Protocol

SafeBoosC-III

Safeguarding the brain of our smallest children

– an investigator-initiated, pragmatic, open label, multinational randomized phase III clinical trial evaluating treatment based on near-infrared spectroscopy monitoring versus treatment as usual in premature infants

Trial phase	Phase III
ClinicalTrials.gov:	NCT03770741
Copenhagen Trial Unit number:	SafeBoosC-DP-363
Protocol date (version):	27.03.19 (PROTOCOL_Version 1.2.2)
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Study site:	Multicentre; international

Revision history

Version	Author	Date	Major changes
1.0 draft	The SafeBoosC-III Trial Group	30.05.2018	Initial version
2.0 draft	The SafeBoosC-III Trial Group	11.06.2018	• Addition of 6.4, 6.7 and allowance of applying for deferred consent • Changes to secondary outcomes and addition of exploratory outcomes
2.1 draft	The SafeBoosC-III Trial Group	13.08.2018	• Addition of exploratory outcome 'other brain injuries' and 'major neonatal morbidities count' • Significant changes to 1.4.2 • Addition of Appendix G, H, I • Power calculations for 'other brain injuries' and 'major neonatal morbidities count'
1.0 protocol	The SafeBoosC-III Trial Group	01.10.2018	• Components of 'other brain injuries' added to the component 'severe brain injury' in the primary outcome • SUSAR reporting has been removed • Time to start monitoring has been prolonged from three to six hours • Addition of explanatory variables (see appendix I)
1.1 protocol	The SafeBoosC-III Trial Group	23.10.2018	• New Portuguese National Coordinator/Steering Committee Member • Addition of text in 1.2.4, 4.5 and 5.1.1 regarding the importance of starting NIRS monitoring as soon as possible after birth
1.2 protocol	The SafeBoosC-III Trial Group	12.11.2018	• Text change in figure 2 (<6 instead of <3) • Change in text (summary, 5.1.2, 8.2) so that not only CE-marked, but all devices approved for clinical use in newborn infants can be used in the trial

			• Addition of 'weight at discharge' as explanatory variable (see appendix I) • Addition to 7.2 that doctor enrolling participant is also responsible for the prescription of NIRS monitoring • Change in 12.2.2, External GCP monitoring • Addition of blinding procedure in relation to cUS interpretation • Addition of sepsis as explorative outcome • Addition that triplets and quadruplets should not be included in the study • Addition of predefined SAE's
1.2.1 Protocol	The SafeBoosC-III Trial Group	14.01.2019	• 5.1.2 Editing of available NIRS-devices • 12.2.2 Removal of severe brain injury from source data verification • 14.1 Editing of legal aspects, additional funding will be applied for • 14.2 Addition of 'no conflict of interest' • 16.3.4 Staff members scoring brain images will be blinded to group allocation • 16.6 Removal of "Serious adverse reactions will be reported directly to the sponsor within 24 hours." • Web-based training and certification will be offered, not demanded, i.e. clinical sites can start randomizing patients without all staff members being certified.
1.2.2	The SafeBoosC-III Trial Group		• Front page Clinicaltrials.gov no. added • 16.9 Addition of "gender" as explanatory variable as a control measure of successful randomization

			• Synchronizing eCRF and Appendix I • 6.6 Change of times for data assessment • 10.2 Removal of statement regarding exclusion of triplets and quadruplets • 6.2 Change in order of exploratory outcomes • Removal of Netherlands as participating country • Removal of UK coordinator from protocol • 16.5 and 6 Minor text editing • 16.1 removal of table 1 in Appendix A

Summary

Background

Each year, 50,000 extremely preterm infants are born in high-income countries with access to neonatal intensive care. Of these, 10,000 will die and a further 10,000 will suffer cerebral palsy or moderate-to-severe neuro-cognitive disability. Time spent outside normal cerebral oxygenation ranges (time with hypoxia or hyperoxia) is associated with poor neurological outcome in children and adults. Monitoring of cerebral oxygenation may reduce the risk of cerebral complications, but no such effects have yet been demonstrated in preterm infants in large randomised clinical trials. The recently completed SafeBoosC phase II trial was conducted at eight sites in eight European countries. 166 extremely preterm infants were randomised to visible monitoring of cerebral oxygenation by near-infrared spectroscopy (NIRS) combined with an evidence-based treatment guideline (experimental group) versus blinded NIRS and treatment as usual (control group). The trial found that NIRS monitoring in combination with an evidence-based treatment guideline successfully reduced the burden of hypoxia and hyperoxia from 81% to 36%hours during the first three days of life ($p<0.001$). Furthermore, the proportion of severe brain injury assessed by central reading of serial cranial ultrasound was 12.5% in the experimental group versus 23.4% in the control group, RR 0.53 (95% CI: 0.26 to 1.08). Mortality was 14% in the experimental versus 25% in the control group, RR 0.50 (95% CI: 0.29 to 1.00). No other evidence has been identified. Based on these preliminary findings, we are planning a phase III randomised clinical trial; the SafeBoosC-III trial.

Objectives

The overall objective of the SafeBoosC-III trial is to investigate the benefit and harms of treatment based on near-infrared spectroscopy monitoring compared with treatment as usual. The hypothesis is that treatment based on near-infrared spectroscopy monitoring for extremely preterm infants during the first 72 hours of life will result in a reduction in severe brain injury or death at 36 weeks postmenstrual age.

Trial design

The SafeBoosC-III trial will be an investigator-initiated, randomized, multinational, pragmatic phase-III clinically open trial with a two-parallel group design. Sixteen-hundred extremely preterm infants will be included within 24 months at 50 neonatal intensive care units (NICUs) across 20 countries (less than two children per month per unit). Data management and statistical analysis will be blinded.

Eligibility

Eligible infants will be born before 28 weeks of postmenstrual age; decision is made to provide full life support; signed informed consent (unless the NICU has chosen to use ‘opt-out’ or deferred consent as consent method); and cerebral NIRS oximeter placed within 6 hours after birth.

Interventions

Participants in the experimental group will be monitored during the first 72 hours of life with a cerebral NIRS oximeter, placed within six hours after birth, and treated according to an evidence-based treatment guideline. Participants in the control group will not undergo cerebral oxygenation monitoring and will be treated as usual. Each participant will be followed up at 36 weeks postmenstrual age.

Outcomes

The primary outcome will be a composite of severe brain injury (cerebral haemorrhage grade III or IV, cystic periventricular leukomalacia, cerebellar haemorrhage, post-haemorrhagic ventricular dilatation or cerebral atrophy) detected on any of the serial cranial ultrasound scans that are routinely performed in these infants up to that age or death until 36 weeks of postmenstrual age.

Exploratory outcomes will be a score of the presence of bronchopulmonary dysplasia, retinopathy of prematurity stage 3+, and severe brain injury as defined in the primary outcome, as well as bronchopulmonary dysplasia, retinopathy of prematurity stage +3, necrotizing enterocolitis stage 2 or higher using the modified Bell's staging and/or focal intestinal perforation, and late-onset sepsis (>72 hours after birth) defined as treatment with antibiotics for at least five days, as isolated outcomes.

Sample size

We have calculated our sample size based on the primary outcome with an alpha of 5%, a power of 90%, and a ratio of 1:1 between intervention groups. In the SafeBoosC-II trial, the proportion of trial participants in the control group with death or severe brain injury was approximately 34%. Assuming the same proportion in the SafeBoosC phase III trial control group and using 22% relative risk reduction as anticipated intervention effect, we will need to randomise a total of 1,600 participants.

Safety

Oximeters approved for clinical use in newborn infants will be used. Predefined serious adverse events and serious adverse reactions will be reported separately. An independent Data Monitoring and Safety Committee will be established to monitor the safety of the trial participants during the trial.

Ethical considerations

Approval from the relevant ethics committees and/or Institutional Review Boards is mandatory for every participating centre. Written parental informed consent will be obtained prior to randomisation unless the ethics committee has granted permission to use deferred informed consent or prior informed assent. The trial will be conducted in compliance with the guidelines of the Declaration of Helsinki in its latest form and of the International Conference on Harmonisation Good Clinical Practice. Procedures will be established to prevent and minimise risk of complications for participants, such as complications related to the device handling. The evidence-based treatment guideline includes only interventions that are commonly used during intensive care for this population.

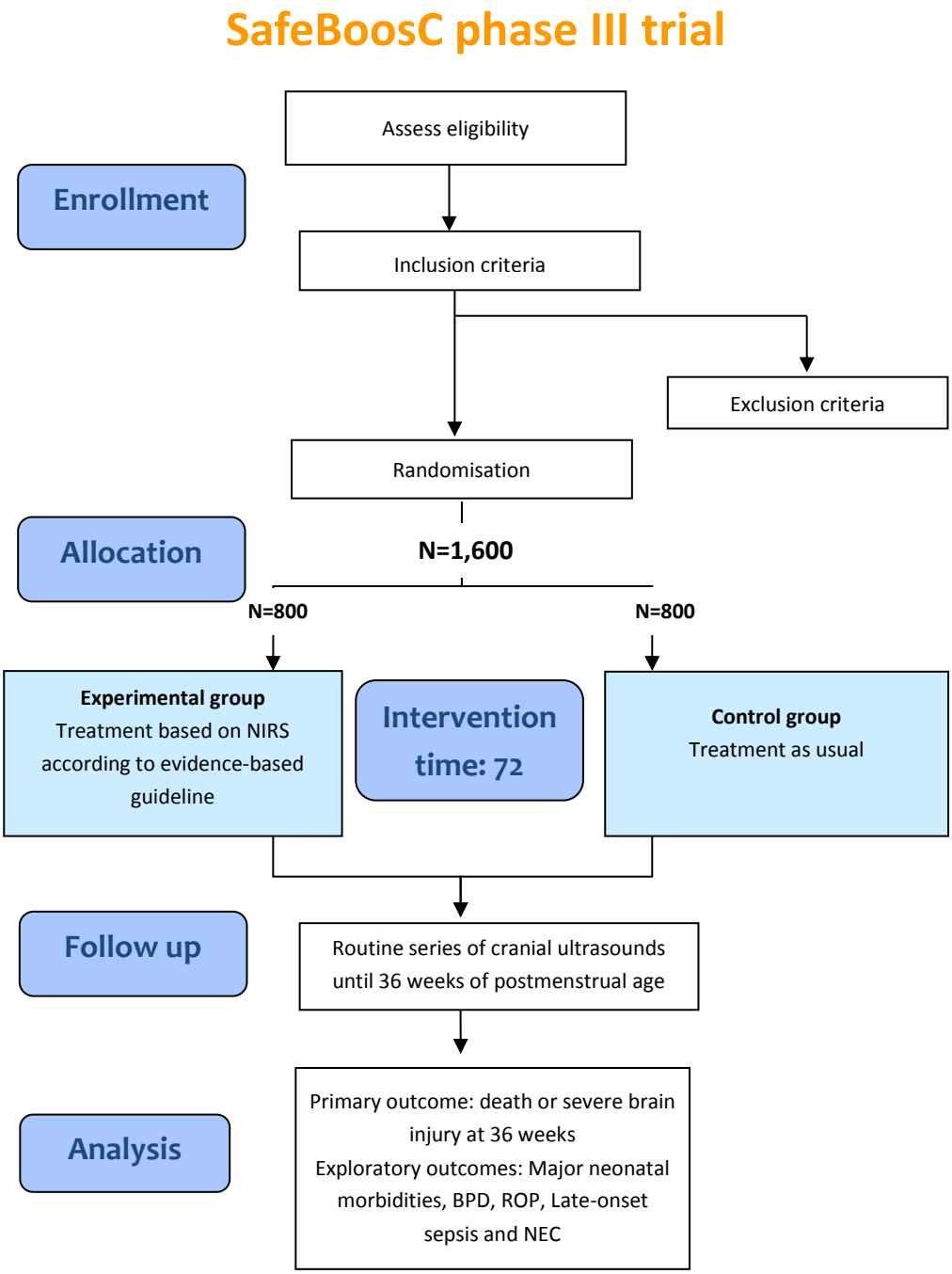
Trial duration

Recruitment will begin in April of 2019 and will be completed within 24 months (April of 2021).

