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| Title | Early biomarkers of brain injury and cerebral hypo- and hyperoxia in the SafeBoosC II trial |
| Authors | Plomgaard, Anne M.;Alderliesten, Thomas;Austin, Topun;van Bel, Frank;Benders, Manon;Claris, Olivier;Dempsey, Eugene M.;Fumagalli, Monica;Gluud, Christian;Hagmann, Cornelia;Hyttel-Sorensen, Simon;Lemmers, Petra;van Oeveren, Wim;Pellicer, Adelina;Petersen, Tue H.;Pichler, Gerhard;Winkel, Per;Greisen, Gorm |
| Publication date | 2017-03-22 |
| Original Citation | Plomgaard, A. M., Alderliesten, T., Austin, T., van Bel, F., Benders, M., Claris, O., Dempsey, E., Fumagalli, M., Gluud, C., Hagmann, C., Hyttel-Sorensen, S., Lemmers, P., van Oeveren, W., Pellicer, A., Petersen, T. H., Pichler, G., Winkel, P. and Greisen, G. (2017) 'Early biomarkers of brain injury and cerebral hypo- and hyperoxia in the SafeBoosC II trial', PLoS ONE, 12(3), e0173440 (15pp). doi: 10.1371/journal.pone.0173440 |
| Type of publication | Article (peer-reviewed) |
| Link to publisher's version | http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0173440 - 10.1371/journal.pone.0173440 |
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| Download date | 2026-11-05 16:51:24 |
| Item downloaded from | https://hdl.handle.net/10468/4108 |




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RESEARCH ARTICLE

# Early biomarkers of brain injury and cerebral hypo- and hyperoxia in the SafeBoosC II trial

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**Citation:** Plomgaard AM, Alderliesten T, Austin T, van Bel F, Benders M, Claris O, et al. (2017) Early biomarkers of brain injury and cerebral hypo- and hyperoxia in the SafeBoosC II trial. PLoS ONE 12 (3): e0173440. https://doi.org/10.1371/journal.pone.0173440

**Editor:** Umberto Simeoni, Centre Hospitalier Universitaire Vaudois, FRANCE

**Received:** February 22, 2016

**Accepted:** February 19, 2017

**Published:** March 22, 2017

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**Data Availability Statement:** The data is sensitive patient data and is stored and analysed under the restrictions of the Danish Data Protection Agency. According to the data management plan, a de-anonymised dataset with gestational age and birth weight recorded into two groups each will be stored at the Danish Data Archive as soon as the two-year follow-up data is cleaned and locked. After January 2018 data from the SafeBoosC II study will be available upon request through the Danish Data Archive: mailbox@sa.dk. In the interim, a dataset used for the present analysis can

## Abstract

### Background

The randomized clinical trial, SafeBoosC II, examined the effect of monitoring of cerebral oxygenation by near-infrared spectroscopy combined with a guideline on treatment when cerebral oxygenation was out of the target range. Data on cerebral oxygenation was collected in both the intervention and the control group. The primary outcome was the reduction in the burden of cerebral hypo- and hyperoxia between the two groups. In this study we describe the associations between the burden of cerebral hypo- and hyperoxia, regardless of allocation to intervention or control group, and the biomarkers of brain injury from birth till term equivalent age that was collected as secondary and explorative outcomes in the SafeBoosC II trial.

### Methods

Cerebral oxygenation was continuously monitored during the first 72h of life in 166 extremely preterm infants. Cranial ultrasound was performed at day 1,4,7,14, and 35 and at term. Electroencephalogram (EEG) was recorded at 64h. Blood-samples taken at 6 and 64 hours were analysed for the brain injury biomarkers; S100beta, brain-fatty-acid-binding-protein, and neuroketal. All analyses were conducted post hoc.

### Results

Significantly more infants with a cerebral burden of hypoxia within the 4$^{th}$ quartile versus infants within quartile 1–3 were diagnosed with severe intracranial haemorrhage (11/39 versus 11/117, p = 0.003), had low burst rate on EEG (12/28 versus 21/103, p = 0.015), or died

be obtained from the authors at request. Note that the grouping of gestational age and birth weight will make it impossible to make an exact reproduction of the results of the statistical analysis. Please contact amplomgaard@gmail.com.

**Funding:** This work was funded by the Danish Council for Strategic Research (DKK 11,100,105). Grant number URL: http://ufm.dk/forskning-og-innovation/tilskud-til-forskning-og-innovation/hvem-har-modtaget-tilskud/2011/bevillinger-fra-det-strategiske-forskningsrad-programkomiteen-for-individ-sygdom-og-samfund-november-2011. HaemoScan provided support in the form of salary for author WvO, but did not have any additional role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The specific roles of this author are articulated in the 'author contributions' section.

**Competing interests:** Author WvO received funding from HaemoScan, a commercial company, for this study. There are no patents, products in development or marketed products to declare. This does not alter our adherence to all the PLOS ONE policies on sharing data and materials.

(14/41 versus 18/123, p = 0.006), whereas none of these events were significantly associated with cerebral hyperoxia. The blood biomarkers were not significantly associated with the burden of cerebral hypo- or hyperoxia.

## Conclusions

The explorative analysis showed that early burden of cerebral hypoxia, but not hyperoxia was significantly associated with low brain electrical activity and severe intracranial haemorrhage while none of the three blood biomarkers were associated with the burden of either cerebral hypo- or hyperoxia.

## Introduction

Extremely preterm infants have an immature cardiorespiratory system and cerebral autoregulation can be impaired, especially during the first days of life [1,2]. This makes the developing brain of the preterm infant susceptible to fluctuations in the cerebral blood flow (CBF) [3] and may cause episodes of cerebral hypo- and hyperoxia. Near-infrared spectroscopy (NIRS) is a non-invasive method for estimating tissue oxygenation. NIRS measures the ratio of the concentrations of oxygenated haemoglobin to total haemoglobin on an absolute scale with a range of 0% to 100% [4]. Changes in cerebral NIRS-values are correlated to CBF [5]. A number of biomarkers–characteristics that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [6]—may be present if the brain is suffering from hypo- or hyperoxia. Severe intraventricular haemorrhage (IVH grade III) and periventricular haemorrhagic infarction (PVHI) mainly develop within the first 3 days of life [7], during the period of transition from intra- to extra-uterine life when the brain is especially vulnerable. Low cerebral oxygenation, as estimated by NIRS, during this transition has been associated with higher grades of intraventricular haemorrhage and lower 2-year developmental quotients [8–10]. In addition high values of NIRS in animal studies are associated with brain injury [11], as confirmed in human asphyxiated term new-borns [12]. Cerebral NIRS monitoring is currently used in some neonatal intensive care units as part of the standard of care for extremely preterm infants and infants with hypoxic-ischemic encephalopathy. Yet it remains to be determined if monitoring cerebral oxygenation combined with clinical interventions when cerebral oxygenation levels are out of range actually prevents cerebral injury, improves neurological outcome, and/or increases the survival of the extremely preterm infants [13].

The phase II randomized clinical trial, SafeBoosC II, demonstrated that it is possible to reduce the burden of cerebral hypoxia during the first 72 hours of life [14]. The SafeBoosC II study was, however, not powered to detect differences in clinical outcomes [15]. In the present post hoc analysis, we use the SafeBoosC II data to explore the association between the burden of cerebral hypo- and hyperoxia regardless of trial allocation to intervention or control group, and the secondary and explorative outcomes of the trial (all being potential biomarkers of brain injury or death): namely serial cranial ultrasound (cUS), electroencephalographic (EEG) measures, and blood molecular markers.

