time (PAAT), right ventricular ejection time (RVET),
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40 218 PAAT:RVET ratio, intraventricular septum configuration, left ventricular end-systolic
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42 219 eccentricity index, tricuspid regurgitation, peak velocity of tricuspid regurgitation, tricuspid
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44 220 annular plane systolic excursion, transductal shunting direction, interatrial shunting direction,
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46 221 and right ventricular systolic to diastolic duration ratio.³⁴
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53 223 Secondary outcomes that will be reported in the total population:

- 54 224 - Estimated maternal blood loss during delivery;
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56 225 - Time interval between birth and start respiratory support;
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3 226 - Apgar scores;
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5 227 - Umbilical cord pH;
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8 228 - Temperature at admission to the intensive care unit;
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10 229 - Respiratory support during resuscitation;
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12 230 - Mortality.
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17 232 Secondary outcomes that will be reported in the total population and in the subgroup of
18
19 233 survivors separately:
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21 234 - Presence of pulmonary hypertension requiring therapy on day 7, 14, 21, 28, and at
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23 discharge;
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26 236 - Treatment for pulmonary hypertension;
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28 237 - Use of inotropes and fluid therapy;
29
30 238 - Presence of early onset and late onset sepsis;
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33 239 - Surgical characteristics;
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35 240 - Presence of hyperbilirubinemia requiring therapy;
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37 241 - Presence of neurological complications;
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40 242 - Respiratory support during hospitalisation;
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42 243 - Presence and severity of bronchopulmonary dysplasia;
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44 244 - Number of days on the intensive care unit.
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49 246 Postpartum haemorrhage is considered a safety parameter, because PBCC will result in later
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51 247 cord clamping times than are currently used for infants with a CDH.
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56 249 **Data collection**
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3 250 All outcome variables will be collected by local physicians and will be entered in a password
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5 251 protected online database (Castor EDC). Data access will be granted to the principal
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7 252 investigators of all participating centres. On request the collected data will be available.
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11 254 **Informed consent**

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14 255 Informed consent will be obtained before birth and the procedure will be explained to the
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16 256 parents by the investigators during a specific antenatal counselling session, followed by a time
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18 257 of reflection for the parents.
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22 259 **Data and Safety Monitoring**

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26 260 The data and safety monitoring board will conduct two interim statistical analyses on safety
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28 261 during the course of this study, after approximately 25% and 50% of the total required patients
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30 262 have completed their primary outcome. The only stopping condition will be concerns regarding
31
32 263 safety outcomes. The safety analysis will include, but will not be restricted to, serious adverse
33
34 264 events and the context-specific safety outcomes listed as secondary outcomes
35
36 265 (bronchopulmonary dysplasia, sepsis, cerebral complications, need for extracorporeal
37
38 266 membrane oxygenation). An annual safety report of all context-specific serious adverse events
39
40 267 will be presented to the data and safety monitoring board and approving ethics committee. All
41
42 268 other serious adverse events will be reported to the approving ethics committee in accordance
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44 269 with their guidelines.
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50 271 **Sample size estimates**

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52
53 272 The background incidence of pulmonary hypertension in infants with a CDH can be estimated
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55 273 based on historical cohorts. The largest registry available is the CDH Study Group registry
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57 274 consisting of data from 70 participating centres in 13 countries. A recent review of 3367 patients
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3 275 of this cohort (2007-2014) reports a 69.7% incidence of pulmonary hypertension in the first
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5 276 week after birth (median of 0 days (0-8)).³⁵ As this is the first human clinical study evaluating
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7 277 PBCC with pulmonary hypertension as primary outcome, we cannot estimate the effect size.
8
9
10 278 Thus, we suggest using a clinically relevant change in incidence of pulmonary hypertension to
11
12 279 determine the sample size. We consider that a relative decrease by one third in the incidence of
13
14 280 pulmonary hypertension in the first 24 hours after birth is realistic and is significant enough to
15
16 281 influence change in the neonatal management of infants with a CDH. Based on the background
17
18 282 incidence of pulmonary hypertension, we calculated that at least 140 infants (70 in each group)
19
20 283 are needed to detect a 1/3 reduction, with 80% power and 0.05 significance level. It will be
21
22 284 difficult to estimate the number of cases that will have the umbilical cord clamped earlier than
23
24 285 the times within the PBCC protocol. However, based on the results from two small human
25
26 286 feasibility studies, it can be expected that we will have good overall adherence to the protocol.
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288 **Statistical analyses**

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35 289 The effect of PBCC on the primary outcome (pulmonary hypertension) will be analysed in the
36
37 290 intention-to-treat population. The intention-to-treat population is defined as all patients that
38
39 291 were randomised to a particular treatment arm, independent of protocol deviations. The effect
40
41 292 will be analysed using multivariable logistic regression analysis with pulmonary hypertension
42
43 293 as dependent variable and treatment allocation, severity of pulmonary hypoplasia, and treatment
44
45 294 centre as independent variables. Per protocol analysis for the primary outcome will be employed
46
47 295 as secondary analysis. The per protocol population is defined as all randomised patients who
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49 296 completed the protocol for the arm they were assigned to, had the primary endpoint measured,
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51 297 and had no major protocol violations.
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56 298 The primary analysis will be a complete case analysis. By protocol, the independent
57
58 299 variables in this multivariable analysis will be present in all cases. The dependent outcome
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3 300 could however be missing in the rare event that evaluation has not been performed in the first
4
5 301 24 hours after birth. We will perform a sensitivity analysis by imputing missing values in the
6
7 302 primary outcome by using the 'worst case' observed in cases in which the primary outcome
8
9 303 was assessed.

10
11
12 304 If more than 20% of values on a secondary outcome are missing, we will remove that
13
14 305 variable from analyses. If no more than 20% of a secondary outcome are missing, we will use
15
16 306 multiple imputation to deal with the missing data. Additionally, we will perform a sensitivity
17
18 307 analysis by replacing missing values with the 'worst case' observed in patients with available
19
20 308 data. For secondary outcomes we will calculate risk ratios or odds ratios with 95% confidence
21
22 309 intervals.

23
24
25 310 All data will be tested for normality using a Shapiro-Wilk test. Normally distributed data
26
27 311 will be presented as mean \pm standard deviations, not-normally distributed data as medians
28
29 312 (interquartile ranges). Statistical significance is set at $p < 0.05$, using two-sided tests. Statistical
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31 313 analyses will be performed using the computing environment R (R Core Team (2020), Vienna,
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33 314 Austria).

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38 39 40 316 **ETHICS AND DISSEMINATION**

41
42 317 Central ethical approval was obtained from the Medical Ethical Committee of the Erasmus MC,
43
44 318 Rotterdam, The Netherlands (METC 2019-0414). Local ethical approval will be obtained by
45
46 319 submitting the protocol to the regulatory bodies and local institutional review boards. The study
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48 320 will be conducted according to the principles of the Declaration of Helsinki and international
49
50 321 rules and regulations on personal data protection. The results of this study will be disseminated
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52 322 via peer-reviewed publications.