Perspectives

If the experimental intervention proves successful, we may save at least 2,000 extremely preterm infants every year from death or a life with a handicap due to brain injury in high-income countries. The ensuing health economics impact running into billions of Euros saved annually.

SafeBoosC phase III trial flow diagram



Source: adapted from the CONSORT Statement, 2010

Table of contents

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SafeBoosC-III is led by a Steering Committee comprising the coordinating investigator/sponsor, the national coordinators and two representatives from Copenhagen Trial Unit. Decisions will be by simple majority.

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The executive committee is responsible for day-to-day management and will comprise the Coordinating investigator, the project manager, co-investigators and two representatives from Copenhagen Trial Unit.

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List of abbreviations

BPD	Bronchopulmonary dysplasia
cUS	Cranial ultrasound
eCRF	Electronic case report form
FiO_2	Fraction of inspired oxygen
GCP	Good clinical practice
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
NIRS	Near-infrared spectroscopy
O_2	Oxygen
pCO_2	Partial pressure of carbon dioxide
PDA	Patent ductus arteriosus
ROP	Retinopathy of prematurity
$rStO_2$	Regional tissue oxygen saturation
SAR	Serious adverse reactions
SafeBoosC	Safeguarding the brain of our smallest children
SpO_2	Pulse oximeter oxygen saturation

1. Introduction and background

1.1 The population and condition

Infants born more than 12 weeks preterm (extremely preterm) carry a high risk of death and long-term cerebral impairment. Currently, mortality is about 20% and about 25% live with either cerebral palsy or low intelligence quotient (1). Every year 50,000 extremely preterm infants are born in high-income countries. This means that 10,000 of these infants die and 10,000 survive with a significant psychomotor impairment. Psychomotor impairment is a major cause of reduced quality of life and increased costs of medical care, rehabilitation, and special education in this population (2). With increasing life expectancy, this is an important problem.

Unfortunately, prevention of preterm birth and its consequences has not been successful; the rate of extremely preterm birth is stable or even increasing. Although there are risk factors, such as multiple pregnancy, and previous preterm birth, most extremely preterm births occur in otherwise normal and healthy women (3).

1.2 Pathophysiology

1.2.1 The transition from fetal to infant circulation

The transition from fetal to neonatal life is a particular problem in the extremely preterm infant. In fetal life, blood circulation includes only minimal perfusion of the lungs due to a large right-to-left shunt through the foramen ovale of the heart and through the ductus arteriosus from the pulmonary artery to the descending aorta. At birth, increased oxygenation results in systemic vasoconstriction and increasing arterial blood pressure (4). Also, following initial lung expansion and oxygen exposure, pulmonary vascular resistance drops, causing left-to-right shunting across the ductus arteriosus, which increases the need for left ventricular output (5). Since the immature myocardium is intolerant to increased afterload and demonstrates poor ability to increase stroke volume, the above transition may cause low systemic blood flow, which may expose the brain to low cardiac output states (4).

1.2.2 Cerebral autoregulation

Autoregulation is the ability to keep the organ blood flow constant despite fluctuations in perfusion pressure. It is accomplished by regulation of the arterial tone, so that low perfusion pressure results in vasodilation and high perfusion results in vasoconstriction. On the systemic level, organs such as the brain, heart, and adrenal glands are vital, and autoregulation maintains normal organ blood flow when systemic blood flow is low, while non-vital organs (e.g. skin and kidney) vasoconstrict to direct circulating blood to the vital organs.

Cerebral autoregulation has limited capacity and is thought to be particularly fragile in the immature brain (1). Pressure passive flow is a state where blood flow is directly affected by blood pressure. It is hypothesized that large fluctuations in cerebral blood flow may result in cerebral haemorrhages due to rupture of the immature blood vessels. A major current limitation is that it has not been possible to identify the threshold in systemic blood pressure below which cerebral perfusion begins to fall (6).

1.2.3 The vulnerable brain

All the organs are immature when an infant is born more than 12 weeks preterm. The immaturity and functional limitations of the lungs, heart, intestine, kidneys and endocrine system all contribute to the acute problems of extremely preterm birth (2,7). The brain is critical, however, in the sense that brain damage results in death or in

neurodevelopmental deficits such as cerebral palsy, cognitive deficits, attention deficit disorder, and major psychiatric disorders, which have long-term consequences for the affected children.

The most easily identifiable type of brain damage in extremely preterm infants is intraventricular haemorrhage (8). Intraventricular haemorrhage severity varies: the mildest form, grade I haemorrhage, is confined to the subependymal germinal matrix, grade II is a germinal matrix haemorrhage with extension into a normal-sized ventricular system, grade III is an intraventricular haemorrhage with ventricular dilatation, and the most severe form of intraventricular haemorrhage, grade IV, is haemorrhage directly involving the brain parenchyma. Severe intraventricular haemorrhage (e.g. grade III or IV) is associated with a high probability of death or cerebral palsy and may result in hydrocephalus (9). Hydrocephalus requiring surgical treatment carries a poor neurodevelopmental prognosis. Periventricular leucomalacia is a non-haemorrhagic white matter injury. In the mildest form, the condition is non-cystic and predicts poor psychomotor development. The most severe form of periventricular leucomalacia is when the condition becomes cystic 2-5 weeks after the damage is induced and is a strong predictor of cerebral palsy (3).

A normal ultrasound predicts a normal neuromotor outcome in 74% to 96% and a normal cognitive outcome in 77% to 97% of extremely preterm infants (10,11).
For intraventricular haemorrhage grade I-II, the probability of cerebral palsy is 9% (95% confidence interval 4-22%) and for intraventricular haemorrhage grade III 26% (95% CI 13% to 45%) (12). Grade IV intraventricular haemorrhage predicts an abnormal neurodevelopmental outcome with a positive predictive value of 47% (95% CI 31% to 64%) (13). Cystic periventricular leucomalacia is predictive of cerebral palsy with a positive predictive value of 77% (95% CI 59% to 89%) (13). Cerebellar haemorrhage predicts abnormal neuromotor and cognitive outcome with a positive predictive value of 71% (95% CI 42% to 90%) (11).

1.2.4 Mechanisms of brain damage in preterm infants and oxygenation of the brain

There are multiple mechanisms of the brain damage in extremely preterm infants. Some of the mechanisms are evoked before birth or even before the start of delivery, such as that due to a foetal inflammatory response induced by infection ascending to the foetal membranes (14). Also, late effects such as insufficient nutrition during the first months of life may play a role (15).

The following postnatal factors have been shown or are thought to be associated with brain injury: respiratory distress syndrome (16), hypocapnia due to inadvertent hyperventilation (17), hypotension (18), perturbations in arterial and venous pressure (19), and also low cerebral blood flow (20). An important common mechanism for these associations is disturbance of cerebral blood flow partly due to impaired cerebral autoregulation as described above (21).

Thus, cerebral hypoxia in extremely preterm infants can be due to brain perfusion disturbances resulting from insufficient blood pressure, cardiac dysfunction, or suboptimal mechanical ventilation. It can also be due to insufficient oxygen content in the blood, or to combinations thereof. There are medical treatments for each of these problems that are already used in routine clinical practice. Selecting the optimal treatment, however, requires novel patient monitoring information, a good understanding of pathophysiology, as well as the ability to put both into a clinical context.

The first days hours and days after birth are likely to be of particular importance to the preterm infant. This is the period of change from a state of low oxygen pressure ('Mount Everest in-utero') to a state of 'normoxaemia'. Moreover, the circulatory adaption to birth presents its own issues as described above. Thus, fluctuations in systemic blood flow are common during the first days of life.

1.3 Current clinical management

1.3.1 General management

Current standard of care of the extremely premature infants during their first 72 hours involves a number of different parallel interventions:

- *Respiratory support:* the use of continuous positive airway pressure or mechanical ventilation is almost universal, and surfactant is often administered within the first 24 hours.
- *Haemodynamic support:* Fluid boluses, inotropes, or vasopressors are used to treat hypotension, although the blood pressure threshold for treatment is controversial (22). A patent ductus arteriosus is, if deemed deleterious, treated with indomethacin, ibuprofen or acetominophen, or as a last resort surgically.
- *Fluid balance/nutrition:* Close observation of hourly and daily estimations of intake and output, scheduled fluid administration, and blood sugar monitoring is routine in preterm neonates. Most infants initially receive full parenteral nutrition and will slowly be introduced to breast milk.
- *Monitoring*: Invasive and non-invasive blood pressure monitoring, continuous pulse oximetry, transcutaneous partial pressure of carbon dioxide (pCO_2), and electrocardiographic monitoring with frequent measurements of arterial blood gases, electrolytes and temperature.

Treatment of extremely premature infants has certainly improved over the last three decades. However, since the optimal arterial blood pressure, the optimal arterial oxygen content, as well as the optimal pCO_2 level are uncertain and likely to be variable, and many possible interventions are with little evidence. Furthermore, while still more comprehensive monitoring is implemented in the intensive care of premature infants, an end-organ monitoring with sufficiently high time resolution to guide evidence-based treatment interventions is lacking. Near infrared spectroscopy (NIRS) has the potential to become that monitor of the brain.

1.3.2 Use and recommendations for cerebral oximetry in preterm infants

Cerebral oximetry by NIRS is used routinely in extremely preterm infants during the first days of life in some NICUs across the world (23). It is likely that the use will become more common as evidence in other patient groups becomes stronger (24). The potential benefit of clinical use is evident and easily conveyed by device manufacturers, yet specific clinical benefits remain unproven (25). Many medical devices (e.g. monitors, ventilators with new features) have entered routine clinical practice on similar grounds. There is therefore, for the time being, a 'window of opportunity' to examine if the treatment based on near-infrared spectroscopy monitoring improves patient-relevant outcomes of this vulnerable group of infants. Therefore, a robust randomised clinical trial is warranted.

1.4 Near-infrared spectroscopy (NIRS) to measure oxygenation

1.4.1 Tissue oxygenation versus haemoglobin-oxygen saturation in arterial and venous blood

Near-infrared spectroscopy (NIRS) is a non-invasive technology that enables estimation of the tissue oxygenation (26). NIRS uses the relative transparency of human tissue to light in the near-infrared region of the

electromagnetic spectrum. NIRS oximeters provide an absolute value of tissue oxygenation ($rStO_2$) expressed as ratio of oxygenated to total haemoglobin in the tissue underlying a given monitoring sensor.

NIRS is based on the same principles as the widely used pulse oximetry, but whereas pulse oximetry uses only a pulsatile signal and thereby selectively measures arterial blood, NIRS measures the light attenuation of the tissue as a whole and the estimate of oxygen haemoglobin saturation is influenced by the blood in all types of vessels. This means that venous blood contributes more to the NIRS signal than arterial blood simply because, anatomically, venous blood has a greater volume within tissues. The ratio of venous:arterial contribution is generally considered to be 75:25, although this has been found to differ between and within infants (27). It is thus not surprising that cerebral tissue oxygenation has shown only a fair correlation with the saturation in cerebral venous blood drawn from the jugular bulb (28). The Bland-Altman limit of agreement is ±15% to 20% (29,30). It also has to be stressed that $rStO_2$ is volume-weighted across areas with high or low oxygen extraction whereas jugular bulb saturation is flow-weighted, thus very good agreement is unlikely.

1.4.2 Tissue oxygenation as a measure of cardiac output

Intensive care routinely uses pulse oximetry to dose oxygen and respiratory support to ensure appropriate blood oxygenation and monitoring of heart rate and blood pressure as indirect measures of cardiac function. In contrast, monitoring of cardiac output or tissue blood flow is not routinely feasible in preterm infants.

Tissue oxygenation is a direct measure of the oxygen demand-supply balance and correlates with cardiac output (31) and low tissue oxygenation is a predictor of vital organ failure (26). As the distance from the skin to the brain surface is less than 5 mm in newborn preterm infants, NIRS is particularly suitable for monitoring cerebral oxygenation in these patients. Therefore, monitoring of cerebral oxygenation may serve a dual purpose as a general indicator of cardiac output and a direct indicator of oxygen sufficiency of the brain, the last being of particular relevance as brain injury is a problem in itself in neonates.