## Patients and methods

### Infant characteristics and study design

SafeBoosC II is a multicentre randomised clinical feasibility trial [15]. A total of 166 extremely preterm infants were included in the SafeBoosC II study before 3 hours of age: 86 infants were

randomised to the experimental group (cerebral NIRS monitoring in combination with an evidence based intervention guideline [16] for NIRS values out of range (55–85%)) and 80 infants to the control group (blinded collection of NIRS values combined with treatment as usual). The inclusion criteria were infants born more than 12 weeks before term (gestational age <27 weeks and six days) with a decision to provide full life support and the possibility to start cerebral NIRS monitoring within three hours after birth. Written informed consent from the parents was mandatory before inclusion and randomisation. The randomisation was web based and handled by The Copenhagen Trial Unit. The generated allocation sequence of 1:1 with block sizes 4 and 6 in random order concealed for the investigators. The intervention period was 72 hours. The 2y-followup of the infants is on going. The infants were recruited from 8 European countries each represented by one neonatal intensive care unit (June 2012 to December 2013). The trial is registered at ClinicalTrial.gov, NCT01590316, the protocol is available in full at http://www.safeboosc.eu.

## The burden of cerebral hypo- and hyperoxia

The primary outcome of the SafeBoosC II trial was the burden of hypo- and hyperoxia. This was calculated as the time spent below or above the target limits multiplied by the mean deviation from the lower (55%) or the upper limit (85%) during the first 72 hours of life, expressed in percentage hours (%hours). The burden was computed from un-edited NIRS-values and extrapolated to 72 hours, without knowledge of any other outcomes of the trial [14].

## Cranial ultrasound

On day 1 (anytime during the first 24 hours of life), 4 (± 1), 7 (± 1), 14 (± 1), and 35 (± 1) and at term equivalent age (week 38 to 44) standardized cUS (6 coronal and 5 sagittal images through the anterior fontanel and one through the mastoid window) was performed. The images were anonymised and uploaded to a central server. The images were centrally analysed by two experts (CH and MB) using the software program OsiriX version 6.0 (Pixmeo, Geneva, Switzerland). The process of the central scoring is described in detail elsewhere [17]. IVH grade III, PVHI, post haemorrhagic ventricular dilatation, porencephalic cysts, cystic periventricular leukomalacia, cerebral atrophy at term, stroke and cerebellar haemorrhage at one or more of the scans were classified as severe brain injury, and thereby as severe adverse outcome.

## Electroencephalogram

EEG was analysed in 133 infants, the median age at EEG-recording was 65 hours postnatal and the median time of recording was 2 hours [18]. Electrodes were placed at P3 and P4 position according to the international 10-20-system. Needle, disc or hydrogel electrodes were used according to local practice. The electrode impedance was less than 20kΩ during the recording. If the child was treated with morphine, other opioids, or sedative medications this was documented.

All EEG analysis was performed in Matlab version R2014b using custom build programmes (MathWorks, Natick, Massachusetts, USA), without knowledge of the medical history of the infant. The analysis of the EEGs is described in detail elsewhere [18].

The raw EEG was band pass filtered (0.5–30 Hz) using a zero phase filter and converted into range-EEG (rEEG) [19]. Artefacts in the rEEG were independently visually identified by two of the authors (GG and AMP) blinded to the clinical history of the infant. The EEG analysis was conducted on the remaining artefact-free data.

Burst rate was calculated as the number of bursts per minute. A burst was defined as nested (high frequency) oscillations within large slow-wave depolarisations using an extraction algorithm based on the co-occurrence of a slow (0.5–2 Hz) wave and higher (8–22 Hz) frequency oscillation, as described by Hartley et al. [20]. Consecutive events occurring within 0.5 seconds of one another were counted as one and events of duration less than 4/22 of a second were discounted [20]. Burst rates were significantly affected (decreased burst rate) by the use of morphine and EEG-recordings with an online filtration at 2–15 Hz (decreased burst rate) [18]. Therefore the burst rates were adjusted for these variables. Adjusted burst rate within the 1$^{st}$ quartile was considered an adverse outcome.

Spectral-analyses were conducted using Matlab routines (Neurospec 2.0, Neurospec.org). The EEG data was segmented into epochs of 2 seconds with an overlap of 50% (1 second). After fast Fourier transformation the 95% spectral edge frequency (SEF95) for each infant was defined as the frequency between 0.5 and 30 Hz, below which 95% of the power was present. SEF95 was significantly affected by the EEG sampling frequency (high sampling frequency higher SEF95) [18]. Therefore the SEF95 was adjusted for this. Adjusted SEF95 within the 1$^{st}$ quartile was considered an adverse outcome.

## Blood biomarkers

At the age of 6 and 64 hours (±1 h) 1 ml of blood was drawn from 123 infants with an indwelling arterial or venous line [18]. After inclusion of the last patient, the samples were shipped and analysed centrally (HaemoScan, Groningen, The Netherlands) without knowledge of the medical history of the infant. *S100beta* (50 μl) was assessed by ELISA: clone 1B2 monoclonal antibody (Abnova, Taipei, Taiwan) and biotinylated clone 8B10 (Hytest, Turku, Fi). Intra-assay variance is 4.6% and the lower level of quantification (LLOQ) is 39 pg/ml. *Brain fatty acid binding protein (BFABP)* (50 μl) was determined by ELISA with BFABP polyclonal capture antibodies and monoclonal detection antibody (HaemoScan). Intra-assay variance is 6.4% and the LLOQ is 150 pg/ml. *Neuroketal* (60 μl) was performed by competitive enzyme immunoassay (HaemoScan). Intra-assay variance is 10% and the LLOQ is 4.1 pg/ml. The laboratory analyses are described in detail elsewhere [18]. An increase in the biomarker concentration from 6 to 64 hours was considered as a marker of potential cerebral injury during the intervention period, therefore and adverse outcome was defined as an increase in the absolute value of the biomarker concentration from 6 hours to 64 hours within the 4$^{th}$ quartile.

## Statistics

The median and inter quartile range of burden of cerebral hypo- and hyperoxia was determined. The infants were divided in groups according to a burden within or below the 4$^{th}$ quartile of the burden of cerebral hypo- and hyperoxia, respectively. The infant characteristics were compared between the burden-groups using the chi-square test or independent t-test as appropriate. Odds ratios with 95% confidence intervals were determined for adverse outcomes for infants within the 4$^{th}$ quartile of the burden of hypo- or hyperoxia versus infants in quartile 1–3. Thereafter univariate correlation analysis was conducted to determine the patient characteristics associated with the composite outcome of severe brain injury or death. Finally, a multiple logistic regression was used with the composite outcome as dependent variable, centre, gestational age above or below 26 weeks (the stratification variables used in the randomized SafeBoosC II trial), and intervention as forced entry independent variables, and the patient characteristics which had significant correlation to the composite outcome as independent variables in a backward stepwise elimination procedure (P-out 0.1).

The between twins inter-cluster correlation for the burden of hypo-and hyperoxia was low (0.02) [14]. Therefore, there was no need to exclude one infant from each twin-cluster thus data from all infants were included in the analysis.

None of the analyses reported here were specified in the SafeBoosC-II study protocol. The dichotomisation between one quartile and the three other quartiles was copied from previous work on biomarkers in our group [21], and chosen before any correlations were calculated.

The statistics was performed using IBM SPSS Statistics for Windows Version 20.0 (Armonk, New York, USA).