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57 58 324 **Trial status**

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3 325 Currently five university medical centres are enrolling patients. The first patient was included
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5 326 on 11th of May, 2020, and by 23rd of June 2021, 13 patients had been included. In 2021, two
6
7 327 additional international centres will be added. Final inclusion is expected in 2023. The current
8
9 328 article is based on protocol version 1.5 (15-03-2021).
10
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14 330 **Abbreviations**

16 331 CDH: congenital diaphragmatic hernia; PAAT: pulmonary artery acceleration time; PBCC: physiological-based
17
18 332 cord clamping; RVET: right ventricular ejection time.
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20 333

22 334 **Acknowledgements**

24 335 Not applicable.
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26 336

28 337 **Patient consent for publication**

30 338 Not required.
31

32 339

34 340 **Funding**

36 341 This trial is supported by a grant from Sophia Children's Hospital Foundation (SSWO, grant S19-12). This
37
38 342 funding source did not have any further role in the writing of the protocol or the decision to submit it for
39
40 343 publication.
41

42 344

44 345 **Availability of data and material**

46 346 The datasets of the current study will be available from the corresponding author on reasonable request, after
47
48 347 publication of the main results.
49

50 348

51 349 **Ethics approval and consent to participate**

53 350 Central ethical approval was obtained from the Medical Ethical Committee of the Erasmus Medical Centre,
54
55 351 Rotterdam, The Netherlands (METC 2019-0414). Local ethical approval has been obtained at all participating
56
57 352 centres. Oral and written information will be given to all eligible candidates. Informed consent will be obtained
58
59 353 from the parents of all study participants.
60

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3 3544
5 355 **Authors' contributions**

6
7 356 PLJD and EJJHO wrote the first draft of the study protocol. All authors participated in critical revision of the
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9 357 protocol. EJJHO and PLJD drafted the manuscript. All authors reviewed and edited the manuscript and approved
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11 358 the final version of the manuscript. All authors approved the final manuscript as submitted and agreed to be
12
13 359 accountable for all aspects of the work.

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16 361 **Consent for publication**17
18 362 Not applicable.19
20 36321
22 364 **Competing interests**

23
24 365 ABTP and SH are members of the advisory committee of Concord Neonatal B.V.. However, the authors declare
25
26 366 that the research was conducted in the absence of any commercial or financial relationships that could be
27
28 367 construed as a potential conflict of interest.

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Table 1 Primary outcome

Pulmonary hypertension is present if at least 2 of the following 4 criteria are present or if the infant requires extracorporeal membrane oxygenation in the first 24 hours after birth:

(1)	Right ventricular systolic pressure $\geq 2/3$ systemic systolic pressure*
(2)	Right ventricle dilatation/septal displacement or right ventricular dysfunction +/- left ventricular dysfunction*
(3)	Difference between preductal and postductal oxygen saturation $>10\%$ **
(4)	Oxygenation index >20 **

*on first ultrasound in first 24 hours after birth

**highest values measured during first 24 hours after birth

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For peer review only

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3 456 **Figures**
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5 457 **Figure 1** Fetal-to-neonatal transition in congenital diaphragmatic hernia.
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8 458 **(A)** Clamping the umbilical cord prior to lung aeration has been established and, thus, prior to
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10 459 the pulmonary vascular resistance (PVR) has decreased, increases the arterial blood pressure
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12 460 (ABP, afterload) and decreases the preload to the left ventricle. As a result, the cardiac output
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14 461 decreases. **(B)** Clamping the umbilical cord after lung aeration has been established and, thus,
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16 462 after the PVR has decreased, will result in a more stable transition. In that case, the left
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18 463 ventricular afterload and preload remain stable.
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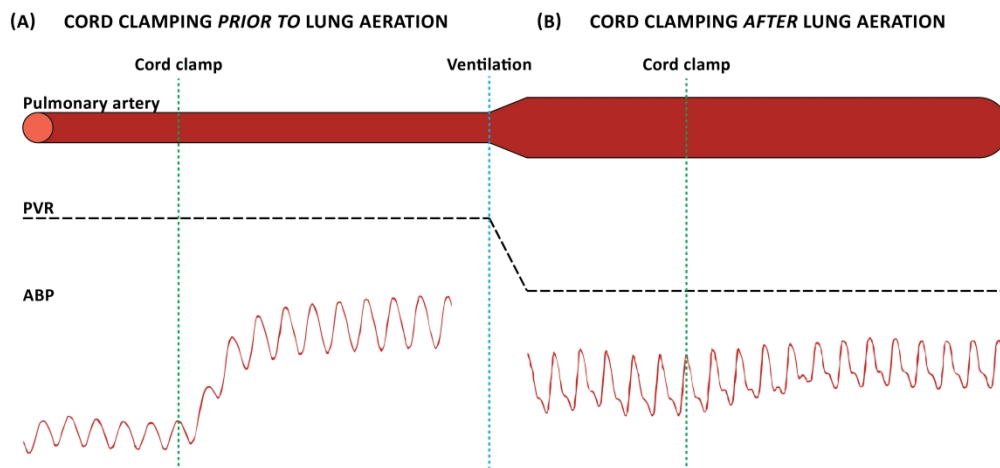
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24 465 **Figure 2** Trial flowchart.
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26 466 The flowchart depicts the steps from the screening of a subject until the evaluation of the
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28 467 primary outcome of the trial.
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33 469 **Figure 3** Position of the Concord Birth Trolley.
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35 470 The Concord Birth Trolley is positioned at the left side of the mother. The infant is then
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37 471 stabilised while the umbilical cord is still intact. The Concord Birth Trolley is fully equipped
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39 472 for stabilisation of infants that are born with a congenital diaphragmatic hernia.
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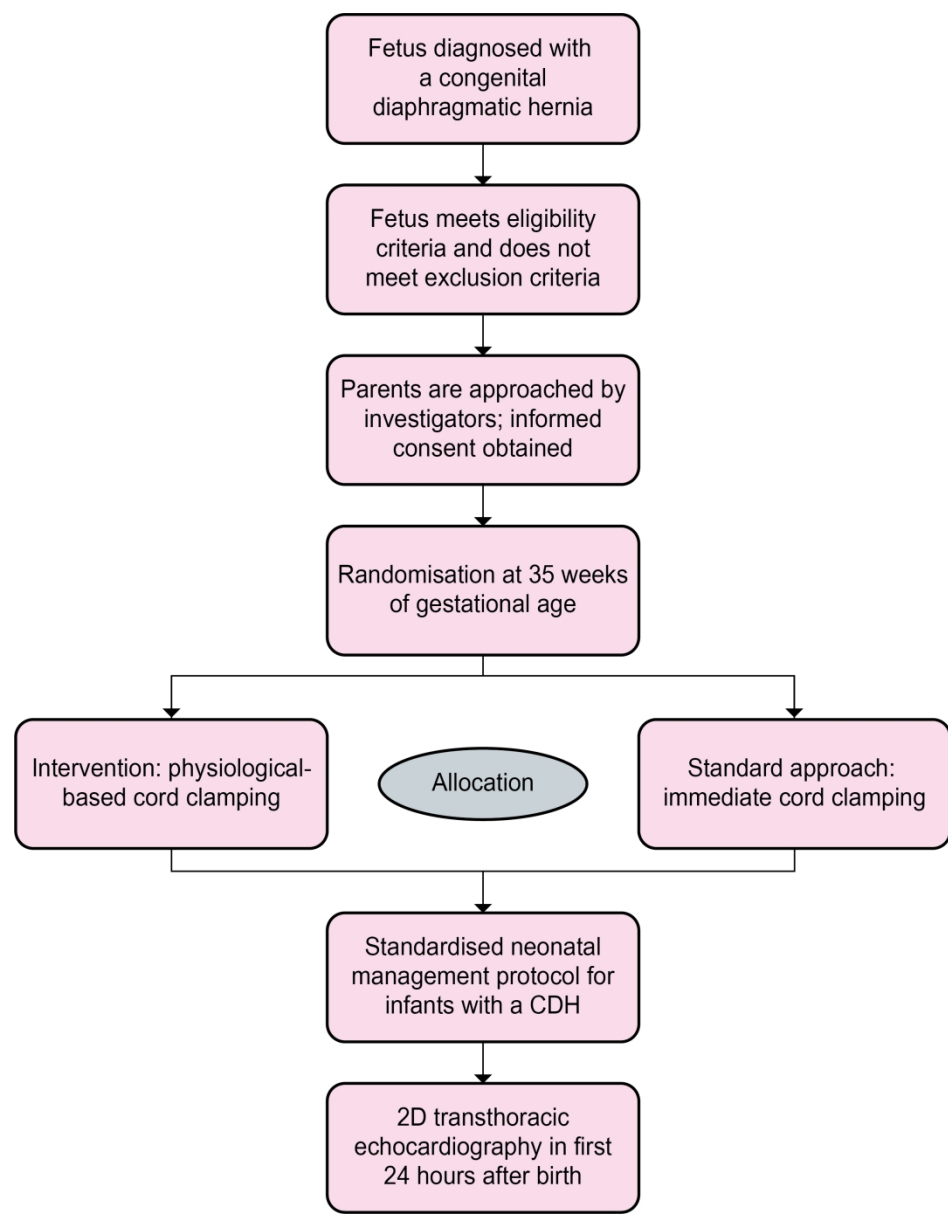