1.4.3 Regional cerebral oxygenation saturation in preterm infants

During the first 3 days of life in more than 400 preterm babies (born at gestational age <32weeks), Lemmers and van Bel found the rStO2 normal range to be 55% to 85% (mean ±2 standard deviations) (personal communication). These data were collected using the INVOS 4100/5100 with the Adult Somasensor® and constituted the basis of the normal ranges in the SafeBoosC phase II trial, i.e. 55% to 85%. Subsequently, a publication with normal ranges of cerebral StO2 in preterm infants has been published, indicating that values may be lower in the most immature infants, in particular during first day of life (32). These values, however, are 'statistically normal', not necessarily biologically normal. It is not evident that optimal StO2 should be correlated with gestational age. The risk of death and brain injury decrease as gestational age increase, and the SafeBoosC-II trial used 55% as the threshold for intervention. Therefore, the SafeBoosC-III trial will use the same threshold as a lower normal value.

1.4.4 NIRS devices and the reliability of monitoring cerebral oxygenation

There is no 'reference standard' for tissue oximetry and different NIRS devices differ in absolute values of $rStO_2$ (33).This is particularly relevant to the lower limits of oxygenation, which is relevant for the SafeBoosC-III trial. Therefore, we have determined the values that correspond to the 55% threshold in the INVOS 5100c with the Adult Somasensor® for Nonin SenSmart X-100 neonatal, CAS Medical FORE-SIGHT Elite, INVOS Neo, and Hamamatsu NIRO-200NX in a blood-lipid phantom (34,35).

1.5 Evidence of the value of cerebral oximetry in preterm infants and trial rationale

1.5.1 Cerebral oxygenation as a physiological variable

We have identified a variety of observational studies that document how cerebral oximetry in extremely preterm infants gives meaningful physiological data. Wolf and Greisen reviewed 36 studies in neonates that all contribute to an understanding of oxygen delivery-consumption balance in this population (36).

1.5.2 Trials of clinical benefit in preterm infants

We have developed a framework for the assessment of the clinical benefit in preterm infants, in the form of a Cochrane Systematic Review (25) The only published randomized clinical trial focusing on the effect of cerebral monitoring outside the delivery room is the SafeBoosC phase-II feasibility trial (www.safeboosc.eu) (37). The conclusion is that the grade of evidence for recommending cerebral oximetry in this population is very low.

In the SafeBoosC-II trial, visible NIRS monitoring of cerebral oxygenation combined with an evidence-based treatment guideline during in the first 72 postnatal hours was compared with non-visible NIRS monitoring and standard care (24). In this trial, 166 extremely preterm infants were enrolled in eight European countries at sites in Lyon, Madrid, Copenhagen, Cork, Utrecht, Graz, Milan, and Cambridge. The primary outcome of the burden of cerebral hypoxia and hyperoxia (calculated as the area under the curve outside the target range of 55% to 85%) was successfully reduced in the experimental group (**Figure 1**) (38).

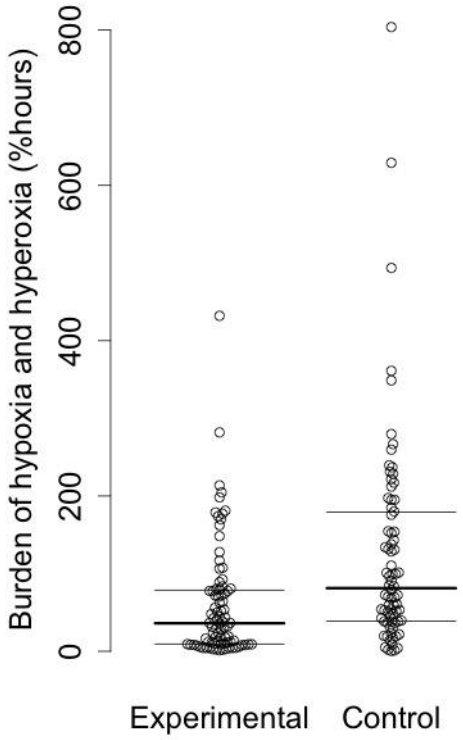

Figure 1.

The SafeBoosC-II trial demonstrated that the burden of hypoxia and hyperoxia was reduced to a median of 36%hours in the experimental group versus a median of 81%hours in the control group (p<0.001) (38). This was due to less burden of hypoxia in the experimental group (median 17%hours versus 54%hours, p=0.0012), while the burden of hyperoxia was low and did not differ significantly between study groups (38). There were also trends towards reduced incidence of severe brain injury and reduced mortality though SafeBoosC II was not powered to demonstrate these outcomes (38). The clinical interventions that occurred in the NIRS-open group included a significant number with likely effects on cardiac output or cerebral blood flow (39).

The risks of bronchopulmonary dysplasia and retinopathy of prematurity, however, tended to be increased. We have published a number of different outcomes related to the SafeBoosC II feasibility study. These include brain injury on cranial ultrasound and magnetic resonance imaging (40) along with biomarkers of brain injury (41). All publications are available in full at www.safeboosc.eu.

The benefit of NIRS as a short-term monitoring tool in the delivery suite has also been addressed (42). This small pilot trial showed that it was feasible to use NIRS as an adjunct to monitor adaptation in the delivery suite to reduce cerebral hypoxia, and a larger randomized trial with patient-relevant outcomes has been funded.

1.5.3 Trials of clinical benefit in adults

Evidence for the benefits of cerebral oximetry is also limited in adults. A systematic review of evidence of clinical utility for cerebral oximetry in adults during coronary surgery concluded that, with data from 47 trials including more than 5,000 participants, the methodological quality of the trials was low and therefore clinical benefits and harms remain uncertain. However, the majority of the studies in the systematic review reported positive results regarding clinical efficacy of NIRS (43). This conclusion was unchanged in 2015 (24). Recently, a Cochrane review confirmed this uncertainty, mainly due to the low incidence of clinically significant negative events (44). Despite the lack of clear evidence on the clinical benefits, NIRS is increasingly used in perioperative care for cardiac patients (45).

1.6 Justification for a large trial to evaluate clinical benefits and harms

1.6.1 The need for a trial in extremely preterm infants

Preterm infants have a significantly increased risk of death or survival with moderate-to-severe neurodevelopmental deficit. In extremely preterm infants, the risk of this composite outcome is as high as 35% to 45%. While this group only constitutes less than 0.5% of all births, the contribution to infant mortality and to the prevalence of cerebral palsy exceeds 10%. There is accumulating evidence that hypoxia is a significant cause of mortality as well as brain injury. Thus, monitoring of cerebral oxygen saturation levels during the first days after birth has the potential to address a significant health problem in a high-risk population. Although the overall risk is high, there are other relevant causes and only a moderate risk reduction can be expected. Therefore, a trial to address this therapeutic question must be large in scope.

On the other hand, NIRS monitoring may theoretically cause harm. There are at least three reasons why large-scale clinical uptake of NIRS monitoring without clear evidence of benefit would be unfortunate. First, although near-infrared light in itself is safe, the sensor may cause skin marks, but more importantly, hypoxic values may result in unnecessary and potentially dangerous changes in cardiorespiratory support. Second, the placement of

yet another sensor to the small body of an extremely preterm infant is disturbing, and these patients are already stressed by care procedures. Third, if NIRS monitoring is without beneficial effects it will result in a significant waste of time while incurring significant monetary costs if NIRS is used clinically.

1.6.2 The need for combining monitoring of cerebral oxygenation with an evidence-based intervention guideline

The patient-relevant benefit of a diagnostic method depends on a consistent use of the information gained to improve clinical management and apply relevant treatment or interventions in a timely manner. As monitoring of intensive care patients is on a minute-to-minute basis, treatment guidelines need to be designed for use by staff on duty around the clock.

Treatment based on near-infrared spectroscopy monitoring should, as a first step, be compared with 'treatment as usual'. Blinding is not possible using a 'sham' instrument showing random values because random values may also do harm by false reassurance and/or by causing inappropriate interventions. Using the blinded monitoring system used in the control group of the SafeBoosC-II trial has, after sincere consideration, been assessed too difficult and too labour intensive.

1.7 Assessment of brain injury with cranial ultrasound

Cranial ultrasound is a standard tool for diagnosing conditions such as haemorrhage and hypoxic-ischaemic lesions. Furthermore, signs of brain atrophy at term-equivalent age are associated with poor neurodevelopmental outcomes in preterm infants (46).

2. Trial objective and hypothesis

The objectives of this phase III trial are to examine the benefits and harms of treatment based on near-infrared spectroscopy monitoring in order to reduce cerebral hypoxia during the first 72 hours of life in extremely preterm infants compared with treatment as usual (standard treatment).

The hypothesis is that the application of treatment based on near-infrared spectroscopy monitoring will decrease a composite outcome of severe brain injury or death at 36 weeks postmenstrual age.

3. Trial design

This is an investigator-initiated, open-label, randomised, multinational, pragmatic phase III clinical trial with a two-parallel group design that will enroll 1,600 extremely preterm infants from 20 countries

3.1 Randomisation

Participants will be randomised into either the experimental group or the control group. The ratio of allocation will be 1:1. The allocation sequence will be computer-generated with a varying block size and stratified by NICU and gestational age (lower gestational age (<26 weeks) versus higher gestational age (≥26 weeks)) and will be concealed for all investigators. Randomisation will be centralised and web-based at the Copenhagen Trial Unit.

Singleton infants will be randomised individually. Multiple birth infants will be randomised as a 'pair' or a 'group', i.e. all siblings will be allocated to the same treatment group. In centres where only one or two cerebral monitoring devices are available, it may not be possible to include all infants from multiple births. Thus, only one of a pair or only one or two infants of triplets may be included. The sibling(s) enrolled will be the one(s) born last.

The issues of multiple births with regard to sample size and statistical analysis are described in section 10, 'Statistical plan and data analysis'.

3.2 Timeline

Cerebral monitoring will start within six hours after birth and the intervention will last until 72 hours postnatal, as these are the most critical hours. Each participant will be followed up at 36 weeks postmenstrual age. (**Figure 2**).

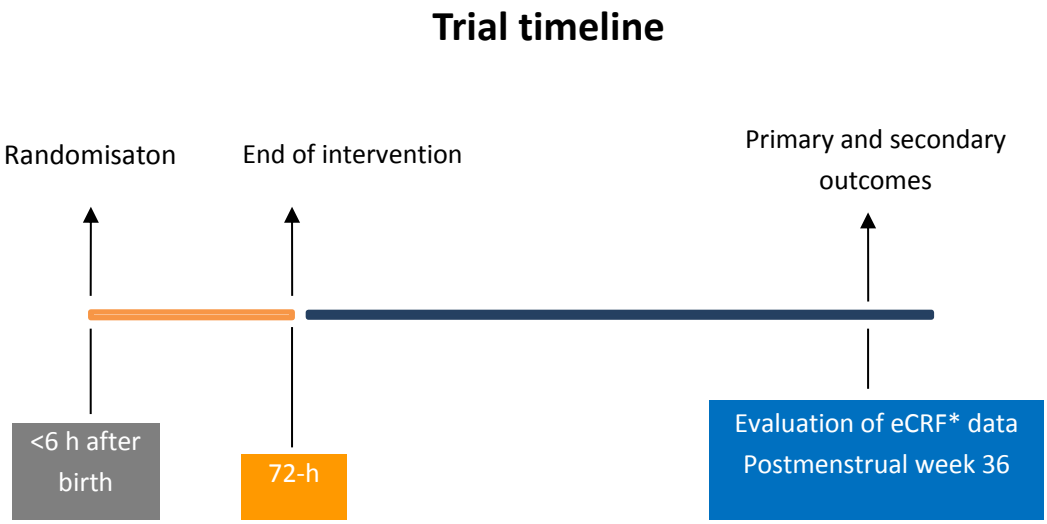

Figure 2. SafeBoosC-III trial timeline. Adverse events and clinical outcomes are assessed until 36 weeks postmenstrual age.

* eCRF: electronic case report form.