## Ethics

The SafeBoosC phase II trial was approved by each hospital's research ethics committee (Hopital Femme Mere Enfants, Lyon, France; Rigshospitalet, Copenhagen, Denmark; La Paz University Hospital, Madrid, Spain; Cork University Maternity Hospital, Cork, Ireland; Wilhelmina Children's Hospital, Utrecht, The Netherlands; Medical University of Graz, Graz, Austria; Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; and Rosie Hospital, Cambridge University Hospitals, United Kingdom), and where required (Austria, Denmark, and France) by the competent authority responsible for medical devices. Written informed parental consent was mandatory before inclusion in the trial. The trial was 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.

## Results

One-hundred-and-sixty-six infants were included in the SafeBoosC II trial, Fig 1. Cerebral oximetry data was missing for two infants due to technical issues (n = 1) and withdrawal of consent (n = 1).

The median (min—max) burden of cerebral hypoxia was 30.6%hours (0.7–803.9). The limits for hypoxia quartile 4 were 99.3–803.9%hours and for quartile 1–3; 0.7–99.2%hours. The median (min—max) burden of cerebral hyperoxia was 1.2%hours (0.0–223.0). The limits for quartile 4 were 14.2–223.0%hours and for quartile 1–3; 0.0–14.1%hours. As expected according to the primary result of the SafeBoosC II trial [14], there were significantly fewer infants form the intervention group with a burden of hypoxia in the $4^{th}$ quartile, than in quartile 1–3, whereas the burden of cerebral hyperoxia within the $4^{th}$ quartile versus quartile 1–3, was unaffected by allocation in the SafeBoosC trial, Table 1. Male sex was associated with less cerebral hypoxia and more cerebral hyperoxia. Furthermore, gestational age was positively associated with cerebral hyperoxia whereas the other baseline characteristics were not associated with either cerebral hypo- or hyperoxia.

Serial ultrasound scans of 155 infants were available for evaluation by central reading. Twenty-seven (27/155) had severe brain injury, of which 22 had IVH grade III or PVHI. The remaining five infants had stroke (n = 2), cerebral atrophy (n = 2), or cerebellar haemorrhage (n = 1). Cerebral hypoxia was significantly associated with severe brain injury and death, Table 2. There was no significant association between cerebral hyperoxia and severe brain injury and death, Table 3. Cerebral hypoxia was significantly associated with early occurrence (day 1 to 4) of severe intracranial haemorrhages whereas there was no association between cerebral hypoxia and later occurrence (day 5 to day 14) of severe intracranial haemorrhages, Table 2. Cerebral hypoxia was also significantly associated with low EEG burst rates at 64 hours of age whereas there was no association between cerebral hypoxia and SEF95 at 64 hours of age. Of the 14 infants with early severe IVH ten (10) infants had EEG measurement and of these five (5) infants had burst rates within the $1^{st}$ quartile, for the infants without severe

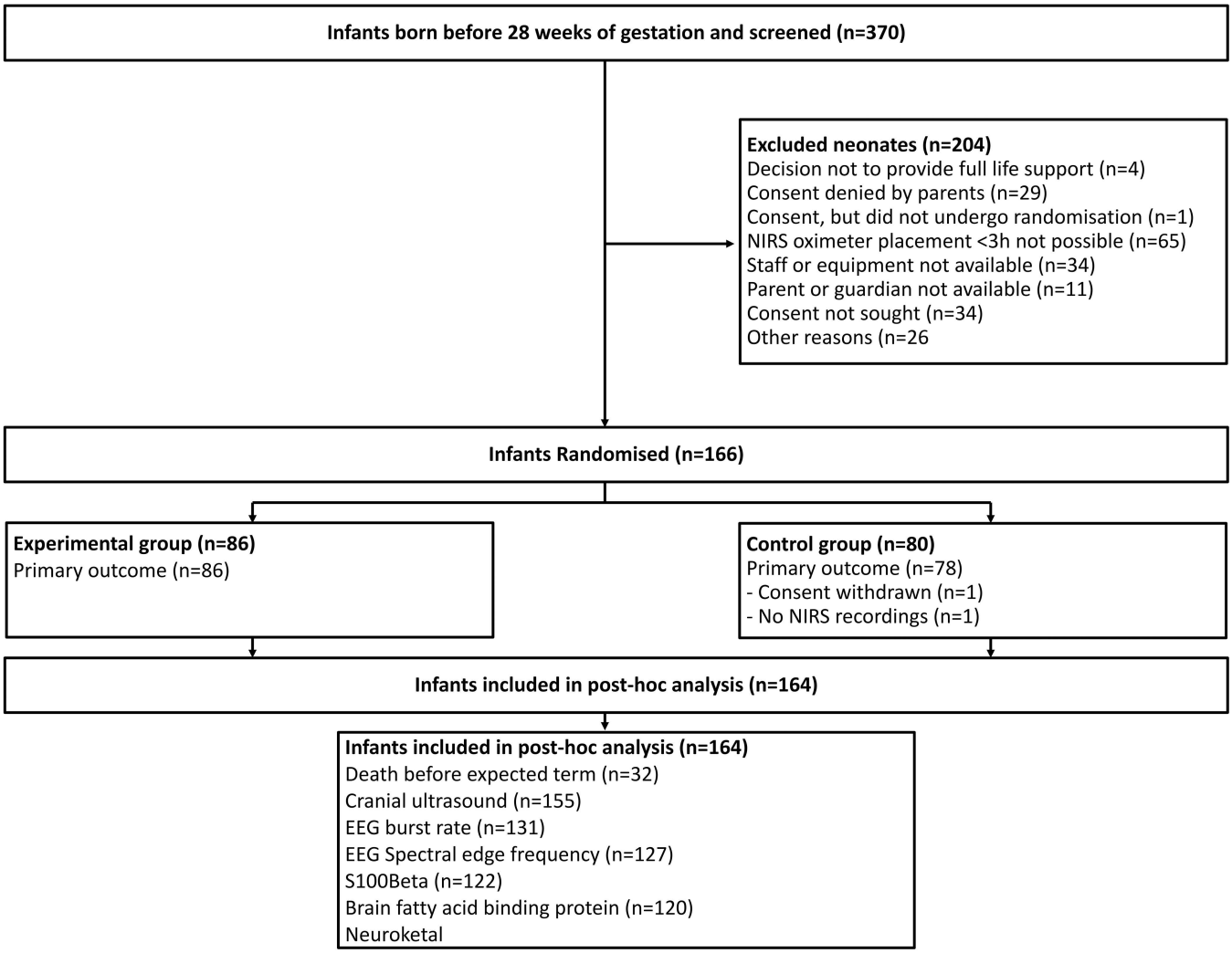



**Fig 1. Flowchart.** Flow of participants through the SafeBoosC II study.

https://doi.org/10.1371/journal.pone.0173440.g001

haemorrhages the number of recorded EEGs was 115 and hereof 26 had burst rates within the 1st quartile (chi-square analysis between groups p = 0.12). The three plasma-biomarker-levels were not associated with cerebral hypoxia, Table 2. Cerebral hyperoxia was not associated with EEG burst rate, SEF95 or plasma biomarkers, Table 3. The odds ratios (ORs) and 95% confidence interval for adverse outcomes are illustrated in Figs 2 and 3.