Fetal-to-neonatal transition in congenital diaphragmatic hernia.

887x420mm (118 x 118 DPI)

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Trial flowchart.

574x733mm (118 x 118 DPI)



Position of the Concord Birth Trolley.

472x232mm (79 x 79 DPI)

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STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	1-15
Protocol version	3	Date and version identifier	14
Funding	4	Sources and types of financial, material, and other support	14
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 15
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	11

1 Introduction

2				
3	Background and	6a	Description of research question and justification for undertaking the trial, including summary of relevant	4-6
4	rationale		studies (published and unpublished) examining benefits and harms for each intervention	
5				
6		6b	Explanation for choice of comparators	4-6
7				
8	Objectives	7	Specific objectives or hypotheses	5-6
9				
10	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group),	6-7
11			allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	
12				
13				

14 Methods: Participants, interventions, and outcomes

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16	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	6
17			be collected. Reference to where list of study sites can be obtained	
18				
19	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and	6-7
20			individuals who will perform the interventions (eg, surgeons, psychotherapists)	
21				
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be	7-9
23			administered	
24		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose	11
25			change in response to harms, participant request, or improving/worsening disease)	
26		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence	12
27			(eg, drug tablet return, laboratory tests)	
28		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
29				
30	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood	9-10
31			pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg,	
32			median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen	
33			efficacy and harm outcomes is strongly recommended	
34	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for	9-10
35			participants. A schematic diagram is highly recommended (see Figure)	
36				
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1	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11-12
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4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	N/A
5				
6	Methods: Assignment of interventions (for controlled trials)			
7	Allocation:			
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10	Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	7
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16	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	7
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20	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7
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24	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A
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27		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
28				
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31	Methods: Data collection, management, and analysis			
32				
33	Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10-11
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39		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	12-13
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1	Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10-11
2				
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5	Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	12-13
6				
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8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	12-13
9				
10		20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	12-13
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14	Methods: Monitoring			
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16	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	11,14,15
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22		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	11
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25	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
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28	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	11
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32	Ethics and dissemination			
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34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	13
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37	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	13
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1	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	11
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4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	11
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7	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	10-11
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10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
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13	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	10-11
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16	Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
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20	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	13
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24		31b	Authorship eligibility guidelines and any intended use of professional writers	13
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26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
27				
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29	Appendices			
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31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
32				
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34	Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	8-9
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*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Physiological-based cord clamping versus immediate cord clamping for infants born with a congenital diaphragmatic hernia (PinC): study protocol for a multicentre, randomised controlled trial

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2021-054808.R1
Article Type:	Protocol
Date Submitted by the Author:	22-Feb-2022
Complete List of Authors:	Horn-Oudshoorn, Emily; Erasmus Medical Center Knol, Ronny; Erasmus Medical Center te Pas, Arjan; Leiden University Medical Center Hooper, Stuart B.; Monash University Cochius - den Otter, Suzan; Erasmus Medical Center Wijnen, Rene; Erasmus Medical Center Crossley, Kelly; Monash University Rafat, Neysan; University Medical Centre Mannheim Schaible, Thomas; University Medical Centre Mannheim de Boode, Willem ; Radboud University Medical Center Debeer, Anne; KU Leuven University Hospitals Leuven Urlesberger, Berndt; Medical University of Graz Roberts, Calum; Monash University Kipfmueller, Florian; University of Bonn Reiss, Irwin; Erasmus Medical Center DeKoninck, Philip; Erasmus Medical Center; Monash University
Primary Subject Heading:	Paediatrics
Secondary Subject Heading:	Obstetrics and gynaecology
Keywords:	NEONATOLOGY, PERINATOLOGY, Respiratory physiology < THORACIC MEDICINE, OBSTETRICS

SCHOLARONE™
Manuscripts

1 **Physiological-based cord clamping versus immediate cord clamping for**
2 **infants born with a congenital diaphragmatic hernia (PinC): study protocol**
3 **for a multicentre, randomised controlled trial**

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5 Rene M.H. Wijnen⁴, Kelly J. Crossley³, Neysan Rafat⁵, Thomas Schaible⁵, Willem P. de Boode⁶, Anne Debeer⁷,
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26 Word count: 2742 words

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2
3 **27 ABSTRACT**
4

5 **28 Introduction:** Pulmonary hypertension is a major determinant of postnatal survival in infants
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7
8 **29** with a congenital diaphragmatic hernia (CDH). The current care during the perinatal
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10 **30** stabilisation period in these infants might contribute to the development of pulmonary
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12 **31** hypertension after birth – in particular umbilical cord clamping before lung aeration. An ovine
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14 **32** model of diaphragmatic hernia demonstrated that cord clamping after lung aeration, called
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16 **33** physiological-based cord clamping (PBCC), avoided the initial high pressures in the lung
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18 **34** vasculature while maintaining adequate blood flow, thereby avoiding vascular remodelling and
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20 **35** aggravation of pulmonary hypertension. We aim to investigate if the implementation of PBCC
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22 **36** in the perinatal stabilisation period of infants born with a CDH could reduce the incidence of
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24 **37** pulmonary hypertension in the first 24 hours after birth.