3.3 Blinding

Due to the nature of the experimental intervention, no blinding can be done for the clinical staff and the parents. Outcome assessment of mortality will not be blinded, but the diagnosis and classification of brain injury and the entry of this into the eCRF, will be done by a person that is blinded to group allocation. Principal investigators must develop a local procedure description that describe how this is done. The local procedure description must be approved by the sponsor. Furthermore, mortality will be checked by Good Clinical Practice (GCP) through source data verification in all patients. The data managers, statisticians and those drawing conclusions will be blinded to treatment allocation. Data management will be blinded. Two blinded statisticians at The Copenhagen Trial Unit will independently perform all statistical analyses and the two statistical reports will be published as supplemental material. Discrepancies between the two reports will be discussed by the Steering Committee. The

two intervention groups will be coded ‘A’ and ‘B’. Two conclusions will be drawn: one assuming ‘A’ is the experimental group and ‘B’ is the control group — and one assuming the opposite.

4. Participants

We will include infants based on the following inclusion and exclusion criteria.

4.1 Inclusion criteria

- Infants born with postmenstrual age less than 28 weeks
- Signed informed consent, unless the NICU has chosen to use ‘opt-out’ or deferred consent as consent method.

4.2 Exclusion criteria

- Missing written parental informed consent (if the ‘opt-out’ method is used for consent, lack of a record that the clinical staff have explained the trial and the ‘opt-out’ consent process to parents and/or a record in the infant’s clinical file of parents’ decision to opt-out, are exclusion criteria)
- Decision not to conduct full life support
- No possibility to place cerebral NIRS oximeter within six hours after birth

4.3 Participation in other trials

Participants included in the SafeBoosC-III trial can participate in any other study or intervention on the condition that the trial does not:

- allow clinical staff access to cerebral oximetry in the control group from inclusion in SafeBoosC-III to the end of intervention period 72 hrs after birth, or;
- exclude a treatment that would be clearly indicated by the SafeBoosC-III evidence-based treatment guideline during the intervention period.

All partners are encouraged to design ancillary studies and draw on data collected by SafeBoosC-III if not compromising the blinding of assessors or the equipoise of the trial. Ancillary studies must seek approval by the SafeBoosC Steering Committee.

4.4 Participant discontinuation and withdrawal

The participants’ parents are free to withdraw their infant’s participation from the intervention or from the SafeBoosC-III trial entirely at any time, and this will not have any consequences for the infant’s further treatment. Reasons for discontinuation, if offered by the parents, will be documented. When possible, the parents will be asked if they will allow their child’s data to be used in analysis.

The attending clinician can withdraw any participant from the trial intervention at any time in case there are safety concerns. Reasons for withdrawal will be documented. There are no pre-specified criteria for discontinuation of participants from the trial.

Discontinuation of participants from the trial will not result in replacement with new participants.

4.5 Recruitment feasibility

The feasibility of recruitment within three hours after birth was proven in the SafeBoosC-II trial, where 166 infants were recruited across eight European countries at eight NICUs (38). In total, 370 preterm infants were screened for eligibility during a 20-month period. In this phase III trial, we have prolonged the enrolment period from three to six hours after birth, similar to what is used in another neonatal intervention – therapeutic hypothermia for hypoxic-ischaemic encephalopathy after birth asphyxia. We believe this will make the trial relevant in settings where antenatal transfer to a perinatal centre is used less often.

Extremely preterm infants are expected to be included at about 50 neonatal intensive care units (NICUs) in about 20 countries. The 93 units that took part in a previous funding application have rates of admission of between 15 and 90 extremely preterm infants per year. The total admissions were estimated to be 3,000 infants per year. The trial should therefore have a good chance of recruiting 1,600 participants within two years. Sites that expect to enroll at least 15 participants per year within the two-year recruitment period will take part. Inclusion of new NICUs after the common start date will be done ad hoc, considering expected contributions and time remaining.

5. Interventions

5.1 Experimental group

The experimental group will receive evidence-based treatment based on near-infrared spectroscopy monitoring during the first 72 hours of life (see **Appendix B**).

5.1.1 Monitoring by cerebral oximetry

Monitoring of cerebral oxygenation by NIRS will be applied as soon as possible after birth and within six hours. The sensor may be moved or replaced as often as indicated by the signal quality indicator, instability of the signal, readings judged to be unreliable, or for routine inspection of underlying skin.

5.1.2 Devices

All commercially available cerebral oximeters that are approved for clinical use in newborn infants may be used. The aim is to use several different devices to generate results of generic value. There are now six~~four~~ commercially available devices in use (INVOS, NIRO, Fore-Sight, Sensmart, O3 and Egos). The appropriate intervention threshold for the devices, defined as the rStO2 value that reflects the same level of brain tissue oxygenation as a rStO2 of 55% using the INVOS adult sensor, has been defined (34). For any new device, the appropriate threshold will be determined as described in **Appendix A** 'Calibration'.

5.1.3 Treatment based on near-infrared spectroscopy monitoring

An evidence-based treatment guideline recommending adjustments of respiratory and cardiovascular support will be followed to keep cerebral oxygenation above 55% (47). Since SafeBoosC II showed a very low burden of hyperoxia and since monitoring had no effect on this, SafeBoosC-III will not target cerebral hyperoxia and therefore the interventions in case of hyperoxia were removed from the treatment guideline. The treatment guideline is detailed in **Appendix B** and will be used in all centres. Clinical staff will undergo web-based training and certification prior to caring for trial participants. As this is a pragmatic trial, we will not require a specific certification rate in order for NICUs to participate in the trial. However, we will aim at a 70%

certification rate in all participating NICUS. Since the web-based training and certification program is a trial quality measure to ensure quality of data and patient care, data on certification rates will be collected and published in a paper regarding the development and implementation of the web-based training and certification program for SafeBoosC III. The principal investigator at each NICU is responsible for listing relevant clinical staff that are expected to use the web-based training and accreditation system as well as providing on-sites information, supervision, and support.

5.1.4 Treatment as usual

Treatment offered to newborn infants in the control group (see below) will be offered equally in the experimental group (e.g. antibiotics; nutrition; etc.).

5.2 Control group

The control group participants will be treated according to standard clinical practice, hereafter referred to as 'treatment as usual'. These treatments will follow local guidelines and practices.

5.3 Concomitant medication/treatment

There is no specified 'per-protocol' concomitant medication or treatment, as any other aspects of treatment not defined in the evidence-based treatment guideline is per choice of the treating physician and the treating team as based on local protocols and guidelines.

6. Outcomes

6.1 Primary outcome

The primary outcome will be a composite of severe brain injury detected on any one of a series of cranial ultrasound scans that are routinely performed in these infants up to that age or death. Severe brain injuries will be cerebral haemorrhage grade III or IV, cystic periventricular leukomalacia, post-haemorrhagic ventricular dilatation, cerebellar haemorrhage or cerebral atrophy (for details see **Appendix C**).

6.2 Exploratory outcomes

The exploratory outcomes will be:

- A score of the presence of major neonatal morbidities associated with neurodevelopmental impairment later in life (48): bronchopulmonary dysplasia, retinopathy of prematurity as defined below, and severe brain injury as defined in the primary outcome.
- Bronchopulmonary dysplasia defined as oxygen or ventilator/continuous positive airway pressure (CPAP) requirement at the time of assessment.
- Retinopathy of prematurity stage 3+ and above at any time until the time of assessment.
- ~~Necrotising enterocolitis stage 2 or higher using the modified Bell's staging system and/or focal intestinal perforation at any time until the time of assessment.~~
- Late-onset sepsis (>72 hours after birth) defined as treatment with antibiotics for at least five days
- Necrotising enterocolitis stage 2 or higher using the modified Bell's staging system and/or focal intestinal perforation at any time until the time of assessment.

Brain injury is diagnosed by cranial ultrasound and defined as done in the protocol. Other diagnoses used as exploratory outcomes are made as per routine in each NICU.

6.3 Later follow-up

This protocol follow-up ends by week 36. However, longer follow-up is certainly relevant and we want to encourage that this will happen at the individual sites. Therefore, we have developed and attached **appendix H** – an informational sheet describing possible outcomes for later follow-up studies and how these studies could be done.

6.4 Outcome assessment tools

The special outcome assessment tool used in the trial is the cranial ultrasound (cUS).

6.5 Brain injury scoring

The reading of cranial ultrasound images and brain injury scoring for primary outcome (see **appendix C)** will be performed by experienced clinical staff at local centers and entered into the eCFR at 36 weeks of postmenstrual age. These staff members will be blinded to group allocation.

6.6 Explanatory variables

To be able to compare characteristics between groups we will obtain additional clinical data as per **Appendix I**.

Data will be drawn from clinical records at end of intervention period and at 36 weeks postmenstrual age ~~the same time as primary and secondary outcomes~~. Explanatory variables primarily consist of a subset of variables usually reported to the neonatal network databases VON or eNewborn.

7. Data collection and trial assessment schedule

7.1 Collection of trial data

7.1.1 Case report form

Trial data will be collected using an electronic case report form (eCRF) as the primary data entry point (see **Appendix F)**. The eCRFs will be designed in collaboration between the Data Manager at the Copenhagen Trial Unit and the sponsor (see section 11.1).

7.2 Trial assessment schedule

When an infant has been enrolled in the trial, this must be documented in the infant's clinical file. This is highly important for filling out the eCRF at 36 weeks PMA or discharge to home. The doctor enrolling the participant in the trial must also be responsible for prescribing NIRS monitoring, if the participant is randomised to the experimental group.

There will be three time points for eCRF data entry: consent/randomisation, end of monitoring by 72 hours of age, and at 36 weeks of postmenstrual age.

Primary and exploratory outcomes will be assessed at week 36+0 postmenstrual age as documented in the infant's clinical files. If the infant has been discharged to a step-down unit, data should be sought from that unit. If this is not possible, data should be used until the date of discharge to the step-down unit. In case that last entry in the infant's clinical files is before week 36+0, for example due to discharge home, the date must be reported in the eCRF.

8. Assessment of safety

8.1 Adverse events and reactions

8.1.1 Definitions

Adverse events: any undesirable medical event occurring to a participant during a clinical trial, whether or not considered related to the trial intervention.

Adverse reaction: any adverse event related to the trial intervention.

Serious adverse event: any event of death, severe brain injury, necrotising enterocolitis, bronchopulmonary dysplasia, retinopathy of prematurity, sepsis, as defined under primary and explorative outcomes.

Serious adverse reactions (SAR): any adverse reaction (related to the trial intervention) that results in death, is life-threatening, requires prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, or requires intervention to prevent permanent impairment or damage, including:

- Physical mishaps associated with managing the oximeter and sensors.
    - Severe skin damage
    - Critical displacement of endotracheal tubes caused by cerebral oximetry monitoring
    - Critical displacement of endovascular lines caused by cerebral oximetry monitoring
- Clinical mismanagement based on data from the cerebral oxygenation monitoring (see **Appendix B)**
    - Interventions aiming at improving respiratory status
    - Interventions aiming at improving cardiovascular status
    - Interventions aiming at improving oxygen transport

8.1.2 Classification of causality

Serious Adverse Reactions means any Serious Adverse Event considered to be a response to NIRS monitoring or a change in clinical management that was the result of this monitoring, for which there is not any better, alternative explanation.

8.1.3 Reporting of adverse events and reactions

Only SARs and SAEs will be reported separately. Expedited reporting will not be used.

8.1.4 Justification for recording and reporting

The preterm patient population is a seriously ill group. Most adverse events may be of a serious nature with or without the SafeBoosC-III trial intervention, and both intervention groups are expected to have a very high proportion of serious adverse events. It is therefore not feasible, nor meaningful, to record and report all adverse events. Therefore, we have decided only to record and report the predefined SAE's and SAR's as described in the protocol. The predefined SAE's have been chosen since they cover the major neonatal morbidities seen in our study population.

8.1.5 Timelines for recording and reporting

The site investigators will report the SARs (mortality and neonatal morbidities) through the eCRF at 72 hours of age and SAE's at 36 weeks postmenstrual age.

The sponsor will inform all investigators in the case of unexpected patterns of SARs and SAE's. Ethics committees will be informed as required.

8.2 Cerebral oximetry NIRS monitoring device

The devices used are approved for clinical use in newborn infants and will be used according to the user manual provided by the manufacturer. Furthermore, all clinical staff will be offered web-based training and certification that covers the principles of NIRS cerebral oximetry, practical application, as well as pathophysiology, and relevant interventions in the case of low cerebral oxygenation.