Correlation analyses of the following variables showed a significant correlation ($p<0.05$) between gestational age, birth weight, clinical chorioamnionitis, surfactant, mechanical ventilation, use of vasopressors, blood transfusions, and the burden of cerebral hypoxia within the 4th quartile on one hand and the composite outcome of severe brain injury or death on the other. These variables were included in the multiple logistic regression model with backward stepwise elimination. As described in the methods, centre, gestational age below 26 weeks, and intervention were forced-entry variables in the model. The following variables remained statistically significant: intervention (OR (95% CI) 0.29 (0.12–0.69; p = 0.003,)), gestational age below 26 weeks (3.33 (1.38–8.06; p = 0.007)), use of vasopressors (3.26 (1.26–8.44; p = 0.014)), and blood transfusion (2.97 (1.22–7.23; p = 0.016)). There was no centre-effect.

**Table 1. Baseline characteristics and treatment during the first 72 hours of life according to burden of cerebral hypo- or hyperoxia split in the three lowest quartiles and the highest quartile.**

| | *Burden of hypoxia* | | | *Burden of hyperoxia* | | |
|---|---|---|---|---|---|---|
| | **Quartile 1 to 3** | **Quartile 4** | | **Quartile 1 to 3** | **Quartile 4** | |
| | **n = 123** | **n = 41** | **P-value** | **n = 123** | **n = 41** | **P-value** |
| **Baseline characteristics** | | | | | | |
| Gestational age (week), mean (SD) | 26.4 (1.2) | 26.5 (1.5) | 0.78 | 26.3 (1.3) | 27.7 (1–0) | 0.35 |
| Gestational age below 26 weeks | 38 (31) | 14 (34) | 0.7 | 42 (34) | 10 (24) | 0.025 |
| Birth weight (gram), mean (SD) | 847 (211) | 875 (207) | 0.47 | 849 (208) | 872 (216) | 0.54 |
| Male sex | 65 (53) | 13 (32) | 0.02 | 53 (43) | 25 (61) | 0.047 |
| Twins | 21 (17) | 12 (29) | 0.09 | 23 (19) | 10 (24) | 0.43 |
| Antenatal steroids full course | 82 (67) | 31 (78) | 0.2 | 88 (72) | 25 (61) | 0.18 |
| Prolonged rupture of membranes | 40 (33) | 17 (36) | 0.21 | 42 (34) | 15 (38) | 0.72 |
| Maternal chorioamnionitis | 6 (5) | 5 (13) | 0.1 | 9 (8) | 2 (5) | 0.59 |
| APGAR-score <5 points at 5 minutes | 21 (17) | 8 (20) | 0.69 | 20 (16) | 9 (23) | 0.38 |
| Umbilical arterial pH, mean (SD) | 7.32 (0.1) | 7.31 (0.1) | 0.62 | 7.32 (0.1) | 7.29 (0.1) | 0.13 |
| SafeBoosC II intervention group | 74 (60) | 12 (29) | 0.001 | 67 (54) | 19 (46) | 0.37 |
| **Treatment during the first 72h of life** | | | | | | |
| Surfactant treatment | 90 (73) | 35 (85) | 0.11 | 90 (73) | 35 (85) | 0.11 |
| Mechanical ventilation | 79 (64) | 30 (73) | 0.29 | 80 (65) | 29 (70) | 0.5 |
| Patent ductus arteriosus treatment | 17 (14) | 4 (10) | 0.52 | 15 (12) | 6 (15) | 0.71 |
| Use of vasopressors/inotropes | 22 (18) | 16 (40) | 0.004 | 29 (24) | 9 (22) | 0.79 |
| Any red blood cell transfusion | 31 (26) | 18 (45) | 0.025 | 41 (35) | 8 (20) | 0.07 |
| Corticosteroids | 4 (3) | 4 (10) | 0.1 | 6 (5) | 2 (5) | 0.99 |

Values are numbers (percentages) unless stated otherwise. P-values have not been corrected for multiple comparisons.

https://doi.org/10.1371/journal.pone.0173440.t001

## Discussion

This post hoc analysis shows that early burden of cerebral hypoxia, but not hyperoxia, is associated with a reduction of brain electrical activity, severe brain injury (especially early IVH grade III and PVHI), and death. There were no significant associations between the burden of cerebral hypo- and hyperoxia and the three blood biomarkers. Multiple logistic regressions showed significant associations between intervention, gestational age below 26 weeks, use of vasopressors, and blood transfusion on one hand and the composite adverse outcome severe brain injury or death on the other.

Low cerebral oxygenation has previously been associated with IVH and lower developmental quotients at 2-year follow up [8–10]. In a study by Noori et al, involving 22 extremely preterm infants, lower levels of cerebral oxygenation, cardiac output, and cerebral hypoperfusion were found prior to the development of IVH II and PVHI [8]. In term piglet models, however, while low cerebral oxygenation when accompanied by low cerebral blood flow caused permanent brain damage, prolonged cerebral hypoxia alone seemed to be of less importance [22,23]. Piglets had to be exposed to cerebral hypoxia as low as 30–35% for several hours before significant histological damage appeared [23].

In the present analysis the burden of cerebral hypoxia was associated with decreased EEG burst rates. This is in agreement with a previous demonstration of an association between low cerebral blood flow and suppressed EEG in preterm infants [24]. EEG burst rate is decreased in infants with severe IVH [25,26] and suppressed EEG is correlated to adverse developmental outcome [27,28]. In the present analysis we did not find significantly more infants with low burst rates and early severe IVH grade III or PVHI.

**Table 2. Distributions of early and late adverse outcomes of cranial ultrasound, EEG variables, blood biomarkers, term diagnoses, and death according to the *burden of cerebral hypoxia* within or below the 4th quartile.**

| Burden of hypoxia | Quartile 1–3 | Quartile 4 | | | | |
|---|---|---|---|---|---|---|
| | n = 123 | n = 41 | P-value | *P-value | OR | (95% CI) |
| Cranial ultrasound | | | | | | |
| IVH 3–4 day 1–4 | 6/117 | 8/38 | 0.003 | 0.045 | 4.93 | (1.59–15.30) |
| IVH 3–4 day 5–14 | 5/111 | 3/31 | 0.63 | >0.95 | 2.27 | (0.51–10.09) |
| IVH 3–4 at any time | 11/117 | 11/39 | 0.003 | 0.045 | 3.77 | (1.49–9.63) |
| Severe brain injury—any time | 14/116 | 13/39 | 0.002 | 0.03 | 3.64 | (1.53–8.69) |
| EEG variables time 64h | | | | | | |
| Burst-rate in the 1st quartile | 21/103 | 12/28 | 0.015 | 0.23 | 2.93 | (1.20–7.12) |
| Spectral edge frequency in the 1st quartile | 15/99 | 5/27 | 0.67 | >0.95 | 1.27 | (0.42–3.88) |
| Plasma biomarkers difference between 6 and 64h | | | | | | |
| S100beta increase in 4th quartile | 25/92 | 6/30 | 0.43 | >0.95 | 0.67 | (0.25–1.83) |
| BFABP increase in 4th quartile | 24/89 | 8/31 | 0.90 | >0.95 | 0.94 | (0.37–2.39) |
| Neuroketal increase in 4th quartile | 22/92 | 9/30 | 0.51 | >0.95 | 1.36 | (0.55–3.41) |
| Other major adverse outcome at term age | | | | | | |
| Necrotising enterocolitis | 13/123 | 6/41 | 0.48 | >0.95 | 1.45 | (0.51–4.10) |
| Retinopathy of prematurity | 18/123 | 4/41 | 0.43 | >0.95 | 0.63 | (0.20–1.98) |
| Bronchopulmonary dysplasia | 52/104 | 14/27 | 0.86 | >0.95 | 1.08 | (0.46–2.51) |
| Death | 18/123 | 14/41 | 0.006 | 0.09 | 3.03 | (1.34–6.84) |
| Combined adverse outcome | | | | | | |
| Death or severe brain injury | 28/117 | 20/41 | 0.003 | 0.045 | 3.03 | (1.44–6.38) |
| Death or IVH 3 or 4 | 25/118 | 19/41 | 0.002 | 0.03 | 3.21 | (1.51–6.84) |

Values are given as numbers of events / numbers of infants investigated for event.