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28 **38 Methods and analysis:** We will perform a multicentre, randomised controlled trial in infants
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30 **39** with an isolated left-sided CDH, born at ≥ 35.0 weeks. Before birth, infants will be randomised
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32 **40** to either PBCC or immediate cord clamping, stratified by treatment centre and severity of
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34 **41** pulmonary hypoplasia on antenatal ultrasound. PBCC will be performed using a purpose-built
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36 **42** resuscitation trolley. Cord clamping will be performed when the infant is considered respiratory
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38 **43** stable, defined as a heart rate >100 bpm, preductal oxygen saturation $>85\%$, while using a
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40 **44** fraction of inspired oxygen of <0.5 . The primary outcome is pulmonary hypertension diagnosed
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42 **45** in the first 24 hours after birth, based on clinical and echocardiographic parameters. Secondary
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44 **46** outcomes include neonatal as well as maternal outcomes.

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48 **49 Ethics and dissemination:** Central ethical approval was obtained from the Medical Ethical
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50 **50** Committee of the Erasmus MC, Rotterdam, The Netherlands (METC 2019-0414). Local ethical
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52 approval will be obtained by submitting the protocol to the regulatory bodies and local
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3 51 **Trial registration:** Netherlands Trial Register, NL7853. Registered on 3rd of July, 2019.

4
5 52 ClinicalTrials.gov, NCT04373902. Registered on 14th of April, 2020.

6
7 53 **Keywords:** Physiological-based cord clamping, congenital diaphragmatic hernia, perinatal

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9 54 stabilisation, pulmonary hypertension, birth defect, resuscitation.

10
11 55 **Word count:** 300 words.

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17 57 **STRENGTHS AND LIMITATIONS OF THIS STUDY**

18
19 58 - This is the first randomised controlled trial evaluating the effect of physiological-based
20
21 59 cord clamping (PBCC) on the incidence of pulmonary hypertension in the first 24 hours
22
23 60 after birth in infants with a congenital diaphragmatic hernia (CDH).

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25 61 - To detect a clinically relevant difference in mortality a significantly larger sample size
26
27 62 would be required, but pulmonary hypertension is an adequate proxy as it is a major
28
29 63 contributor to mortality in infants with a CDH.

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31 64 - Treatment allocation cannot be blinded in this trial; to account for this, objective
32
33 65 echocardiographic parameters are used to objectify the primary outcome.

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35 66 - Real-time monitoring of physiological parameters will improve our understanding of
36
37 67 the physiological changes occurring during the perinatal stabilisation period in infants
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39 68 with a CDH.

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41 69 - Although a multicentre trial has inherent disadvantages, collaboration is essential given
42
43 70 that CDH is a rare disease; the use of a relatively early primary outcome may decrease
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45 71 the impact of centre-specific differences.

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72 INTRODUCTION

73 A congenital diaphragmatic hernia (CDH) is a birth defect characterised by incomplete closure
74 of the diaphragm. Abdominal organs herniate into the fetal thorax and interfere with lung
75 development, thereby contributing to the development of pulmonary hypoplasia.¹⁻⁸ Pulmonary
76 hypoplasia translates in delayed lung aeration after birth, thereby requiring prompt resuscitation
77 and respiratory support. In anticipation of this requirement, the umbilical cord is usually
78 clamped immediately after birth so that the infant can be transferred to a resuscitation table.

79 Despite extensive respiratory support, infants with a CDH face significant mortality (around
80 30% in most series) and long-term morbidity, with many survivors suffering from chronic
81 respiratory problems and pulmonary hypertension.⁹⁻¹² The aetiology of pulmonary hypertension
82 in infants with a CDH is multifactorial. Abnormal structural development of the vasculature,
83 altered vasoreactivity, and progressive vascular remodelling are considered important factors
84 in developing and maintaining high perfusion pressures in the lungs.¹³⁻¹⁵ Postnatal left
85 ventricular systolic dysfunction correlates with outcomes in infants with a CDH and also
86 contributes to the development of pulmonary hypertension.^{16 17} Pulmonary hypertension can
87 develop in the first hours after birth and can persist for weeks to even months. The presence of
88 severe pulmonary hypertension at 1 month of life is associated with a 56% mortality rate prior
89 to discharge.¹⁸ Current treatment options for pulmonary hypertension are limited and mainly
90 consist of pulmonary vasodilator drugs with varying responses and the use of extracorporeal
91 membrane oxygenation.¹⁹

92 Currently, immediate cord clamping is performed in almost all infants born with a CDH.
93 Before cord clamping, oxygenated blood in the umbilical veins shunts to the left atrium via the
94 ductus venosus and foramen ovale, thereby guaranteeing venous return to the left ventricle of
95 the heart.^{20 21} Thus, clamping the cord separates the infant from both its oxygen source as well
96 as the blood flow required to maintain left ventricular preload.^{20 21} In addition, left ventricular

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3 97 afterload increases when the low-resistance circulation of the placenta is removed.^{20 21} As a
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5 98 result, cardiac output decreases. In term neonates with normal lung development, lung aeration
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7 99 causes the pulmonary vascular resistance to decrease and the pulmonary blood flow to increase,
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10 100 allowing the lungs to take over from the placenta in providing gas exchange (oxygenation) and
11
12 101 maintaining cardiac output.^{20 21}

13
14 102 In contrast, most infants born with a CDH are faced with a complicated transition from the
15
16 103 fetal to the neonatal phenotype. Due to the abnormally developed pulmonary vasculature and
17
18 104 hypoplastic lungs, lung aeration is delayed and, thus, the pulmonary vascular resistance does
19
20 105 not decrease sufficiently to accommodate the entire output of the right ventricle.²¹⁻²³ Pulmonary
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22 106 vascular pressures then increase and potentially result in a reactive vasospasm triggering
23
24 107 vascular remodelling, perpetuated by ongoing hypoxic pulmonary vasoconstriction.²² When
25
26 108 lung aeration is established prior to clamping the cord, called physiological-based cord
27
28 109 clamping (PBCC), the lungs will already have taken over the placental function before the cord
29
30 110 is clamped, thereby avoiding the hypoxia and high pulmonary arterial pressures that can occur
31
32 111 after immediate cord clamping (Figure 1). Recently, in an ovine model of a diaphragmatic
33
34 112 hernia, we have confirmed that PBCC resulted in significantly lower pulmonary arterial
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36 113 pressures while maintaining higher pulmonary blood flows up to 20 and 120 minutes after birth
37
38 114 respectively.²² PBCC thus has the potential to influence the functionality of the pulmonary
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40 115 vessels.

