Early Oxygen-Utilization and Brain Activity in Preterm Infants

Maria Luisa Tataranno1,2*, Thomas Alderliesten1*, Linda S. de Vries1‡, Floris Groenendaal1‡, Mona C. Toet1‡, Petra M. A. Lemmers1‡, René E. Vosse van de3‡, Frank van Bel1‡, Manon J. N. L. Benders1,4*

1 Dept. of Perinatology and Brain Center Rudolph Magnus, University Medical Center Utrecht, Utrecht, The Netherlands, 2 Dept. of Molecular and Developmental Medicine, University of Siena, Siena, Italy, 3 Dept. of Medical Technology and Clinical Physics, University Services, University Medical Center Utrecht, Utrecht, The Netherlands, 4 Centre for the Developing Brain, King’s College London, London, United Kingdom

☯ These authors contributed equally to this work.
‡ These authors also contributed equally to this work.
* m.benders@umcutrecht.nl

Abstract

The combined monitoring of oxygen supply and delivery using Near-InfraRed spectroscopy (NIRS) and cerebral activity using amplitude-integrated EEG (aEEG) could yield new insights into brain metabolism and detect potentially vulnerable conditions soon after birth. The relationship between NIRS and quantitative aEEG/EEG parameters has not yet been investigated. Our aim was to study the association between oxygen utilization during the first 6 h after birth and simultaneously continuously monitored brain activity measured by aEEG/EEG. Forty-four hemodynamically stable babies with a GA < 28 weeks, with good quality NIRS and aEEG/EEG data available and who did not receive morphine were included in the study. aEEG and NIRS monitoring started at NICU admission. The relation between regional cerebral oxygen saturation (rScO2) and cerebral fractional tissue oxygen extraction (cFTOE), and quantitative measurements of brain activity such as number of spontaneous activity transients (SAT) per minute (SAT rate), the interval in seconds (i.e. time) between SATs (ISI) and the minimum amplitude of the EEG in μV (min aEEG) were evaluated. rScO2 was negatively associated with SAT rate (β=-3.45 [CI=-5.76- -1.15], p=0.004) and positively associated with ISI (β=1.45 [CI=0.44-2.45], p=0.006). cFTOE was positively associated with SAT rate (β=0.034 [CI=0.009-0.059], p=0.008) and negatively associated with ISI (β=-0.015 [CI=-0.026--0.004], p=0.007). Oxygen delivery and utilization, as indicated by rScO2 and cFTOE, are directly related to functional brain activity, expressed by SAT rate and ISI during the first hours after birth, showing an increase in oxygen extraction in preterm infants with increased early electro-cerebral activity. NIRS monitored oxygenation may be a useful biomarker of brain vulnerability in high-risk infants.
Introduction

Neuro-monitoring tools such as Near-InfraRed Spectroscopy (NIRS) and amplitude integrated EEG (aEEG) are becoming part of daily clinical care in many neonatal intensive care units (NICUs) [1]. NIRS utilizes infrared light to monitor regional cerebral oxygen saturation (rScO2), in a mixed venous-capillary-arterial compartment [2]. NIRS can therefore be used to estimate cerebral oxygenation, and also as a surrogate for cerebral perfusion [2]. In addition, when combined with arterial oxygen saturation (SpO2), the cerebral fractional tissue oxygen extraction (cFTOE) can be calculated ((SpO2-rScO2)/SpO2), which can be used as an estimator of oxygen utilization of the brain [2,3]. On the other hand, aEEG is an excellent tool for continuous, non-invasive assessment of cerebral activity [1]. Routinely, aEEG tracings are classified based on the background pattern [4]. Recently, quantitative approaches for digital aEEG and raw EEG signals have been introduced [5]. These approaches classify spikes in cerebral activity as bursts or the equivalent spontaneous activity transients (SATs), which are likely to be crucial for brain development [6,7]. This classification enables the calculation of the intervals between bursts, called inter SATs intervals (ISI) and the SATs per minute (SAT rate) [8, 5]. Studies in humans have already shown that both the SAT rate and ISI, and quantitative aEEG parameters give valuable information about brain function of preterm infants during early phases of neonatal intensive care [8, 9, 10, 11, 12]. In addition, especially these aEEG variables have been shown to be associated with brain growth and development, and also with neurodevelopmental outcome [5, 8–10, 13]. Hence, the simultaneous assessment of NIRS and (a)EEG couples monitoring of oxygen supply and delivery to cerebral activity and could therefore yield new insights into brain metabolism and detect potentially vulnerable situations. In the past, studies on the combination of NIRS with aEEG/EEG monitoring, showed that a higher cFTOE was associated with a narrower aEEG bandwidth suggesting more oxygen utilization to meet higher metabolic demand in case of a more mature aEEG/EEG [14]. However, the relationship between NIRS and quantitative EEG parameters such as SAT rate or ISI has not yet been investigated. Therefore, the aim of the current study was to compare the pattern of oxygen delivery and utilization, as determined by NIRS, to the simultaneously acquired quantification of the cross-cerebral digital aEEG/EEG signal during the first 6 h after birth.