* P-value after Bonferoni correction for multiple comparisons.

https://doi.org/10.1371/journal.pone.0173440.t002

The three blood biomarkers measured in the SafeBoosC II trial were not significantly associated with either cerebral hypo- or hyperoxia. We did not register whether the samples were drawn from arterial or venous line, therefore we are unable to determine whether the concentrations of the blood biomarkers differ between arterial and venous blood.

S100beta is a calcium binding protein present in high concentration in Schwann cells and astrocytes [29] and is released to the systemic circulation after cerebral damage. S100beta is an established marker of brain injury in adult trauma patients [30] and the levels of S100beta in blood and urine from term and preterm infants has been associated with the severity of both hypoxic ischemia and IVH [31–33]. And in a study including 64 term and late preterm infants the levels of S100beta was significantly negatively correlated to NIRS-values [34]. However a study by Rogers et al [35] including 130 extremely preterm infants reporting no associations between the S100beta levels and severe intracranial haemorrhages. S100Beta has also been investigated as a potential biomarker of brain injury in paediatric patients undergoing cardiac surgery–the infants with unfavourable neurological outcome 12 months after surgery had significantly lower cerebral NIRS values during surgery, whereas the levels of S100Beta did not differ [36]. We evaluated the effects of the accumulated burden of hypoxia during the first 72 hours of life rather than correlating specific NIRS-values to the levels of S100beta, and therefore we cannot determine whether the S100beta levels measured in the SafeBoosC II study were correlated to the NIRS-levels. We found no differences between the levels of S100beta between the 4th quartile of cerebral hypoxia where the proportion of infants with severe IVH was highest vesus the three lowest quartiles of cerebral hypoxia. The conflicting results of the

**Table 3. Distributions of early and late adverse outcomes of cranial ultrasound, EEG variables, blood biomarkers, term diagnoses, and death according to the *burden of cerebral hyperoxia* within or below the 4th quartile.**

| Burden of hyperoxia | Quartile 1–3 | Quartile 4 | | | | |
|---|---|---|---|---|---|---|
| | n = 123 | n = 41 | P-value | *P-value | OR | (95% CI) |
| Cranial ultrasound | | | | | | |
| IVH 3–4 day 1–4 | 11/116 | 3/39 | 0.74 | >0.95 | 0.80 | (0.21–3.01) |
| IVH 3–4 day 5–14 | 6/100 | 2/36 | 0.98 | >0.95 | 0.92 | (0.18–4.79) |
| IVH 3–4 at any time | 17/117 | 5/39 | 0.79 | >0.95 | 0.87 | (0.30–3.52) |
| Severe brain injury—any time | 21/117 | 6/38 | 0.76 | >0.95 | 0.86 | (0.32–2.31) |
| EEG variables time 64h | | | | | | |
| Burst-rate in the 1st quartile | 27/95 | 3/36 | 0.17 | >0.95 | 0.50 | (0.19–1.35) |
| Spectral edge frequency in the 1st quartile | 13/91 | 7/35 | 0.43 | >0.95 | 1.50 | (0.54–4.14) |
| Plasma biomarkers difference between 6 and 64h | | | | | | |
| S100beta increase in 4th quartile | 24/93 | 7/29 | 0.86 | >0.95 | 0.92 | (0.35–2.41) |
| BFABP increase in 4th quartile | 23/92 | 9/28 | 0.45 | >0.95 | 1.42 | (0.57–3.58) |
| Neuroketal increase in 4th quartile | 25/93 | 6/29 | 0.5 | >0.95 | 0.71 | (0.26–1.95) |
| Other major adverse outcome at term age | | | | | | |
| Necrotising enterocolitis | 17/123 | 2/41 | 0.12 | >0.95 | 0.32 | (0,07–1,45) |
| Retinopathy of prematurity | 20/123 | 2/41 | 0.064 | >0.95 | 0.26 | (0.06–1.18) |
| Bronchopulmonary dysplasia | 55/97 | 11/34 | 0.015 | 0.23 | 0.37 | (0.16–0.83) |
| Death | 26/123 | 6/41 | 0.36 | >0.95 | 0.64 | (0.24–1.68) |
| Combined adverse outcome | | | | | | |
| Death or severe brain injury | 38/120 | 10/38 | 0.53 | >0.95 | 0.77 | (0.34–1.75) |
| Death or IVH 3 or 4 | 35/120 | 9/39 | 0.46 | >0.95 | 0.73 | (0.31–1.69) |

Values are given as numbers of events / numbers of infants investigated for event.

* P-value after Bonferoni correction for multiple comparisons.

https://doi.org/10.1371/journal.pone.0173440.t003

associations between S100beta and severe intracranial haemorrhages make the clinical value of this biomarker questionable and further research is needed to evaluate whether S100beta is suitable as an early biomarker of brain injury in extremely preterm infants.

BFABP, a brain-specific marker, is rapidly released from astrocytes as a response to ischemia, mechanical and oxidative brain damage [37]. BFABP was chosen as a potential biomarker for brain injury in the SafeBoosC II trial, as it is known to rapidly increase in hypoxic-ischaemic stroke patients, and the high levels persist for several days after an event [37]. BFABP might be a more sensitive marker of minor traumatic brain injury than S100beta [38] and is elevated in patients with various neurodegenerative diseases[39]. A study involving 57 patients undergoing cardiac surgery reports that neither S100beta nor BFABP had any clear prognostic value for postoperative cognitive dysfunction [40]. We found no association between BFABP and the burden of cerebral hypo- or hyperoxia–this might be because of the different nature of the acute and severe local hypoxic-ischemia occurring in stroke patients compared to the global relative cerebral hypoxia measured in the SafeBoosC II trial. Our study ads information to the current litterateur of BFABP and brain injury–suggesting that there is no associations between the burden of cerebral hypoxia at 72h of age and the increase of the levels of BFABP from 6 to 64h of life. Further research on the associations between BFABP and brain injury in extremely preterm infants is needed.

Neuroketals are compounds produced by free radical induced peroxidation of docosahexenoic acid, have been associated with white matter damage on MRI in preterm infants [41] and are solely present in the brain [42]. Neuroketal is mainly released as a reaction to cerebral