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42 116 Two recent feasibility studies described the concept of initiating respiratory support prior to
43
44 117 cord clamping in infants with a CDH.^{24 25} Both studies confirmed that this approach was feasible
45
46 118 and had promising effects on the cardiovascular adaptation in the first hours after birth, although
47
48 119 neither studies were powered to detect differences in outcomes.^{24 25} Hence, the logical next step
49
50 120 is a randomised clinical trial to determine the true benefit of PBCC for infants with a CDH.²⁶
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52 121 We hypothesise that implementing a non-invasive intervention (such as PBCC) during the
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3 122 perinatal stabilisation period avoids initial high pulmonary perfusion pressures that initiate a
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5 123 vasoreactive response, thereby reducing the risk of pulmonary hypertension. The primary aim
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7 124 of this study is to investigate if the implementation of PBCC in the stabilisation period of infants
8
9 125 born with a CDH reduces the incidence of pulmonary hypertension in the first 24 hours after
10
11 126 birth, a clinically relevant outcome in these infants. The secondary aim of this study is to
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13 127 perform real-time monitoring of physiological parameters, which will improve our
14
15 128 understanding of the physiological changes occurring during the perinatal stabilisation period
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17 129 in this population of infants.
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131 **METHODS AND ANALYSIS**

132 **Study design**

133 The PBCC in CDH (PinC) trial is an international randomised controlled trial, that will be
134 conducted in multiple academical centres in Europe and Australia. Infants will be randomised
135 to either PBCC or immediate cord clamping (Figure 2), whereas ongoing management will be
136 according to a consensus-based postnatal management protocol.¹⁹
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138 **Patient and public involvement**

139 Patients were not involved in the design of this study.
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141

141 **Patient population**

142 We will include infants diagnosed with an isolated left-sided CDH on prenatal ultrasound with
143 gestational age at delivery ≥ 35.0 weeks. Exclusion criteria are right-sided and bilateral CDH,
144 antenatal diagnosed major associated structural or genetic abnormalities, high urgency
145 caesarean section (intended interval to delivery < 15 min), cases that have been treated during
146 pregnancy with experimental drug therapy aiming to decrease the occurrence of pulmonary
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3 147 hypertension, twin pregnancies in which the infant diagnosed with a CDH is born first, multiple
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5 148 birth >2, and placental abnormalities (anterior placenta praevia, placental abruption).
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10 150 **Randomisation**

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12 151 Participants will be randomised using Castor EDC, an electronic data capture system that uses
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14 152 a computer-generated randomisation list and, thus, ensures concealment of allocation. Infants
15
16 153 will be randomised 1:1 to either PBCC or the current standard approach of immediate cord
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18 154 clamping. Allocation will be stratified by predicted lung size (determined by observed/expected
19
20 155 lung-to-head ratio and liver position, graded as mild/moderate/severe lung hypoplasia,
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22 156 measured between 20-26 weeks or at the initial visit) and by treatment centre, using variable
23
24 157 random permuted block sizes (4-8).²⁷
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30 159 **Study procedures**

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33 160 Providing adequate respiratory support immediately after birth while performing PBCC
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35 161 requires a resuscitation table near the mother. To facilitate this approach, several trolleys have
36
37 162 been developed and we will preferably use the Concord Birth Trolley (Concord Neonatal B.V.,
38
39 163 Leiden, The Netherlands). This trolley was purpose-built for PBCC and has shown excellent
40
41 164 feasibility in preterm infants.²⁸ The trolley is fully equipped for stabilisation of infants with a
42
43 165 CDH. In all infants, we will use a monitor that records vital parameters during stabilisation.
44
45 166 Prior to the start of the study, all caregivers involved in delivery room care will be trained using
46
47 167 the Concord.
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51 168 In PBCC, the Concord will be placed next to the bed of the mother and all equipment will
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53 169 be checked before the second stage of labour has started (Figure 3). The infant will be placed
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55 170 on the platform of the Concord immediately after birth, avoiding any traction or pressure on the
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57 171 cord and avoiding heat loss by radiation heating. The umbilical cord will not be clamped until
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3 172 the infant is considered respiratory stable, which is defined as the presence of a heart rate >100
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5 173 bpm and preductal oxygen saturation >85%, while using a fraction of inspired oxygen of <0.5.
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7 174 Oxytocin administration will be postponed until after cord clamping if there are no obstetric
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9 175 concerns. To both guarantee an optimal placental-to-fetal transfusion as well as avoid excessive
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11 176 maternal blood loss, the minimum and maximum times of cord clamping are three and ten
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13 177 minutes after birth, respectively.²⁹ At any time, the attending neonatologist and obstetrician can
14
15 178 decide that PBCC should not be performed or be interrupted. In that case, the infant can be
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17 179 placed on the standard resuscitation table for (further) stabilisation. In this trial, physicians
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19 180 cannot be blinded to treatment allocation. However, we believe that the lack of blinding will
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21 181 not lead to deviations from the intended intervention, hence the influence on the primary
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23 182 outcome will be limited. In the immediate cord clamping group, the cord will be clamped
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25 183 immediately after birth. The infant will then be transferred to the standard neonatal resuscitation
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27 184 table. Thermomanagement during stabilisation is an important focus in both groups since
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29 185 hypothermia is a known trigger for pulmonary hypertension. Normal precautions will be taken
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31 186 to prevent heat loss, such as dry towels, caps, and a radiant warmer. After cord clamping, all
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33 187 infants will be managed according to the standardised neonatal management protocol for infants
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35 188 with a CDH, which is a consensus of current clinical guidelines by the CDH EURO
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37 189 consortium.¹⁹ A 2D echocardiography will be performed within the first 24 hours of life to
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39 190 evaluate the presence or absence of pulmonary hypertension.