Materials and Methods

Patients

In this observational study, patients were selected from a larger longitudinal MRI study cohort of preterm infants with a GA < 28 weeks. MRI’s have been performed as standard clinical care at the Wilhelmina Children’s hospital between 2008 and 2013. Those babies did only receive serial MRI if they were clinically stable (n = 138) “Fig 1”.

Since we were interested in evaluating the physiological correlation between NIRS and aEEG/EEG parameters, only babies with good quality NIRS and aEEG/EEG data available were included in the study “Fig 1”. Permission from the medical ethical review committee of the University Medical Center Utrecht (MERC UMC Utrecht) for the MRI study was obtained. Patient data were anonymized prior to analysis. Since this was a retrospective study, using NIRS and aEEG/EEG monitoring as part of standard clinical care, no written consent or specific ethical approval was required. The MERC UMC Utrecht waived the need for parental consent for the use of medical data. Additional exclusion criteria were: chromosomal or congenital abnormalities, hemodynamic instability, and administration of morphine or other sedative drugs during the selected period [15]. Morphine administration before or during the study period was an exclusion criteria because it has been shown to cause suppression of the aEEG/EEG activity.
Likewise, infants who were hemodynamically instable such as infants with arterial blood pressure less than 10th percentile for birth weight or treated with inotropes during the study period were excluded, as inotropes can alter cerebral blood flow while presumably not affecting cerebral oxygen consumption [17]. Furthermore, neonates were excluded if they had either incomplete NIRS or aEEG/EEG data (minimum 3 hours of monitoring) during the first 6 hours after birth: 44 infants were eligible for the analysis. In all enrolled neonates, NIRS and aEEG monitoring started within 3 hours after birth. One hour of registration of the NIRS and aEEG/EEG signal was chosen between 4-6h postpartum and subsequently the relationship between NIRS and aEEG/EEG was evaluated. A small window was chosen, since the 1st hours after birth brain perfusion and metabolism undergo large changes to adapt to extra-uterine life [18]. Germinal matrix-intraventricular hemorrhage (GMH-IVH) was diagnosed according to the classification of Papile et al [19]. The first cranial ultrasound was routinely performed at admission, within 6 hours after birth and serially repeated till term equivalent age [20].

**NIRS**

A 2-wavelength (730 and 810 nm) near-infrared spectrometer (INVOS 4100–5100; Covidien, Mansfield, MA) was used. A transducer (small adult SomaSensor SAFB-SM; Covidien, Mansfield, MA) containing a light-emitting diode and 2 distant sensors (30 and 40mm) was positioned on the fronto-parietal side of the infant’s head and fixated with an elastic bandage to prevent displacement [2]. rScO2 was calculated from the differential signals obtained from these 2 sensors, expressed as the predominantly venous weighted percentage of oxygenated hemoglobin (oxygenated hemoglobin/total hemoglobin [oxygenated hemoglobin + deoxygenated hemoglobin]) [2, 21]. The rScO2 provides an absolute measure, and has the advantage over measuring oxygenated and deoxygenated hemoglobin of being less sensitive to patient movements. The rScO2 was recorded simultaneously with heart rate, arterial blood pressure, and arterial saturation on the right hand (SpO2). SpO2 was measured using Philips Intellivue MP70 patient monitor containing Nelcor technology. To investigate the balance between oxygen delivery and oxygen consumption, cFTOE was also calculated as (SaO2-rScO2)/SaO2 using an