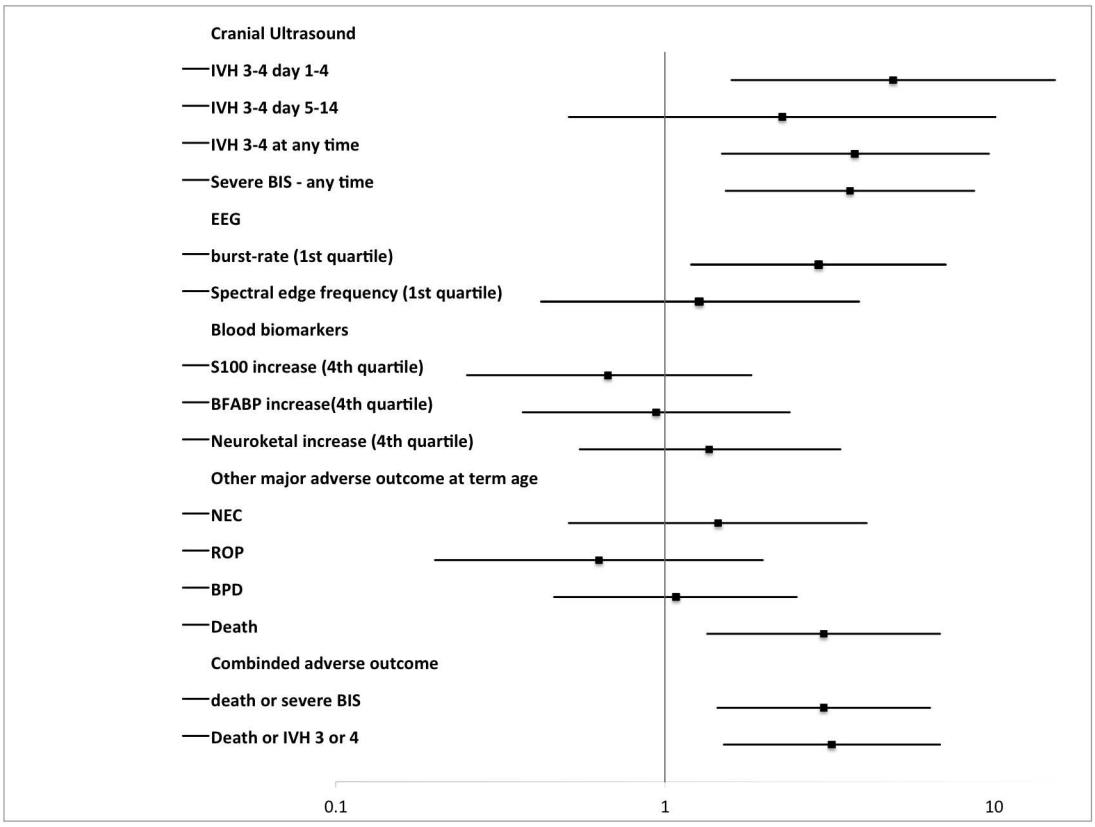



**Fig 2. Risk for adverse outcomes for infants with a *burden of cerebral hypoxia* within or below the 4th quartile.** Odds ratio (OR) and 95% confidence interval.

https://doi.org/10.1371/journal.pone.0173440.g002

oxidative stress, and may due to their reactivity be involved in formation of protein cross-links, which is a common feature in neurodegenerative diseases, where neuroketal is known to be increased [43]. Neuroketal has been reported elevated in cerebrospinal fluid during the first 3 weeks of life infants with white matter damage on MRI performed at term equivalent age [41]. We expected that the levels of neuroketal would be increased in the infants with cerebral hyperoxia in the 4th quartile, but that was not the case. This might be explained by the early measure of neuroketal– 6 to 64 hours of life and the fact that the level of cerebral hyperoxia in the SafeBoosC phase II trial was low [14]. In addition neuroketal is still only an experimental biomarker of cerebral injury and might not be a good marker of early acute brain injury in extremely preterm infants. Whether neuroketal in the future can serve as a marker of later white matter injury in extremely preterm infants remains to be determined.

## Limitations

The explorative post hoc analysis of the SafeBoosC II trial data presents the association between the early cerebral oxygenation and short-term adverse cerebral outcomes and death in extremely preterm infants. However, this study has some limitations. Most importantly, cranial ultrasound was conducted at pre-specified days of life, but the exact timing of IVH grade III and PVHI and other severe brain damages is not available and therefore we cannot know if cerebral hypoxia preceded IVH or vice versa. However, one small study in very preterm infants recently identified a significantly lower regional cerebral oxygenation during the

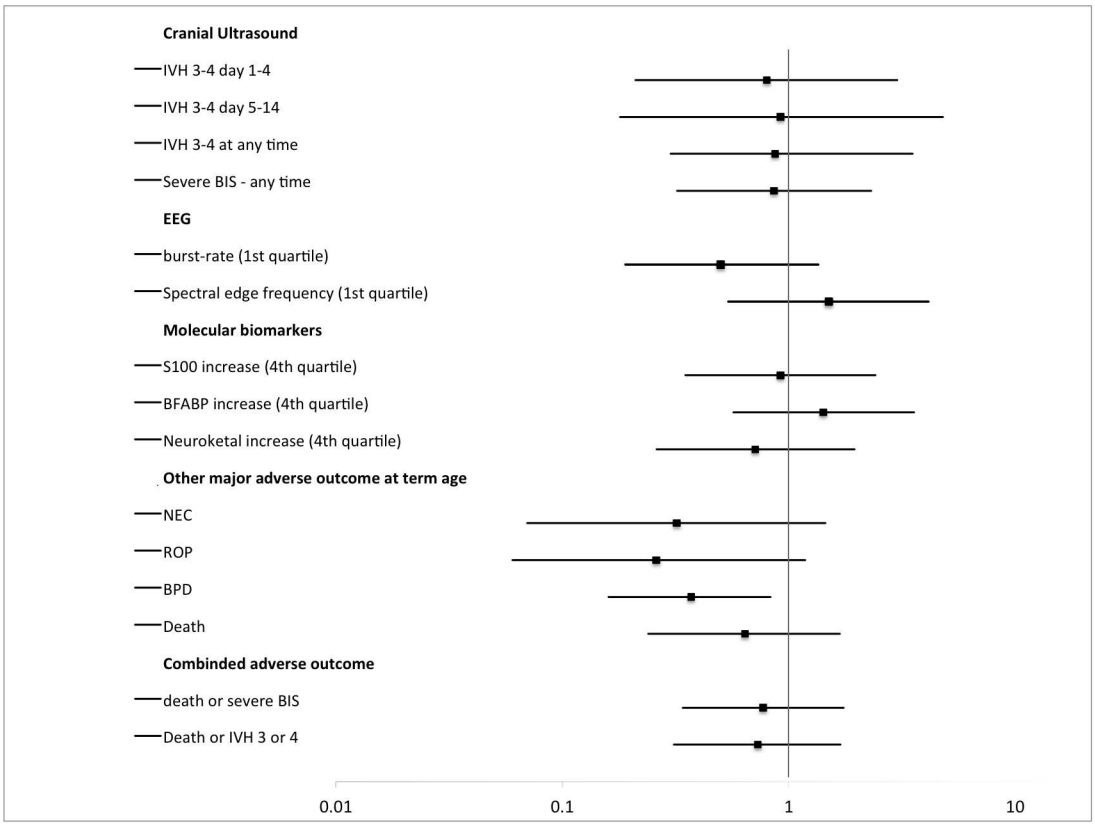



**Fig 3. Risk for adverse outcomes for infants with a *burden of cerebral hyperoxia* within or below the 4th quartile.** Odds ratio (OR) and 95% confidence interval.

https://doi.org/10.1371/journal.pone.0173440.g003

early transition in infants who later developed IVH versus the infants who did not [44]. Similarly, EEG was only recorded once. While the timing at 64 hours of age was expected to assess the potential effects of the accumulated burden over the intervention period, finer details of preceding or concurrent cerebral hypoxia could not be extracted, such as the relative significance of longer periods of moderate hypoxia versus peaks of severe hypoxia. We did not collect data on continuous blood pressure, arterial oxygen saturation, or CBF, which may have contributed to both low cerebral NIRS values and severe brain damage [8,22,23].