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42 191 This trial provides the unique possibility of collecting umbilical cord blood samples
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44 192 from a significant number of infants with a CDH that will have been randomised for two
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46 193 different stabilisation methods. We speculate that the physiological changes during the
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48 194 stabilisation period could trigger the release of biomarkers, such as free oxygen radicals,
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50 195 iron/hepcidin and metabolomics. Free oxygen radicals stimulate pulmonary vasoconstriction
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52 196 and could thus contribute to the occurrence and therapy-resistance of pulmonary hypertension.³⁰
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3 197 These biomolecules also induce lipid peroxidation modifying certain metabolic pathways, such
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5 198 as the endocannabinoid metabolism. Endocannabinoids are an interesting target for further
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8 199 analysis because of their involvement in supporting the fetal-to-neonatal transition.³¹ A second
9
10 200 promising pathway is iron homeostasis, in particular the regulatory protein hepcidin. Iron-
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12 201 deficiency seems to alter smooth muscle cell activity, influence pulmonary vascular function,
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14 202 and, thus, contribute to the severity of pulmonary hypertension.³² Hepcidin treatment in rats
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16 203 with pulmonary hypertension resulted in a decrease in right ventricular systolic pressure and
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18 204 mean pulmonary arterial pressure, and with that in a decrease in pulmonary lesions induced by
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20 205 pulmonary hypertension.³³ Samples will be collected and stored in a Biobank in the Erasmus
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22
23 206 MC. Cord blood will be analysed to detect relevant biomarkers in the prediction of postnatal
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25 207 outcomes. The above-mentioned biomarkers could eventually be used as early predictors of
26
27 208 both short- and long-term outcomes, thereby allowing early interventions and individualised
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29 209 treatments and specialised package of care.
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35 211 **Primary and secondary outcomes**

37 212 The primary study outcome is pulmonary hypertension diagnosed in the first 24 hours after
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39 213 birth combining clinical and echocardiographic parameters (Table 1). As the physician
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41 214 assessing the echocardiogram cannot be blinded to the intervention in all centres, we will collect
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43 215 the following echocardiographic parameters to guarantee objective evaluation of the presence
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45 216 or absence of the echocardiographic parameters: right ventricular systolic pressure, right
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47 217 ventricular size, pulmonary artery acceleration time (PAAT), right ventricular ejection time
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49 218 (RVET), PAAT:RVET ratio, intraventricular septum configuration, left ventricular end-systolic
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51 219 eccentricity index, tricuspid regurgitation, peak velocity of tricuspid regurgitation, tricuspid
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53 220 annular plane systolic excursion, transductal shunting direction, interatrial shunting direction,
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55 221 and right ventricular systolic to diastolic duration ratio.³⁴
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5 223 Secondary outcomes that will be reported in the total population:
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8 224 - Maternal blood loss during delivery, estimated using the volume in the suction device
9
10 225 and on the surgical swabs;
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12 226 - Time interval between birth and start respiratory support;
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14 227 - Apgar scores;
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16 228 - Umbilical cord pH;
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18 229 - Temperature at admission to the intensive care unit;
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21 230 - Respiratory support during resuscitation;
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24 231 - Mortality.

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26 232
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28 233 Secondary outcomes that will be reported in the total population and in the subgroup of
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30 234 survivors separately:

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33 235 - Presence of pulmonary hypertension requiring therapy on day 7, 14, 21, 28, and at
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35 236 discharge;
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37 237 - Treatment for pulmonary hypertension;
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39 238 - Use of inotropes and fluid therapy;
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42 239 - Presence of early onset and late onset sepsis;
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44 240 - Surgical characteristics;
45
46 241 - Presence of hyperbilirubinemia requiring therapy;
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48 242 - Presence of neurological complications;
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51 243 - Respiratory support during hospitalisation;
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53 244 - Presence and severity of bronchopulmonary dysplasia;
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56 245 - Number of days on the intensive care unit.

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3 247 Postpartum haemorrhage is considered a safety parameter, because PBCC will result in later
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5 248 cord clamping times than are currently used for infants with a CDH.
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10 250 **Data collection**
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12 251 All outcome variables will be collected by local physicians and will be entered in a password
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14 252 protected online database (Castor EDC). Data access will be granted to the principal
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16 253 investigators of all participating centres. On request the collected data will be available.
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21 255 **Informed consent**
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23 256 Informed consent will be obtained before birth and the procedure will be explained to the
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25 257 parents by the investigators during a specific antenatal counselling session, followed by a time
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27 258 of reflection for the parents.
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33 260 **Data and Safety Monitoring**
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35 261 The data and safety monitoring board will conduct two interim statistical analyses on safety
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37 262 during the course of this study, after approximately 25% and 50% of the total required patients
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39 263 have completed their primary outcome. The only stopping condition will be concerns regarding
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41 264 safety outcomes. The safety analysis will include, but will not be restricted to, serious adverse
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43 265 events and the context-specific safety outcomes listed as secondary outcomes
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45 266 (bronchopulmonary dysplasia, sepsis, cerebral complications, need for extracorporeal
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47 267 membrane oxygenation). An annual safety report of all context-specific serious adverse events
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49 268 will be presented to the data and safety monitoring board and approving ethics committee. All
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51 269 other serious adverse events will be reported to the approving ethics committee in accordance
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53 270 with their guidelines.
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272 **Sample size estimates**

273 The background incidence of pulmonary hypertension in infants with a CDH can be estimated
274 based on historical cohorts. The largest registry available is the CDH Study Group registry
275 consisting of data from 70 participating centres in 13 countries. A recent review of 3367 patients
276 of this cohort (2007-2014) reports a 69.7% incidence of pulmonary hypertension in the first
277 week after birth (median of 0 days (0-8)).³⁵ As this is the first human clinical study evaluating
278 PBCC with pulmonary hypertension as primary outcome, we cannot estimate the effect size.
279 Thus, we suggest using a clinically relevant change in incidence of pulmonary hypertension to
280 determine the sample size. We consider that a relative decrease by one third in the incidence of
281 pulmonary hypertension in the first 24 hours after birth is realistic and is significant enough to
282 influence change in the neonatal management of infants with a CDH. Based on the background
283 incidence of pulmonary hypertension, we calculated that at least 140 infants (70 in each group)
284 are needed to detect a 1/3 reduction, with 80% power and 0.05 significance level. It will be
285 difficult to estimate the number of cases that will have the umbilical cord clamped earlier than
286 the times within the PBCC protocol. However, based on the results from two small human
287 feasibility studies, it can be expected that we will have good overall adherence to the protocol.