8.3 Data Monitoring and Safety Committee

A Data Monitoring and Safety Committee will be established to monitor mortality, neonatal morbidity, and SARs with 'certain' or 'probably/likely' relationships with the cerebral NIRS oximeter or the application of the evidence-based treatment guideline. The charter for the Data Monitoring and Safety Committee will be written prior to inclusion of participants and prior to any analysis. The trial will not be stopped early because of futility, and Lan-DeMets boundaries will be used at each interim analysis to assess if the thresholds for statistical significance are crossed (49).

8.4 Suspension or premature termination of the trial

The Steering Committee decides about trial discontinuation. If the Steering Committee terminates or suspends the trial, the relevant ethical committees will be provided with a detailed written explanation of the termination or suspension.

The Steering Committee can, upon completion of the analysis of the reason(s) for a suspension, decide to lift the suspension when necessary corrective actions have been implemented. The investigators and ethical committees, will be notified and provided with the relevant data supporting the decision.

Breaking of blinding will not be relevant in this trial, since group allocation is visible.

9. Ethical considerations

Due to the particular pathophysiology of prematurity, the research question can only be answered in this vulnerable population.

To obtain evidence-based knowledge on the benefit and harms of cerebral monitoring using NIRS as part of clinical management of premature infants, a large-scale randomised clinical trial is needed. The SafeBoosC phase II trial served as a feasibility trial for the present large SafeBoosC-III trial.

There is clinical equipoise, which means that there is genuine uncertainty over whether the cerebral oximeter and subsequent treatments will be beneficial or may even be harmful to study participants. Extremely preterm infant show stress reactions related to manipulation. Positioning and re-positioning of the cerebral NIRS oximeter sensors can result in such reactions. There are, however, no data to support substantially more risk or discomfort as compared with no intervention. All interventions proposed in the evidence-based treatment guideline are commonly used in this patient group.

'Treatment as usual', i.e. treatment according to each hospital's standard procedures will be given to the control group. Also, this will be the care given to any participant that is withdrawn, and infants who are not included in the trial.

Multiple births will be randomised together and undergo allocation to the same intervention. This is to avoid parents ascribing differences in the clinical courses of their infants to their participation in this trial.

No clinical site will start randomisation before their eligibility has been confirmed,, and the protocol has been approved by the relevant ethics committee. Any amendments to the protocol will be decided by the Steering Committee, and subject to ethical review before implemented. Written informed consent will be obtained prior to randomisation of any participant. However, the local NICUs have the possibility to apply for 'deferred consent' when obtaining approval of the protocol at their ethical committee. Parents can withdraw their consent for participation at any time. The trial will be conducted in compliance with the guidelines of the Declaration of Helsinki in its latest form and the International Conference on Harmonisation Good Clinical Practice guidelines (International Conference on Harmonization GCP) (50) and the applicable EU regulations and directives.

The trial protocol will be registered at www.clinicaltrials.gov, prior to participant enrolment, and, after completion of the trial, summary data will be entered.

9.1 Informed consent procedure

As cerebral oximetry must be initiated within six hours after birth, it is recommended to seek parental consent before delivery of the infants and confirm it after. Informed consent is required from one or both parents, according to national regulations. The investigator/investigator's delegate (qualified physician or nurse connected to the trial) will make contact and parents will be informed of the trial and given the Parent information sheet (**Appendix D**) for the trial. The information consultation will be held in an undisturbed setting. The parents will be given time to consider as far as possible given the need to begin the intervention within six hours after birth and to ask questions, before a written informed consent (**Appendix E**) will be obtained. Parents will be given a signed copy of the informed consent.

9.2 Deferred informed consent and prior informed assent

Due to the nature of the population, birth can be sudden and neonates will often be in a critical state. Obtaining prior informed consent from the parents within few hours from birth in an ethical way is delicate and complicated. Therefore, we allow and encourage the principal investigators at each NICU, to seek approval for two other consent forms, i.e. deferred informed consent and prior informed assent (see **Appendix G**).

9.3 Risk of complications for participants

The following procedure will be implemented to prevent and/or minimise risk of complication for participants.

Related to devices

Correct reading of the monitor (mainly to prevent false reading which may lead to wrong treatments) is critical. All clinical staff with be trained by e-learning and the investigators will train the local staff, as appropriate and trained staff will closely supervise all participants during the intervention. To minimise skin irritation related to the device, we suggest that the oximeter is moved to a different location every 4 hour.

Related to application of the evidence-based treatment guideline

The guideline has been tested in the SafeBoosC-II trial. The evidence-based treatment guideline is in harmony with current national clinical practices. All staff caring for trial infants will be offered the the web-based training and certification programme.

9.4 Benefit for participants

The participants in both groups will receive careful attention from qualified physicians and hospital staff during the trial.

10. Statistical plan and data analysis

10.1 Sample size estimation

We have calculated our sample size with an alpha of 5%, a power of 90%, and a ratio of experimental trial participants to control trial participants of 1:1. We will use the combined outcome of severe brain injury or death as the primary outcome. The sample size calculation has been calculated for the combined outcome and not the individual components.

In the 2009 EuroNeoNet report from 77 European NICUs, 1826 extremely preterm infants were recorded: the mortality was 33% and severe intracranial haemorrhage was recorded in 15%. In the SafeBoosC-II trial the proportion of trial participants in the control group with the primary outcome was approximately 34% and in the experimental group 26%. Mortality was reduced from 24% to 13% and the rate of severe brain injury from 23% to 13%.

If we randomize 800 infants to the experimental group and 800 infants to the control group, i.e. a total of 1,600 participants, we will be able to demonstrate a reduction from 34% to 26.5%. This corresponds to a 22% relative risk reduction or a 7% absolute risk reduction. We consider this to be a clinically relevant and important benefit,

since mortality is of direct patient relevance and since surviving infants with severe brain injury are at a 40% risk of moderate-to-severe neurodevelopmental deficit. The absolute risk reduction corresponds to a 'number-needed to treat' of only 15 that is very likely to influence clinical practice.

10.2 Twins and their intra-cluster correlation

We will randomise infants from the same twin couple to the same group, either experimental or control. In SafeBoosC-II the intra-class correlation coefficient (ICC) of the burden of hypoxia within pairs of twins was negligible. The ICC for various binary outcomes has been estimated in a previous study; ICC for death before discharge was estimated to 0.00 (-0.04, 0.02) and for intraventricular haemorrhage grade 3 or 4, -0.01 (-0.05, 0.01). This correlates to a design effect of very close to one (51). Therefore, in the primary analysis we will analyse twin data as independent observations. However, due to the possibility that the correlation between the primary outcome within pairs of twins will interfere with the estimation of the treatment effect (52), we will perform additional secondary analyses, taking this effect into consideration. First, we will analyse data where we remove the first and the last infant from twin pairs respectively. This will eliminate any effect of twin correlation, but also reduce the number of participants to approximately n = 1200, since 20-30% of extremely preterm infants are twins. Last, we will analyse data using a generalised estimating equation model (GEE). The main advantage of GEEs is the strong estimation of the standard errors of the parameters, despite not knowing the exact correlation structure (53).

~~Triplets and quadruplets should not be included in the study.~~

10.3 Power estimations

For the exploratory outcomes we have performed the following power calculations.

If we assume a mean difference of 0.12 in the rate of major neonatal morbidities (BPD, ROP, and severe brain injury) with a standard deviation of 0.8, we will be able to detect the difference of 0.12 between the experimental and control group with 90% power at a 5% significance level.

As for BPD, if assuming a prevalence of 40% among extremely preterm infants (54) and a relative risk decrease or increase of 20% in the experimental group, we will be able to detect this difference between the experimental and the control group with 89% power at a 5% significance level.

Assuming a 13% prevalence of ROP stage 3+ among extremely preterm infants and a relative risk decrease or increase of 30% in the experimental group (25), we will be able to detect this difference between the experimental and the control group with 68% power at a 5% significance level.

If we assume an 11% prevalence of NEC stage 2 and 3 among extremely preterm infants and a 17% relative risk decrease or increase in the experimental group, as is the estimate from existing trials (25), we will be able to detect this difference between the experimental and the control group with 23% power at a 5% significance level.

If we assume a 40% prevalence of late-onset sepsis in the control group (55), defined as treatment with antibiotics for at least five days, and a 20% relative risk decrease or increase in the experimental group, we will

be able to detect this difference between the experimental and control group with 91.6% power at a 5% significance level.

10.4 Data analysis and statistical methods

A fully detailed statistical analysis plan will be developed and published before enrolment is started. General principles are outlined below.

The analyses will be intention-to-treat and allocation is blinded to the statistician. The primary analyses for all outcomes' will be adjusted for the stratification variables, i.e. NICU and gestational age (see section 3.1). Secondly, we will present unadjusted analyses.

Analysis of outcomes

Dichotomous outcomes will be summarized as numbers, percentages, odds ratios, and 95% confidence intervals. We will use the generalized linear model using a 'log link' with 'site' as random intercept.

Assessment of components of the primary outcome

We will secondly assess each component of the primary outcome, i.e. severe brain injury and death, separately.

Threshold for significance

The thresholds for significance will be assessed according to the 5-point procedure suggested by Jakobsen et al (49).

Missing outcomes

We will consider using multiple imputation and present best-worst and worst-best case scenarios if it is not valid to ignore missing data (Jakobsen et al. 2017) (56). Best-worst and worst-best case scenarios assess the potential range of impact of the missing data for the trial results (56). In the 'best-worst' case scenario, it is assumed that all patients lost to follow-up in the experimental group have had a beneficial outcome, and all those with missing outcomes in the control group have had a harmful outcome (56). Conversely, in the 'worst- best' case scenario, it is assumed that all patients who were lost to follow-up in the experimental group have had a harmful outcome, and that all those lost to follow-up in the control group have had a beneficial outcome (56).

11. Data management plan

11.1 Data handling and archiving

All participant data are protected in accordance with the Danish Act on processing of personal data and the Danish Health Act. The data flow is outlined in **Appendix F**.

The Copenhagen Trial Unit will provide central, web-based data entry (in eCRF) by the use of OpenClinica, an open-source data management environment that was also used for SafeBoosC-II. Data will be stored in accordance with guidelines issued by the Danish Data Protection Agency, with whom approval of the trial will be sought. As the trial is pragmatic, only clinical data that are already documented in clinical records and usually recorded in neonatal network databases will be used. Only NICU numbers and study numbers will be used to identify participants (i.e. the data kept

at Copenhagen Trial Unit is pseudonymised), while lists of study numbers and personal identifying information (e.g. to allow GCP, data cleansing, and later follow-up) will be kept at the NICUs.

Six months after the acceptance of the publication that presents the primary outcome, the dataset will be transferred to the Danish data archive. Before transfer, subject study numbers, will be removed, NICU numbers will be replaced, genders removed, and birth weight and gestational age recoded into binary variables to minimize the risk of re-identification. Use by other researchers will depend on the permission of the trial Steering Group.
The investigators permit trial-related monitoring, audits, regulatory inspections by providing direct access to the source data and other relevant documents. Trial data will be handled according to regulations of data protection agencies in the respective countries.

12. Quality assurance

The trial will be conducted in compliance with this protocol across all sites. Detailed instructions and Standard Operating Procedures will be developed for specific tasks, as needed. Any major or safety related deviations will be recorded, analysed and reported to the research ethics committees within seven work days. If an investigator refuses to comply with the protocol he/she will be disqualified.

12.1 Eligibility

12.1.1 Sites

To take part in the SafeBoosC-III trial the site must be able to:

- Randomise, i.e. the clinical staff must be in equipoise for the duration of the trial as regards the value of monitoring cerebral oxygenation in extremely preterm infants during the first days of life, to act on hypoxic values and not to use cerebral oximetry for clinical care of infants randomized to the control arm.
- Enrol at least 15 infants per year to the trial, i.e. obtain consent, do web randomization and start NIRS in the experimental group within six hours of birth.
- Provide NIRS device(s) for the SafeBoosC-III trial.
- Organise local Good Clinical Practice (GCP) monitoring.
- If necessary, provide for patient insurance.
- Oximeters and/or sensors and/or expenses of GCP and/or patient insurance may be provided by industry, but principal investigators and clinical staff must make sure that industry has no influence on the data that is collected.