The analyses are based on a dataset from our randomised clinical trial; therefore, the multiple logistic regression models were adjusted for the stratification variables (centre and gestational age below 26 weeks), as well as the randomisation indicator (experimental vs. control group). As the burden of hypoxia was reduced by 50% in the experimental group compared with the control group [14], it was therefore not surprising that adjusting for the randomisation indicator did reduce the statistical significance of the burden of hypoxia. We do not think that this changes the main conclusion of the study. On the other hand, the statistical significance of the randomisation indicator means that the risk of severe brain injury or death in the experimental group of the SafeBoosC-II trial was less in the experimental group than in the control group when adjusted for a number of other factors. However, neither the composite outcome nor the particular statistical analyses were specified in the trial protocol. This post-hoc finding must therefore be interpreted conservatively. Finally, the 2-year follow up of the infants included in the SafeBoosC II trial will further explore if cerebral hypoxia and/or the intervention is related to patient-relevant outcomes such as psychomotor deficit. Larger

randomised clinical trials investigating possible patient-relevant benefits of continuous NIRS monitoring in extremely preterm infants is needed before the method is implemented as standard care in this population.

## Conclusions

Our analysis is currently the largest dataset published on cerebral oxygenation in extremely preterm infants providing data on short-term neurological outcomes. The results support the previous findings of associations between low cerebral oxygenation in extremely preterm infants during the first days of life and EEG suppression, severe intracranial haemorrhage, and death. However, our analyses are exploratory and we were unable to determine which came first: cerebral hypoxia or severe intracranial haemorrhage. We did not find any evidence that cerebral hyperoxia is associated with either death or severe brain damage. We found no associations between the burden of cerebral hypo- or hyperoxia and the three blood biomarkers (S100beta, BFABP, and Neuroketal).

## Supporting information

**S1 Appendix. The SafeBoosC Phase II protocol.**
(PDF)

**S2 Appendix. Consort 2010 Checklist.**
(DOC)

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**Writing – original draft:** AMP GG SHS THP.

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## References

1. Soul JS, Hammer PE, Tsuji M, Saul JP, Bassan H, Limperopoulos C, et al. Fluctuating pressure-passivity is common in the cerebral circulation of sick premature infants. Pediatr Res. 2007; 61: 467–473. https://doi.org/10.1203/pdr.0b013e31803237f6 PMID: 17515873

2. Boylan GB, Young K, Panerai RB, Rennie JM, Evans DH. Dynamic cerebral autoregulation in sick newborn infants. Pediatr Res. 2000; 48: 12–17. https://doi.org/10.1203/00006450-200007000-00005 PMID: 10879794
3. Wong FY, Silas R, Hew S, Samarasinghe T, Walker AM. Cerebral oxygenation is highly sensitive to blood pressure variability in sick preterm infants. PLoS ONE. 2012; 7: e43165. https://doi.org/10.1371/journal.pone.0043165 PMID: 22905222
4. Wolf M, Greisen G. Advances in near-infrared spectroscopy to study the brain of the preterm and term neonate. Clin Perinatol. 2009; 36: 807–34– vi.
5. Wintermark P, Hansen A, Warfield SK, Dukhovny D, Soul JS. Near-infrared spectroscopy versus magnetic resonance imaging to study brain perfusion in newborns with hypoxic-ischemic encephalopathy treated with hypothermia. Neuroimage. 2014; 85 Pt 1: 287–293.
6. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001; 69: 89–95. https://doi.org/10.1067/mcp.2001.113989 PMID: 11240971
7. Bassan H, Benson CB, Limperopoulos C, Feldman HA, Ringer SA, Veracruz E, et al. Ultrasonographic features and severity scoring of periventricular hemorrhagic infarction in relation to risk factors and outcome. Pediatrics. 2006; 117: 2111–2118. https://doi.org/10.1542/peds.2005-1570 PMID: 16740854
8. Noori S, McCoy M, Anderson MP, Ramji F, Seri I. Changes in cardiac function and cerebral blood flow in relation to peri/intraventricular hemorrhage in extremely preterm infants. J Pediatr. 2014; 164: 264–70.e1–3.
9. Sorensen LC, Maroun LL, Borch K, Lou HC, Greisen G. Neonatal cerebral oxygenation is not linked to foetal vasculitis and predicts intraventricular haemorrhage in preterm infants. Acta Paediatr. 2008; 97: 1529–1534. https://doi.org/10.1111/j.1651-2227.2008.00970.x PMID: 18673361
10. Alderliesten T, Lemmers PMA, Van Haastert IC, de Vries LS, Bonestroo HJC, Baerts W, et al. Hypotension in preterm neonates: low blood pressure alone does not affect neurodevelopmental outcome. J Pediatr. 2014; 164: 986–991. https://doi.org/10.1016/j.jpeds.2013.12.042 PMID: 24484771
11. Yiş U, Kurul SH, Kumral A, Cilaker S, Tuğyan K, Genç S, et al. Hyperoxic exposure leads to cell death in the developing brain. Brain Dev. 2008; 30: 556–562. https://doi.org/10.1016/j.braindev.2008.01.010 PMID: 18329209
12. Lemmers PMA, Zwanenburg RJ, Benders MJNL, de Vries LS, Groenendaal F, Van Bel F, et al. Cerebral oxygenation and brain activity after perinatal asphyxia: does hypothermia change their prognostic value? Pediatr Res. 2013; 74: 180–185. https://doi.org/10.1038/pr.2013.84 PMID: 23728382
13. Hyttel-Sørensen S, Støy LS, Greisen G, Als-Nielsen B, Gluud C. Cerebral near-infrared spectroscopy monitoring for prevention of brain injury in very preterm infants (Protocol). Group CN, editor. The cochrane Collaboration, The cochrane Libary. 2015;: 1–12.
14. Hyttel-Sørensen S, Pellicer A, Alderliesten T, Austin T, Van Bel F, Benders M, et al. Cerebral near infrared spectroscopy oximetry in extremely preterm infants: phase II randomised clinical trial. BMJ. 2015; 350: g7635. https://doi.org/10.1136/bmj.g7635 PMID: 25569128
15. Hyttel-Sørensen S, Austin T, Van Bel F, Benders M, Claris O, Dempsey E, et al. A phase II randomized clinical trial on cerebral near-infrared spectroscopy plus a treatment guideline versus treatment as usual for extremely preterm infants during the first three days of life (SafeBoosC): study protocol for a randomized controlled trial. Trials. 2013; 14: 120. https://doi.org/10.1186/1745-6215-14-120 PMID: 23782447
16. Pellicer A, Greisen G, Benders M, Claris O, Dempsey E, fumagalli M, et al. The SafeBoosC Phase II Randomised Clinical Trial: A Treatment Guideline for Targeted Near-Infrared-Derived Cerebral Tissue Oxygenation versus Standard Treatment in Extremely Preterm Infants. Neonatology. 2013; 104: 171–178. https://doi.org/10.1159/000351346 PMID: 23921600
17. Plomgaard AM, Hagmann C, Alderliesten T, Austin T, Van Bel F, Claris O, et al. Brain injury in the international multicentre randomised SafeBoosC phase II feasibility trial: cranial ultrasound and magnetic resonance imaging assessments. Pediatr Res. 2016; 79: 466–472. https://doi.org/10.1038/pr.2015.239 PMID: 26571218
18. Plomgaard AM, van Oeveren W, Petersen TH, Alderliesten T, Austin T, Van Bel F, et al. The SafeBoosC II randomised trial: treatment guided by near-infrared spectroscopy reduces cerebral hypoxia without changing early biomarkers of brain injury. Pediatr Res. 2016; 79: 528–535. https://doi.org/10.1038/pr.2015.266 PMID: 26679155
19. O'Reilly D, Navakatikyan MA, Filip M, Greene D, Van Marter LJ. Peak-to-peak amplitude in neonatal brain monitoring of premature infants. Clin Neurophysiol. 2012; 123: 2139–2153. https://doi.org/10.1016/j.clinph.2012.02.087 PMID: 22608473
20. Hartley C, Berthouze L, Mathieson SR, Boylan GB, Rennie JM, Marlow N, et al. Long-range temporal correlations in the EEG bursts of human preterm babies. PLoS ONE. 2012; 7: e31543. https://doi.org/10.1371/journal.pone.0031543 PMID: 22363669