288

289 **Statistical analyses**

290 The effect of PBCC on the primary outcome (pulmonary hypertension) will be analysed in the
291 intention-to-treat population. The intention-to-treat population is defined as all patients that
292 were randomised to a particular treatment arm, independent of protocol deviations. The effect
293 will be analysed using multivariable logistic regression analysis with pulmonary hypertension
294 as dependent variable and treatment allocation, severity of pulmonary hypoplasia, and treatment
295 centre as independent variables. Per protocol analysis for the primary outcome will be employed
296 as secondary analysis. The per protocol population is defined as all randomised patients who

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3 297 completed the protocol for the arm they were assigned to, had the primary endpoint measured,
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5 298 and had no major protocol violations.
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8 299 The primary analysis will be a complete case analysis. By protocol, the independent
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10 300 variables in this multivariable analysis will be present in all cases. The dependent outcome
11
12 301 could however be missing in the rare event that evaluation has not been performed in the first
13
14 302 24 hours after birth. We will perform a sensitivity analysis by imputing missing values in the
15
16 303 primary outcome by using the 'worst case' observed in cases in which the primary outcome
17
18 304 was assessed.
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21 305 If more than 20% of values on a secondary outcome are missing, we will remove that
22
23 306 variable from analyses. If no more than 20% of a secondary outcome are missing, we will use
24
25 307 multiple imputation to deal with the missing data. Additionally, we will perform a sensitivity
26
27 308 analysis by replacing missing values with the 'worst case' observed in patients with available
28
29 309 data. For secondary outcomes we will calculate risk ratios or odds ratios with 95% confidence
30
31 310 intervals.
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35 311 All data will be tested for normality using a Shapiro-Wilk test. Normally distributed data
36
37 312 will be presented as mean \pm standard deviations, not-normally distributed data as medians
38
39 313 (interquartile ranges). Statistical significance is set at $p < 0.05$, using two-sided tests. Statistical
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41 314 analyses will be performed using the computing environment R (R Core Team (2020), Vienna,
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43 315 Austria).
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49 317 **ETHICS AND DISSEMINATION**

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51 318 Central ethical approval was obtained from the Medical Ethical Committee of the Erasmus MC,
52
53 319 Rotterdam, The Netherlands (METC 2019-0414). Local ethical approval was obtained from the
54
55 320 ethical committees of the University Hospital of Graz, Austria; the Radboudumc University
56
57 321 Medical Center, Nijmegen, the Netherlands; Monash Health, Clayton, Australia; University
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3 322 Hospitals Leuven, Belgium. The study is in the final stage of the review process by the ethical
4
5 323 committees of the University of Bonn, Germany, and the University Medical Center Mannheim,
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7 324 Germany. The study will be conducted according to the principles of the Declaration of Helsinki
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10 325 and international rules and regulations on personal data protection. The results of this study will
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12 326 be disseminated via peer-reviewed publications.
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16 17 328 **Trial status**

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19 329 Currently five university medical centres are enrolling patients. The first patient was included
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21 330 on 11th of May, 2020, and by 23rd of June 2021, 13 patients had been included. In 2021, two
22
23 331 additional international centres will be added. Final inclusion is expected in 2023. The current
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25 332 article is based on protocol version 1.5 (15-03-2021).
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29 30 334 **Abbreviations**

31
32 335 CDH: congenital diaphragmatic hernia; PAAT: pulmonary artery acceleration time; PBCC: physiological-based
33
34 336 cord clamping; RVET: right ventricular ejection time.
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36 337

37 38 338 **Acknowledgements**

39
40 339 Not applicable.
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42 340

43 44 341 **Patient consent for publication**

45
46 342 Not required.
47
48 343

49 50 344 **Funding**

51
52 345 This trial is supported by a grant from Sophia Children's Hospital Foundation (SSWO, grant S19-12). This
53
54 346 funding source did not have any further role in the writing of the protocol or the decision to submit it for
55
56 347 publication.
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59 60 349 **Availability of data and material**

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3 350 The datasets of the current study will be available from the corresponding author on reasonable request, after
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5 351 publication of the main results.

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9 353 **Ethics approval and consent to participate**

10 354 Central ethical approval was obtained from the Medical Ethical Committee of the Erasmus Medical Centre,
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12 355 Rotterdam, The Netherlands (METC 2019-0414). Local ethical approval has been obtained at all participating
13
14 356 centres. Oral and written information will be given to all eligible candidates. Informed consent will be obtained
15
16 357 from the parents of all study participants.

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19
20 359 **Authors' contributions**

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22 360 PLJD and EJJHO wrote the first draft of the study protocol. RK, ABP, SBH, SCMCO, RMHW, KJC, NR, TS,
23
24 361 WPB, AD, BU, CTR, FK, and IKMR participated in critical revision of the protocol. EJJHO and PLJD drafted
25
26 362 the manuscript. RK, ABP, SBH, SCMCO, RMHW, KJC, NR, TS, WPB, AD, BU, CTR, FK, and IKMR
27
28 363 reviewed and edited the manuscript. All authors approved the final manuscript as submitted and agreed to be
29
30 364 accountable for all aspects of the work.

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34 366 **Consent for publication**

35 367 Not applicable.

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39 369 **Competing interests**

40
41 370 ABTP and SH are members of the advisory committee of Concord Neonatal B.V.. However, the authors declare
42
43 371 that the research was conducted in the absence of any commercial or financial relationships that could be
44
45 372 construed as a potential conflict of interest.

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Table 1 Primary outcome

Pulmonary hypertension is present if at least 2 of the following 4 criteria are present or if the infant requires extracorporeal membrane oxygenation in the first 24 hours after birth:

(1)	Right ventricular systolic pressure $\geq 2/3$ systemic systolic pressure*
(2)	Right ventricle dilatation/septal displacement or right ventricular dysfunction +/- left ventricular dysfunction*
(3)	Difference between preductal and postductal oxygen saturation $>10\%$ **
(4)	Oxygenation index >20 **

*on first ultrasound in first 24 hours after birth

**highest values measured during first 24 hours after birth

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For peer review only

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3 461 **Figures**
4

5 462 **Figure 1** Fetal-to-neonatal transition in congenital diaphragmatic hernia.
6