12.1.2 Trial personnel

Prior to initiation, the trial personnel at each site will be offered training via a web-based certification programme in monitoring of cerebral oxygenation by NIRS, the evidence-based treatment guideline, cUS imaging, and uploading of data. Training and certification system will be hosted in a Moodle virtual learning environment (VLE), a commonly used, shareware software. The teaching methodology will be case-based and interactive as far as possible and will cover:

- NIRS monitoring: The principles of measuring cerebral oxygenation by NIRS, basic device operation, application and fixation of the sensor to the head of the baby, care of the sensor and repositioning, the risk of skin marks, the interpretation of measured values, the concept of venous-weighted tissue blood oxygenation,

the use of the tracing to judge the reliability of measured values, artefact recognition and rejection, and the use of alarms.
- Treatment guideline: The rationale of the evidence-based treatment guideline as based on pathophysiologic concepts, the possible clinical interventions, and expected effects with regard to degree, onset, and duration.
- Cerebral ultrasound: Optimal timing of scans, standard transfontanellar views, grading of peri-and intraventricular haemorrhage and of periventricular leucomalacia.

These modules will be available on demand. Also, there will be provision for forums for asynchronous discussion to handle questions and experiences during the project. If necessary, changes in procedures can be handled by modifying the modules and re-training/ re-certification of staff.

12.2 Monitoring

12.2.1 Internal, central monitoring

Internal monitoring will consist of central daily check of recruitment to the trial, and the quality, completeness and timeliness of data entry in the eCRF by the data manager. Statistics of the internal monitoring will be published on the trial website.

In case of problems, the national coordinators will be involved in corrective actions.

12.2.2 External, local monitoring

The trial will be monitored according to the International Conference on Harmonization GCP guidelines, and a detailed monitoring plan will be developed. The following will be monitored locally

- All participants for existence of clinical file, existence of documented informed consent and entry of trial participation in clinical files. Source check will be done for group allocation, gestational age below 28 weeks, and survival or death at 36 weeks postmenstrual age.

Despite severe brain injury being a composite of the primary outcome, it will not undergo source check. This is due to, that the blinded person reviewing brain images or radiology reports will type data directly into the eCRF. Therefore, there will be no source available for verification of eCRF entry.

12.3 Device quality control

Each site is responsible for adhering to the quality control measures described in the oximeter manufacturer's users guidelines.

13. Trial timeframe

Trial stages	Timeframe
Protocol development	October 2014 to September 2018
Protocol finalised	September, 2018
Site selection	Ongoing

Trial stages	Timeframe
Recruitment phase	April 2019 – April 2021
Assessment phase	Primary and secondary outcome April 2019 to April 2021
Final analysis	2021
Publication	2022

14. Legal aspects

14.1 Finance

Funding	Timeframe
EU-OpSTART, DK	July 2014, awarded DKK 75,000.00-
RegionHovedstaden, DK	July 2014, awarded DKK 200,000.00-
Elsass Fonden	May 2018, awarded DKK 2,700,000.00-

The sponsor/coordinating investigator, Professor of Neonatology Gorm Greisen, is the initiator of the SafeBoosC-III project. He has no financial interest in the results of the trial, nor in the NIRS-devices. He is not financially or in any other way involved in the European Union Framework Programme for Research and Innovation. We seek additional local and central funding, including funding from industry sponsors, such sources will not get any influence on the methodology, data, analysis, reporting, or conclusions of the trial. Furthermore, any participating department can seek local/national support from all sources, including dealers of devices, as long such sources will not get any influence on the methodology, data, analysis, reporting, or conclusions of the trial.No financial compensation is foreseen for participating in this trial.

14.2 Conflict of interest

The authors declare that they have no competing interest.

14.3 Participant insurance

Participants will be insured in accordance with existing legislation in their respective countries. Individual NICUs are obliged to finance the participants' insurances. If external funding becomes available, expenses may potentially be covered.

14.4 Publication plan

The trial will be registered on www.ClinicalTrials.gov prior to the randomisation of the first participant. Further summary data of main outcomes will be entered after statistical analyses are conducted. Attempts will be sought to publish all results, positive, neutral, as well as negative, in a peer-reviewed international journals. Authorship will be determined according to the International Committee of Medical Journal Editors. An additional requirement is one author per NICU completing at least 30 participants. Ancillary studies with results

potentially affecting equipoise regard to the value of NIRS, shall not be published before the main publication of SafeBoosC-III.

14.5 Statements of compliance

The randomised clinical trial will be conducted in accordance with this protocol, its Standard Operating Procedures and to the GCP.

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Gorm Greisen

16. Appendices

16.1 Appendix A: Calibration

There are six~~four~~ commercial NIRS devices available to estimate $rStO_2$ (INVOS, NIRO, Fore-Sight, Sensmart, O3 and Egos ~~INVOS, Hamatsu, Foresight and Nonin~~). The algorithms to calculate the $rStO_2$ are often unpublished (57). In practice, $rStO_2$ values vary between instruments (58–62), because they rely on different algorithms (63). Based on (unpublished) data from more than 400 very preterm infants, recorded with the INVOS 5100c using the adult SomaSensor, the normal range of rStO2 in stable preterm infants has been defined as between 55% and 85% during the first 72 hours of life (Petra Lemmers and Frank Van Bel, Utrecht, The Netherlands). The lower value was chosen as the intervention threshold and definition of cerebral hypoxia in SafeBoosC-II and will be used as the only intervention threshold in SafeBoosC-III.

Prior to SafeBoosC-III we have defined the appropriate intervention thresholds for available commercial NIRS devices, corresponding to a rStO2 value of 55% using the INVOS adult SomaSensor. The SOP **'Hypoxic threshold for cerebral oximeters'** which is available on www.safeboosc.eu, presents the appropriate intervention thresholds for NIRS devices approved for use in Newborns~~**Table 1** shows the appropriate intervention thresholds for NIRS devices approved for use in newborns~~ (34).

If new commercial NIRS devices become available during the trial period, the appropriate intervention thresholds for these devices will be determined as well (see method below).

~~**Table 1**~~

~~Hypoxic thresholds corresponding to a 55% rStO2 in INVOS adult. The different subtypes within each NIRS device (e.g. FORESIGHT small/FORESIGHT small band) represent different sensors and therefore show different hypoxic thresholds.~~

~~**NIRS Device**~~	~~**Hypoxic threshold %**~~
~~FORESIGHT small~~	~~66~~
~~FORESIGHT non-adhesive small~~	~~67~~
~~NIRO small~~	~~61~~
~~NIRO small re-usable~~	~~63~~
~~NIRO large~~	~~62~~
~~NIRO large re-usable~~	~~62~~
~~INVOS neo~~	~~63~~
~~SenSmart neo 8004CB-NA~~	~~66~~

The test consists of an accuracy test in a phantom. The phantom is a specially designed container, which is filled with a solution of Intralipid®, human haemoglobin, buffer, glucose, salt (physiological concentration) and yeast. Its optical properties are matched to the head of preterm infants. The oxygenation of its haemoglobin is reduced by the yeast, which consumes oxygen, and increased by bubbling oxygen. The solution is constantly stirred and kept at 37°C. Sensors of four different instruments can be attached simultaneously to the phantom. This enables comparison between the $rStO_2$ of different instruments over the full range of oxygenation. This phantom methodology was developed by University Hospital of Zurich.

Alarm limits will be adjusted for each NIRS device so that it corresponds to 55% as measured by the INVOS 5100c with the small adult probe since this was the device used to define the normal range.

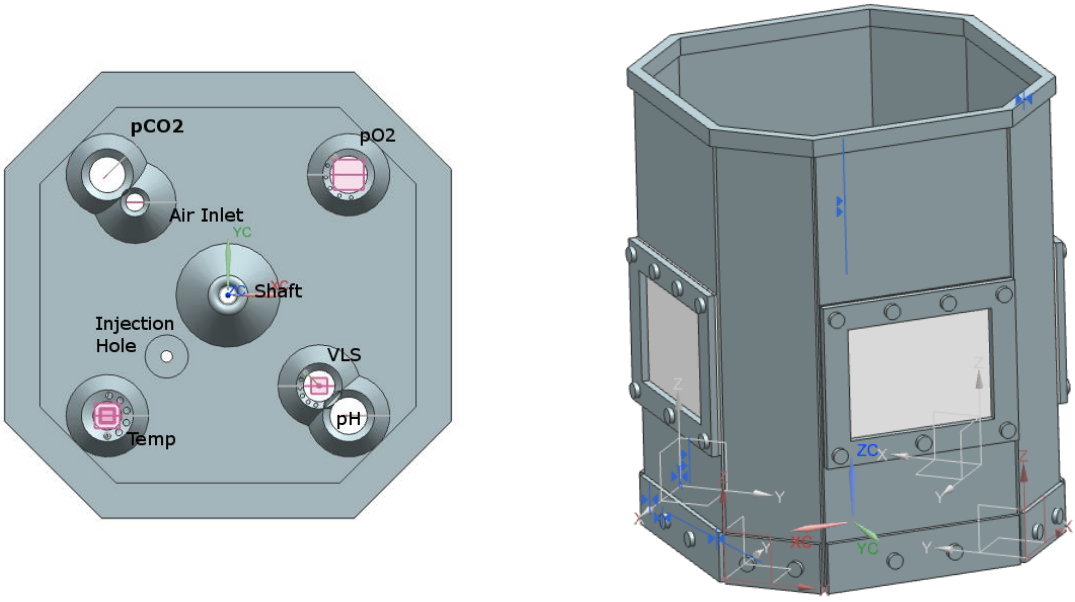

Figure: The phantom container (right) has four windows to place sensors of instruments. The distance between these windows is sufficient to avoid crosstalk between instruments. The container is filled with a solution of Intralipid®, human haemoglobin, buffer, glucose, salt (physiological concentration) and yeast. The solutions is constantly stirred and kept at 37°C. The lid (left) is an airtight cover to achieve equilibrium of the gases in the solution quickly. It allows the placement of different sensors to measure temperature, pH, pCO_2, and take or inset samples.

16.2 Appendix B: Evidence-based treatment guidelines and justifications

SafeBoosC-III Clinical Guidelines

Assessment of cerebral oxygen saturation
Regional cerebral tissue oxygen saturation ($rStO_2$) is a composite measure of tissue oxygen saturation across arterial, capillary and venous beds and reflects a balance between cerebral oxygen delivery (CDO_2) and cerebral metabolic rate ($CMRO_2$). In preterm infants, the $CMRO_2$ is unlikely to vary much and a change in $rStO_2$ largely reflects changes in CDO_2. The factors which influence CDO_2 are arterial oxygen saturation (SaO_2), haemoglobin concentration and cerebral blood flow (CBF).

Establishment of monitoring of cerebral oxygenation
As soon as possible and within 6 hours of age

Period of monitoring of cerebral oxygenation
Until 72 hours after birth

Recommendation for clinical interventions
The thresholds for intervention depends on the oximeter. If StO2 is predominantly below the hypoxic threshold over a 10-minute period or drops acutely and markedly under the threshold, the sensor should be inspected for any potential displacement, and possibly be repositioned. If this does not solve the problem, a decision regarding intervention (modification of cardio-respiratory support) should be made (identified in '•') as listed below and StO_2 reassessed 30 to 60 minutes after the intervention. Generally, only one intervention should be chosen at a time. All the interventions proposed here are commonly used in this patient group.

For each intervention, the level of evidence (I-III) and strength of recommendation (A-E) are given (defined in Tables 1 and 2). For further explanation, see below.

Rationale/aim of interventions: A low $rStO_2$ reflects a low CDO_2. The interventions should be directed to increasing CBF, blood haemoglobin concentration, or SaO_2.