21. Hahn GH, Maroun LL, Larsen N, Hougaard DM, Sorensen LC, Lou HC, et al. Cerebral autoregulation in the first day after preterm birth: no evidence of association with systemic inflammation. Pediatr Res. 2012; 71: 253–260. https://doi.org/10.1038/pr.2011.46 PMID: 22278187

22. Hou X, Ding H, Teng Y, Zhou C, Tang X, Li S, et al. Research on the relationship between brain anoxia at different regional oxygen saturations and brain damage using near-infrared spectroscopy. Physiol Meas. 2007; 28: 1251–1265. https://doi.org/10.1088/0967-3334/28/10/010 PMID: 17906392

23. Kurth CD, McCann JC, Wu J, Miles L, Loepke AW. Cerebral oxygen saturation-time threshold for hypoxic-ischemic injury in piglets. Anesth Analg. 2009; 108: 1268–1277. https://doi.org/10.1213/ane.0b013e318196ac8e PMID: 19299799

24. Greisen G, Pryds O. Low CBF, Discontinuous EEG Activity, and Periventricular Brain Injury in Ill, Preterm Neonates. Brain Dev. 1989; 11: 164–168. PMID: 2751062

25. Soubasi V, Mitsakis K, Sarafidis K, Griva M, Nakas CT, Drossou V. Early abnormal amplitude-integrated electroencephalography (aEEG) is associated with adverse short-term outcome in premature infants. Eur J Paediatr Neurol. 2012; 16: 625–630. https://doi.org/10.1016/j.ejpn.2012.02.008 PMID: 22425390

26. Greisen G, Hellström-Westas L, Lou H, Rosén I, svenningsen NW. EEG depression and germinal layer haemorrhage in the newborn. Acta Paediatr Scand. 1987; 76: 519–525. PMID: 3604671

27. West CR, Harding JE, Williams CE, Nolan M, Battin MR. Cot-side electroencephalography for outcome prediction in preterm infants: observational study. Arch Dis Child Fetal Neonatal Ed. 2011; 96: F108–13. https://doi.org/10.1136/adc.2009.180539 PMID: 20870908

28. Hellström-Westas L, Klette H, Thorngren-Jerneck K, Rosén I. Early prediction of outcome with aEEG in preterm infants with large intraventricular hemorrhages. Neuropediatrics. 2001; 32: 319–324. https://doi.org/10.1055/s-2001-20408 PMID: 11870588

29. Donato R, Sorci G, Riuzzi F, Arcuri C, Bianchi R, Brozzi F, et al. S100B's double life: intracellular regulator and extracellular signal. Biochim Biophys Acta. 2009; 1793: 1008–1022. https://doi.org/10.1016/j.bbamcr.2008.11.009 PMID: 19110011

30. Eskesen V, Springborg JB, Undén J, Romner B. Initial håndtering af minimale, lette og moderate hovedtraumer hos voksne. Ugeskr Læger. 2014; 176: V09130559.

31. Thorngren-Jerneck K, Alling C, Herbst A, Amer-Wahlin I, Maršál K. S100 protein in serum as a prognostic marker for cerebral injury in term newborn infants with hypoxic ischemic encephalopathy. Pediatr Res. 2004; 55: 406–412. https://doi.org/10.1203/01.PDR.0000106806.75086.D3 PMID: 14630979

32. Gazzolo D, Vinesi P, Bartocci M, Geloso MC, Bonacci W, Serra G, et al. Elevated S100 blood level as an early indicator of intraventricular hemorrhage in preterm infants. Correlation with cerebral Doppler velocimetry. J Neurol Sci. 1999; 170: 32–35. PMID: 10540033

33. Gazzolo D, Bruschettini M, Lituania M, Serra G, Bonacci W, Michetti F. Increased urinary S100B protein as an early indicator of intraventricular hemorrhage in preterm infants: correlation with the grade of hemorrhage. Clin Chem. 2001; 47: 1836–1838. PMID: 11568094

34. Tina LG, Frigiola A, Abella R, Tagliabue P, Ventura L, Paterlini G, et al. S100B protein and near infrared spectroscopy in preterm and term newborns. Front Biosci (Elite Ed). 2010; 2: 159–164.

35. Rogers LK, Graf AE, Bhatia A, Leonhart KL, Oza-Frank R. Associations between maternal and infant morbidities and sRAGE within the first week of life in extremely preterm infants. PLoS ONE. 2013; 8: e82537. https://doi.org/10.1371/journal.pone.0082537 PMID: 24324804

36. Sanchez-de-Toledo J, Chrysostomou C, Munoz R, Lichtenstein S, Sao-Avilés CA, Wearden PD, et al. Cerebral regional oxygen saturation and serum neuromarkers for the prediction of adverse neurologic outcome in pediatric cardiac surgery. Neurocrit Care. 2014; 21: 133–139. https://doi.org/10.1007/s12028-013-9934-y PMID: 24203460

37. Wunderlich MT, Hanhoff T, Goertler M, Spener F, Glatz JFC, Wallesch C-W, et al. Release of brain-type and heart-type fatty acid-binding proteins in serum after acute ischaemic stroke. J Neurol. 2005; 252: 718–724. https://doi.org/10.1007/s00415-005-0725-z PMID: 15834650

38. Pelsers MMAL, Hanhoff T, Van der Voort D, Arts B, Peters M, Ponds R, et al. Brain- and heart-type fatty acid-binding proteins in the brain: tissue distribution and clinical utility. Clin Chem. 2004; 50: 1568–1575. https://doi.org/10.1373/clinchem.2003.030361 PMID: 15217991

39. Teunissen CE, Veerhuis R, De Vente J, Verhey FRJ, Vreeling F, van Boxtel MPJ, et al. Brain-specific fatty acid-binding protein is elevated in serum of patients with dementia-related diseases. Eur J Neurol. 2011; 18: 865–871. https://doi.org/10.1111/j.1468-1331.2010.03273.x PMID: 21143341

40. Kok WF, Koerts J, Tucha O, Scheeren TWL, Absalom AR. Neuronal damage biomarkers in the identification of patients at risk of long-term postoperative cognitive dysfunction after cardiac surgery. Anaesthesia. 2016.

41. Inder T, Mocatta T, Darlow B, Spencer C, Volpe JJ, Winterbourn C. Elevated free radical products in the cerebrospinal fluid of VLBW infants with cerebral white matter injury. Pediatr Res. 2002; 52: 213–218. https://doi.org/10.1203/00006450-200208000-00013 PMID: 12149498

42. SAIF2. Isoprostanes as Biomarkers and Mediators of Oxidative Injury in Infantand Adult Central Nervous System Diseases. 2004;: 1–14.

43. Greco A, Minghetti L. Isoprostanes as biomarkers and mediators of oxidative injury in infant and adult central nervous system diseases. Curr Neurovasc Res. 2004; 1: 341–354. PMID: 16181083

44. Baik N, Urlesberger B, Schwaberger B, Schmölzer GM, Avian A, Pichler G. Cerebral haemorrhage in preterm neonates: does cerebral regional oxygen saturation during the immediate transition matter? Arch Dis Child Fetal Neonatal Ed. 2015.