7 463 **(A)** Clamping the umbilical cord prior to lung aeration has been established and, thus, prior to
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9 464 the pulmonary vascular resistance (PVR) has decreased, increases the arterial blood pressure
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11 465 (ABP, afterload) and decreases the preload to the left ventricle. As a result, the cardiac output
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13 466 decreases. **(B)** Clamping the umbilical cord after lung aeration has been established and, thus,
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15 467 after the PVR has decreased, will result in a more stable transition. In that case, the left
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17 468 ventricular afterload and preload remain stable.
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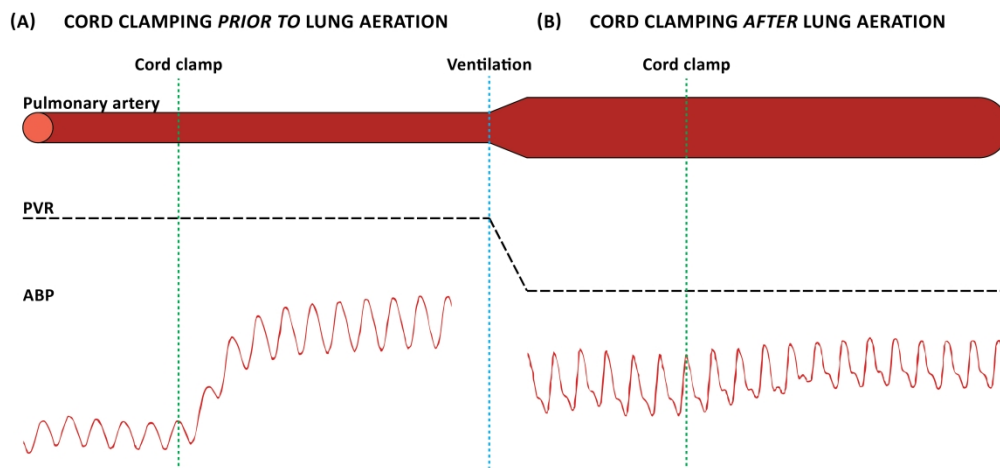
23 470 **Figure 2** Trial flowchart.
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25 471 The flowchart depicts the steps from the screening of a subject until the evaluation of the
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27 472 primary outcome of the trial.
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32 474 **Figure 3** Position of the Concord Birth Trolley.
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34 475 The Concord Birth Trolley is positioned at the left side of the mother. The infant is then
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36 476 stabilised while the umbilical cord is still intact. The Concord Birth Trolley is fully equipped
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38 477 for stabilisation of infants that are born with a congenital diaphragmatic hernia.
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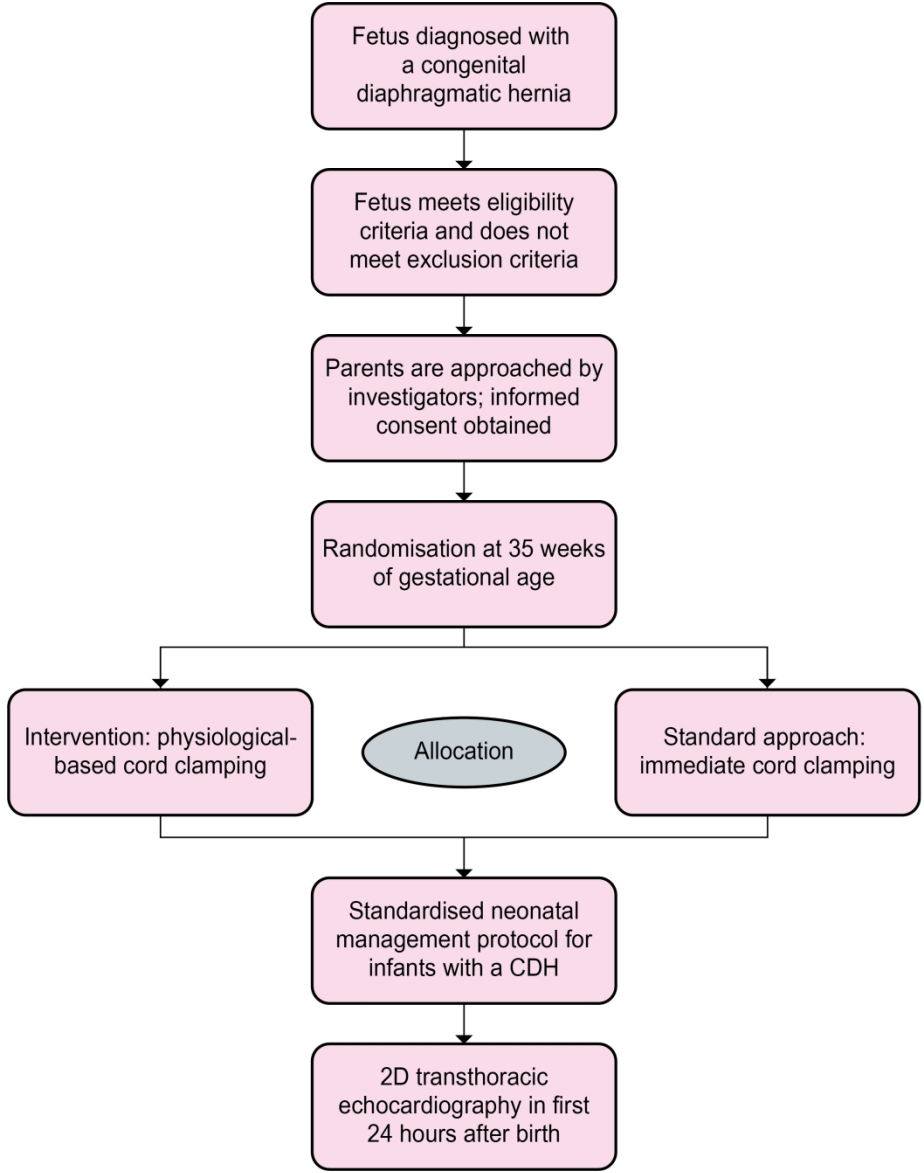


Fetal-to-neonatal transition in congenital diaphragmatic hernia.

887x420mm (118 x 118 DPI)

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Trial flowchart.

628x733mm (118 x 118 DPI)



Position of the Concord Birth Trolley.

472x232mm (79 x 79 DPI)

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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3
	2b	All items from the World Health Organization Trial Registration Data Set	1-15
Protocol version	3	Date and version identifier	14
Funding	4	Sources and types of financial, material, and other support	14
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 15
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	14
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	11

1 Introduction

2				
3	Background and	6a	Description of research question and justification for undertaking the trial, including summary of relevant	4-6
4	rationale		studies (published and unpublished) examining benefits and harms for each intervention	
5				
6		6b	Explanation for choice of comparators	4-6
7				
8	Objectives	7	Specific objectives or hypotheses	5-6
9				
10	Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group),	6-7
11			allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	
12				
13				

14 Methods: Participants, interventions, and outcomes

15				
16	Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will	6
17			be collected. Reference to where list of study sites can be obtained	
18				
19	Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and	6-7
20			individuals who will perform the interventions (eg, surgeons, psychotherapists)	
21				
22	Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be	7-9
23			administered	
24		11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose	11
25			change in response to harms, participant request, or improving/worsening disease)	
26		11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence	12
27			(eg, drug tablet return, laboratory tests)	
28		11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
29				
30	Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood	9-10
31			pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg,	
32			median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen	
33			efficacy and harm outcomes is strongly recommended	
34	Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for	9-10
35			participants. A schematic diagram is highly recommended (see Figure)	
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1	Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	11-12
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4	Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	N/A
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6	Methods: Assignment of interventions (for controlled trials)			
7	Allocation:			
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10	Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	7
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16	Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	7
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20	Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7
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24	Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A
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27		17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
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31	Methods: Data collection, management, and analysis			
32				
33	Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10-11
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38		18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	12-13
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1	Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	10-11
2				
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5	Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	12-13
6				
7				
8		20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	12-13
9				
10		20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	12-13
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14	Methods: Monitoring			
15				
16	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	11,14,15
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22		21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	11
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25	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	11
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28	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	11
29				
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32	Ethics and dissemination			
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34	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	13
35				
36				
37	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	13
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1	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	11
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3				
4		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	11
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7	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	10-11
8				
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10	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
11				
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13	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	10-11
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16	Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
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20	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	13
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24		31b	Authorship eligibility guidelines and any intended use of professional writers	13
25				
26		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
27				
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29	Appendices			
30				
31	Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
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34	Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	8-9
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*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.