<u>Assess cardiovascular status:</u>
Blood pressure below the normal range or low, even in the normal range, consider:

- Vasopressor-inotropes (I/B) (64,65)
- Fluid bolus (normal saline) (I/C) (66,67)
- Decrease mean airway pressure on ventilator or CPAP (III/B) (68–71)

Poor systemic circulation, consider if:
Echocardiography shows low cardiac output and/or low SVC flow, consider:

- Inotropes (I/B) (31,67,72–75)
- Fluid bolus (normal saline) (I/C) (66,67)
- Decrease mean airway pressure (III/B) (68–71)

- Reduce vasopressor (III/ B) (76)

Echocardiography not available but has at least 2 of the following signs:
Lactate > 3.5 mmol/l
Capillary Refill Time > 3 seconds
Urine output < 1 ml/kg/hour
consider:

- Inotropes (I/B) (31,67,72–75)
- Fluid bolus (normal saline) (I/C) (66,67)
- Decrease mean airway pressure (III/B) (68–71)
- Reduce vasopressor (III/ B) (76)

Patent ductus arteriosus, consider:

- Medical treatment (II-2/B) (69,70,77,78)

Assess oxygen transport:
Blood haemoglobin concentration below the normal range or low, even in the normal range, consider:
Red blood cell transfusion (I/B) (79–82)

Assess respiratory status:
SaO2 below the normal range or low, even in normal range, consider:

- Increase FiO_2 (II-1/A) (83) (ATTENTION: be careful not to exceed the upper target threshold of SpO_2)
- Increase mean airway pressure (III/B) (68,84,85)

PCO_2 below the normal range or low, even in normal range, consider:

- Decrease minute ventilation - (II/A) (79,86–88)

Level of evidence and recommendation of intervention
The level of evidence (Table 1) and recommendation for a given intervention (in brackets and Table 2) were graded according to the U.S. Preventive Services Task Force system (89)

Table 1: Hierarchy of research design and level of evidence

Level of evidence	Type of study
I	Evidence obtained from at least one properly randomized controlled trial
II-1	Evidence obtained from well-designed controlled trials without randomization
II-2	Evidence obtained from well-designed cohort or case-control analytic studies, preferably from more than one centre or research group
II-3	Evidence obtained from multiple time series with or without intervention. Dramatic results in uncontrolled experiments (such as the results of the introduction of penicillin treatment in the 1940s) could also be regarded as this type of evidence
III	Opinions of respected authorities, based on clinical experience, descriptive studies and case reports, or reports of expert committees

Table 2: Recommendation grid

	Net benefit			
Quality of evidence	substantial	moderate	small	zero/negative
Good	A	B	C	D
Fair	B	B	C	D
Poor	E	E	E	E
Standard recommendation language	A= Strongly recommended (good evidence that the intervention improves important health outcomes and benefits substantially outweigh harms). B= Recommended (at least fair evidence that the intervention improves important health outcomes and benefits substantially outweigh harms). C= No recommendation for or against routine provision of the intervention (fair evidence that the service can improve health outcomes but the balance of the benefits and harms is too close to justify a general recommendation). D= Recommends against routinely providing the intervention (at least fair evidence that the service is ineffective or that harms outweigh benefits). E= Insufficient to recommend for or against routinely providing the intervention (evidence that the intervention is effective is lacking, of poor quality, or conflicting and the balance of benefits and harms cannot be determined).			

Abbreviations

SVC	*Superior vena cava*
CRT	*Capillary refill time*
FiO_2	*Fraction of inspired oxygen*
$rStO_2$	*Regional cerebral tissue oxygen saturation*
SaO_2	*Saturation of oxygen (arterial blood)*
PCO_2	*Partial pressure of carbon dioxide*
CDO_2	*Cerebral oxygen delivery*
$CMRO_2$	*Cerebral metabolic rate*
CBF	*Cerebral blood flow*

16.3 Appendix C: Procedure for assessment of cranial ultrasound

16.3.1 Outcome measures
16.3.2 Which views to take
16.3.3. When to perform the cUS
16.3.4. Final grading

16.3.1 Outcome measure

Primary outcome: severe brain injury or no severe brain injury (Table 1).

Table 1. Severe brain injury comprises

Severe brain injury
• **Intra-ventricular haemorrhage grade III**
• **Parenchymal/periventricular haemorrhagic infarction (grade IV)**
• **Cystic periventricular leukomalacia (cPVL)** (90)
• **Post-hemorrhagic ventricular dilatation**
• **Cerebellar hemorrhage**
• **Cerebral atrophy**

16.3.2 Which views to take

Examination of infants with cranial ultrasound will be by the local NICUs guidelines. However, views through the anterior fontanelle are recommended; 5 sagittal and 6 coronal (Fig. 1), whereas mastoid imaging is optional (Fig. 2).

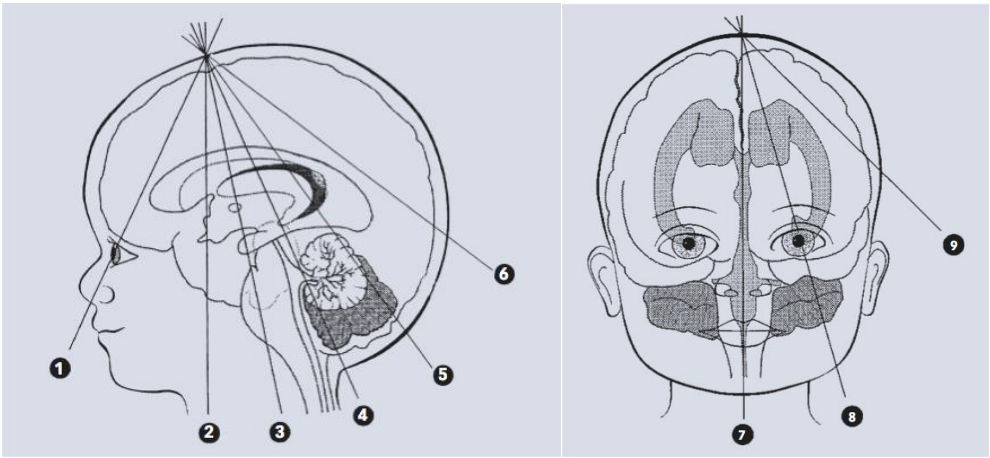

Figure.1. a) Six coronal views (C1-6) and in b) five sagittal views (7-9, S1-S5 respectively) (91)

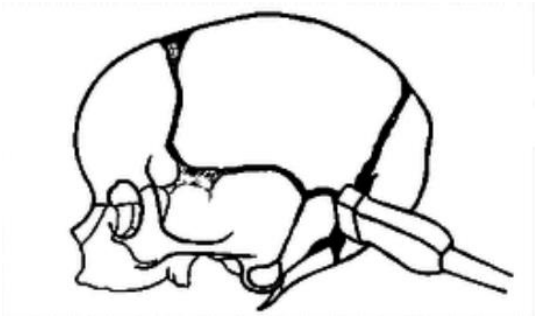

Figure.2. Transducer placement over mastoid fontanelle (92)

Anterior fontanelle	
C1 at the level of the frontal lobes	S1 Midline sagittal
C2 at the level of the frontal horns of lateral ventricles	S2 Angled parasagittal: C shape of the left lateral ventricle
C3 at the level of the third ventricle and the foramen of Monro	S3 Tangential parasagittal: left periventricular white matter and through Sylvian fissure

C4 at the level of the cerebellum largest diameter left-right	S4 Angled parasagittal: C shape of the right lateral ventricle
C5 at the level of the posterior horns with plexus, level of the trigone	S5 Tangential parasagittal: right periventricular white matter and through Sylvian fissure
C6 at the level of the occipital lobes	
Mastoid fontanelle	
Coronal and axial view	

16.3.3 When to perform the cUS

Cranial ultrasound is a standard routine examination in preterm infants and will be performed multiple times up to the follow-up time at 36 weeks postmenstrual or first discharge home, per local NICU guidelines. These are often obtained at birth, 7 postnatal days, and 30 postnatal days or more often as clinically indicated.

16.3.4 Brain injury scoring

At 36 weeks of postmenstrual age or first discharge home, the series of ultrasound scans and the infant's clinical files will be reviewed by experienced clinical staff in the local NICU. If the infant has suffered a brain injury as defined in table 1 and 2, it will be entered into the eCRF. If no brain injury, this will also be entered into the eCRF. These staff members will be blinded to group allocation.

16.4 Appendix D: Parent information sheet

Will be sent out as a separate document later in the process.

16.5 Appendix E: C~~Informed~~ consent – the SafeBoosC phase III trial

Will be sent out as a separate document later in the process.

16.6 Appendix F: SafeBoosC-III Data flow

Web-based Case Report Form

The eCRF will be a web-based solution in the open source clinical trials software OpenClinica®. This will handle the inclusion procedure, the documentation of the stratification and randomisation process, the serious adverse reaction and events and the relevant clinical data from enrolled subjects, including primary and exploratysecondary outcomes and explanatory variables. The data will be entered into the eCRF directly by the medical staff. A form for randomisation/inclusion and the 36 weeks follow-up will be created.

16.7 Appendix G: Consent procedures

The protocol allows the use of prior informed consent or assent (opt-out with enrollment as default), as well as deferred informed consent from one or both parents for the enrollment of infants in the SafeBoosC-III. The principal investigator at each NICU is encouraged to seek approval for the consent form he/she thinks is most appropriate to local conditions.

In the following, the concepts are described with reference to the literature and a list of arguments for either form of consent is provided.

Participants in clinical research must, in principle, voluntarily agree to participate prior to enrollment. The decision to participate must be based on adequate information with regard to the study, including aims, methods, anticipated benefits, potential risks, any discomfort the individual may experience, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, and any other relevant aspects of the study. Furthermore, the participant must be able to process and understand the information, thus being mentally competent (93). If the participant, based on this information, decides to participate in the study, informed consent must be written or at least formally documented and witnessed (93). Regarding children or newborns, parents or legal guardians must provide surrogate informed consent (94,95). The procedure should be as described above.

To improve clinical care, to save the lives or reduce the risks of permanent damage of critically ill children, clinical trials are of utmost importance. However, the practicalities of enrolling children into emergency and intensive care research and the process of obtaining prior informed consent, often conflict. Many of the interventions and treatments in emergency and intensive care research have a short therapeutic window. If clinical staff must obtain a valid informed consent, prior to providing the intervention, this therapeutic window might have closed and the child would miss out on interventions or treatments that would potentially have benefitted the child (96). Even if proper information is given to the parents and there is still time for the intervention, parents of critically ill children might be inadequately prepared to render such decision, due to the acute circumstances and therefore incapable of processing the information and providing valid consent (97,98). Being the parents of a critically ill child is an intense emotional situation, and therefore parents might not want to be approached about clinical research participation at such time and staff may not want to approach them (99).

In this situation, a different consent procedure may be more appropriate. Deferred consent was first described in the literature in 1980 (100). It involves enrolling patients into a trial, i.e. randomizing, applying the allocated interventions and initiating the necessary data collection without seeking prior consent. Instead, informed consent is sought later. If consent is given, the patient can continue in the trial and if not, any intervention will be withdrawn, and data will not be used. Deferred consent is sought from either the patient or, if the patient is unconscious or otherwise unable to provide consent, from a legal proxy, which is often a close family member. In pediatric research, parents or a legal guardian are most typically asked for consent. After 40 years, the use of deferred consent is still controversial and is only accepted in some countries. In 2008, the United Kingdom allowed deferred consent in clinical trials, including pediatric trials, when the following conditions were met: treatment is required urgently, urgent action is required for the purpose of the trial, it is not reasonably practical to obtain consent prospectively and an ethics committee has given approval to the procedure for which the

action is taken (101). Across countries in the European Union, regulations have made it possible to apply to ethical committees for deferred consent in intensive care research, including studies involving minors, if certain criteria are met (102)(see page 33).

A number of studies have evaluated both parental and practitioners view on deferred consent. Most of the studies found that parents in general have a positive attitude towards deferred consent (99,103–106). Studies evaluating practitioner's views also show a general positive attitude towards deferred consent (103,105). One study stratified practitioners into two groups based on experience with deferred consent and found that practitioners with previous experience with deferred consent were positive towards it, while practitioners without deferred consent experience demonstrated a negative viewpoint (96).

We believe that many of the criteria outlined for the use of deferred consent in intensive care research are fulfilled in the SafeBoosC-III trial; NIRS monitoring by protocol has to start within six hours from birth and some of the potential benefit may exist prior to six hours, during the minutes when the infant is placed in the incubator in the NICU when first decisions are made regarding the cardio-respiratory support. Extremely preterm birth causes emotional stress in itself. Many mothers deliver by caesarian section and may be under the effects of anesthesia. The father's attention may be divided between concern for his wife's health and that of his newborn child. However, one issue warrants special consideration: some mothers of preterm neonates have been admitted to the maternity ward for several days or even weeks due to complications of pregnancy and for these women and their partners, there may be numerous opportunities for prior information about potential trials for their newborn to be enrolled in, should she/he be born extremely preterm.

Another option for seeking consent, is the opt-out method with enrollment as default. When using this consent form, prior information of the study will be provided to parents and it will be specifically outlined that their child will be enrolled in the trial, unless they decide to 'opt-out'. Clinical staff must record in the infants clinical file, that they have explained the trial and the 'opt-out' consent process to parents. If no record on this, the child will be excluded from the trial. Full informed 'opt-out' consent is a continuous process, where parents will be able to review their decision on an ongoing basis. Parents decision to 'opt-out' must be recorded in the infants clinical file. The opinion towards this method has been evaluated among research ethics committees, parents and health care professionals and was in general considered valid and appropriate (107).

To help in the decision of what form of consent will be applied for to individual research ethics committees / review board, we have outlined the arguments below.

For deferred informed consent

- The intervention in the SafeBoosC-III trial is already in clinical use. Several NICUs worldwide routinely provide monitoring of cerebral oxygenation to newborn preterm infants and therefore do not think that it is ethically appropriate to randomize to the control arm. Furthermore, the SafeBoosC-III trial does not involve any study-related investigation, such as blood sampling or imaging, that may carry discomfort, inconvenience or risk.
- Parents may be unavailable due to the urgency of extremely preterm birth
- Parents may be unprepared in cases of acute delivery, thereby without prior knowledge about the risks of death, complications, and long-term damage to extremely preterm infants nor the options of diagnosis and treatment.

- Parents may lack capacity to provide a valid informed consent in the emotionally difficult situation or due to the influence of anesthetics (97,108)
- Deferred informed consent, although incompletely providing the opportunity for parents to opt out, allows proper time for information, reflection, and decision.
- Seeking a valid prior informed consent is time-consuming and could potentially delay time-critical interventions, thereby reducing the effect of the intervention (109)
- Vulnerable populations, such as critically ill children, should not be denied the opportunity to participate in research due to difficulties in the informed consent process (110). This is an important issue in pediatric emergency care, since multiple treatments routinely administered as part of clinical guidelines are lacking solid evidence (111).
- In countries with private or non-government health care, patients with lower educations and socioeconomic status might lack access to good health care, and therefore potentially could attempt to access health care by being enrolled, or enroll their children, into clinical trials (108). An asthma trial study found, that parents with lower education and socioeconomic status were more willing to give consent to clinical trials, than higher educated parents. This might create a bias in recruitment, towards lower educated people primarily being enrolled in clinical trials (112).

For prior informed assent (opt-out)

- Parental consent on behalf of children is a proxy consent. Although parents are legal guardians of their children, this does not remove this basic fact. In practice, parents may express difficulties with giving consent on behalf of their children while saying that it would be easy if it were for themselves.
- Assent, combined with a short-form parent information sheet has been argued to ease the decisional burden of patients, when deciding on participation in clinical trials.
- Providing participation as the 'first' choice is similar to the assent/consent provided for clinical care, where the recommendation of treatment is based on the professional judgement that overall benefits exceeds overall risks. For the SafeBoosC-III trial, this means that infants in the experimental arm have a chance of receiving better treatment (there is an a priori expectation of net-benefit) and the infants in the control arm will receive 'treatment as usual', i.e. not being exposed to any trial related discomfort, inconvenience or risk. A short-form parent information sheet can be said to match this purpose. A long-format parental information sheet should be available to the parents who wish to review more information.

For prior informed consent

- The process of prior consent enables the execution of the autonomy of patients and parents (103,110). They are able to say no to all elements of the trial.
- Although the fully informed, fully competent, and fully voluntary, consent may be difficult to achieve in real life, particularly in urgent situations, the requirement of prior informed consent, symbolically, demonstrates the idea of full control to subjects for participation in medical research.
- New ethical questions arise when using deferred consent: how and when to inform parents that their child has been included and what to do when a child included in the trial dies before consent has been sought (113).
- Even though deferred consent might not cause any physical harm to subjects, it can have other consequences, for example causing distrust to both researchers, the ethical review process (97) and the health care system in general.

To explore the different views on consent for trials in newborn infants across the world, we plan ancillary studies. For instance, we want to encourage all NICU's seeking permission to use deferred consent or assent (opt-out) to their local ethical committee. We will evaluate the responses from the research ethics boards and the process leading up to the response. Results will be published in an international peer reviewed journal.

16.8 Appendix H: Later follow-up

The SafeBoosC-III protocol requires follow-up of patients until 36 weeks of postmenstrual age or first discharge home, whichever comes first. SafeboosC-III is a pragmatic trial with minimal extra work for clinical investigators. The clinical outcome at 36 weeks will allow us to determine the effect on the intervention on almost all of the mortality, as well as on the major forms of brain injury, and on short-term morbidity. Follow-up, however, to determine the longer-term effects of neonatal interventions is also of significant value.

Days alive outside hospital and length of hospital stay within one year of age, could be evaluated solely through subjects' clinical files.

Days alive outside hospital

Days alive outside hospital is a relevant outcome to combine the effects on in-hospital mortality, length of hospital stay in survivors, re-admissions and post discharge mortality. This outcome is easily assessed, if data can be extracted from subjects' clinical files.

Criteria's for discharge home may differ considerably among NICUs, some use early discharge with continued electronic monitoring and tube feeding, to reduce inconveniences to families as well as costs. Statistical analysis that is stratified for individual NICUs should correct for this problem.

Length of hospital stay (LOS)

Seen from a social and economic perspective, length of hospital stay is a relevant outcome. Being hospitalized with an extremely preterm infant until term age is physically and mentally demanding for the family. Therefore, decreasing the number of days in hospital is of direct value for families. Furthermore, the cost of caring for an extremely preterm infant until discharge from the NICU, has been estimated to more than 65,000 US dollars, with a mean length of hospital stay at 70 days for survivors born before 28 weeks of age, and even higher in younger infants (114,115). Decreasing length of stay even for just a few days would be of economic impact to hospitals, potentially saving thousands of dollars per infant.

However, length of hospital stay is a problematic outcome due to early mortality as seen within our population. Infants who die early 'save' hospitalized days, which would appear as a benefit (116). Furthermore, increased survival could mean that more immature and more ill infants will survive, potentially with longer recovery time. If treatment based on NIRS-monitoring increases survival, the mean length of stay could potentially be longer in the experimental group. On the other hand, since NIRS monitoring may save infants from early complications, it may potentially reduce the time for recovery. Seen from an economic view point, knowledge of the time of hospitalization (length of stay) in survivors as well as non-survivors are relevant outcomes, as components of the total costs when incorporating NIRS-monitoring in to care standards. All these results will be incorporated in a health economic analysis.

Both outcomes encounter an issue with regard to rehospitalization data, since almost half of extremely preterm infants who survive to discharge home are rehospitalized within the first years of life (114). If rehospitalization to the primary hospital does not happen, it might be difficult for investigators to identify rehospitalization periods at one-year follow-up. If such is the case, another possibility is to contact parents at one or two-year time points to collect further data.

Below, we have outlined two hypothetical sample size estimations, based on assumptions, for the outcomes days alive outside hospital during the first year of life and length of hospital stay in survivors during the first year of life.

Since we do not expect data to be normally distributed, the populations used in the sample size calculations should be downsized by a factor of 0.8 to correct for loss of power, as described by Jakobsen et al. (116). Thus, we must correct the practical sample size accordingly.

Days alive outside hospital during the first year of life
If we assume a minimal important risk difference of five days within the first year of life and a standard deviation of 25 days, we will be able to detect the difference between the two groups with 90% power at a 5% significance level, if we include 657 infants in each group, i.e. a total of 1,314 infants in a follow-up study.

Length of hospital stay during the first year of life in survivors.
We will use a smaller standard deviation than in days alive outside hospital since we expect the mean number of days to be lower (114).

If we assume a minimal important risk difference of five days in length of hospital stay during the first year of life and a standard deviation of 15 days, we will be able to detect the difference between the experimental and the control group with 90% power at a 5% significance level, if we include 237 infants in each group, i.e. a total of 474 infants in a follow-up study.

When analysing these data, the non-parametric Van Elteren test should be used, since we expect data to be non-normally distributed.

Since both outcomes have great power, they would be valid endpoints, despite investigators not being able to include all NICUs participating in the SafeBoosC-III trial. Even if only 1000 of the 1600 infants were included, we would still be able to detect a difference with more than 80% power at a 5% significance level (based on the assumptions above).

We hope that all NICUs will prepare for ancillary follow-up studies by 1) storing the personal information necessary to track all infants and 2) defining the possible sources for follow-up. This is to enable the best possible data for later systematic follow-up.

Neurodevelopmental impairment (NDI)

In most NICUs, extremely preterm infants are usually offered clinical follow-up, at least until the 'walking and talking' developmental stage. Therefore, ancillary follow-up studies based on clinical evaluation at follow-up and data from subjects' clinical files could also be performed.

Neurodevelopmental impairment (NDI) is common in this population. NDI is a significant burden to the children, their families, and to society due to costs of rehabilitation, medical care and special education (117).

In SafeBoosC-III, we assess severe brain injury in the neonatal period. However, despite severe brain injury being a strong predictor for neurodevelopmental impairment (11), some infants with a history of severe brain injury in the neonatal period, do not develop significant sequelae, and some infants without major brain injury diagnosed on brain ultrasound do (118). If treatment based on NIRS-monitoring decreases the incidence of severe brain injury, it would be important to document that the beneficial effect persists into early childhood in the form of better neurodevelopmental outcome, as well as to examine if other less expected benefits or harms can be detected.

Multiple validated methods exist to assess neurodevelopment in early childhood; The Bayley Scales of Infant Development (BSID) (119) is a widely used clinical examination, evaluating five major developmental domains such as cognitive status, language level, motor status, adaptive and social-emotional behaviour. However, BSID is highly time demanding for parents and practitioners and have significant costs. If BSID is not implemented in clinical practice, The Ages & Stages Questionnaire (ASQ) is a validated parental questionnaire covering both motor and mental development. ASQ is an alternative to evaluate neurodevelopment, since the agreement between ASQ and BSID is good (120).

16.9 Appendix I: List of variables to be reported (web-based case report form)

Data needed at randomization

- Birth date and hour
- Gestational age ~~less than 28 weeks or less than 26 weeks~~in weeks
- Method of consent
- Decision to conduct full life support
- Time from birth until cerebral oximetry started (before or after 6 postnatal hours)

Data needed at 72 hours of age

- Gestational age in weeks and days
- Birth weight
- Gender
- Apgar 1 and Apgar 5
- ~~Resuscitation~~
- Age in hours when cerebral oximetry was started
- Type of NIRS device used
- Visible cerebral oximetry monitoring (if control group participant)
- Cerebral oximetry monitoring stopped prematurely with reason
- Severe incidents due to monitoring
- Change of medical management due to cerebral hypoxia in patient record
- Surfactant therapy
- Cardiovascular support (volume, vasopressors, inotropes) before 72 hrs
- Parents discontinue trial participation (yes/no) and reason if yes
- SARs

Data needed at 36 weeks of postmenstrual age (or referring to age at discharge home if that happened before 36 weeks)

- Follow-up date
- Major congenital anomaly
- SAEs~~Rs~~
- Mechanical ventilation, and number of days of mechanical ventilation
- Sepsis
- Patent ductus arteriosus~~Treatment for patent ductus arteriosus~~
- Cranial ultrasound performed before 8 days of age and/or after 35 days of age
- Intraventricular haemorrhage grade 3 or 4
- Cystic periventricular leukomalacia
- Post-haemorrhagic ventricular dilatation
- Cerebellar haemorrhage
- Cerebral atrophy
- Bronchopulmonary dysplasia

- Necrotis~~z~~ing enterocolitis stage 2 or greater via modified Bell's staging system or focal intestinal perforation
- Retinopathy of prematurity stage 3 or higher
- Death before 36 weeks post-menstrual age and before discharge to home
- Classification of cause of death
- Weight at follow-up~~discharge~~ and date for weighing

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