---

**Fig 1.** Flow-chart of study population selection. P1 indicates 0–6 h after birth (study period).

doi:10.1371/journal.pone.0124623.g001
algorithm in the program. An increase in this parameter reflects increased oxygen extraction by brain tissue, whereas a decrease suggests less utilization or increased delivery of oxygen [3].

**aEEG monitoring**

Two-channel rawEEG and aEEG tracings were obtained simultaneously with the NIRS signal in all neonates using BrainZ cerebral function monitors (BRM2 or BRM3, Natus CA, Seattle, USA). Only the cross-sectional signal was used for analysis by subcutaneous needle electrodes in P3-P4 position with a central reference electrode to measure impedance. Needle electrodes were preferred, since they are more suitable for stable long-term recording. In addition, they usually have lower impedance compared to gel electrodes and less handling is required to maintain a good impedance following insertion of the needles. The P3-P4 cross-sectional signal was chosen since it has been proven to be a good predictor for neurodevelopmental outcome [8].

**Data analysis**

In house developed software (SignalBase; version: 7.8; University Medical Center Utrecht, Utrecht, The Netherlands) was used to perform the simultaneous post-processing of the NIRS and EEG data. In each patient a 1 hour epoch (P1) was manually selected between 4 and 6 hours after birth. The recorded aEEG/EEGs were assessed visually to identify marked artifacts, periods of high impedance, and events that were annotated by the nurses (e.g. care, blood sampling). Based on the aEEG, periods were chosen with a more continuous activity, including both active and quiet sleep, and free from suspected electrical discharges and artifacts. The NIRS data was evaluated in a similar way, resulting in the selection of 1h-epochs with “clean” NIRS and EEG/aEEG data. For the EEG/aEEG the following variables were calculated: number of SATs per minute (SAT rate) (rounded to the nearest whole number) also called “bursts” [6, 22], the interval in seconds (i.e. time) between SATs (ISI) (also called “interburst intervals”) [6], both derived from the raw EEG, and the minimum amplitude of the aEEG signal in μV (min aEEG).

The quantification of SATs was done using a nonlinear energy operator (NLEO) contained in the SignalBase software (5). EEG data were recorded at a sampling rate of 256 Hz. In the present cohort 7 patients were registered with BRM 2 and 37 with BRM 3 monitors. These devices had a filter setting of 2Hz and 0.5 Hz respectively. This difference implies a lower sensitivity of BRM2 monitors for low frequencies. To evaluate the possible bias of the different filter settings, 10 patients with BRM3 monitoring were randomly selected and re-sampled with a 2Hz filter. Results were then compared and no significant differences were found between aEEG/EEG variables during P1. The moments of intubation and surfactant administrations were avoided, since this was marked in the events [16, 23]. For the cFTOE calculation the involved signals are the arterial oxygen saturation and the regional oxygen saturation (NIRS). Both signals are first smoothed. This is done by applying two sub sequential filters. For each signal: the first filter is an averaging filter with a Chunk width of 50 seconds and a sample rate equal to the sample rate of its source (rectangular moving average’) and the second filter has a Chunk time of 36 seconds. The sample rate of the second filter is equal to the sample rate of the resulting signal. This will result in a Low pass filter of -6dB at 0.01 Hz and <-30 dB for averaging >0.02 Hz. The smoothed signals are then re-sampled with a re-sampling time of 1 second.

**Statistical analysis**

Clinical data are summarized as mean ± standard deviations (SD), percentages and absolute frequencies where appropriate. The association between NIRS (i.e. rScO2 and cFTOE) and aEEG/EEG parameters (i.e. SAT rate, ISI, min aEEG) was first visualized in dot plots. Correlations were checked using the Spearman correlation test (2-tailed). Afterwards a multivariable
linear regression analysis was performed, adjusting for GA, arterial pCO2 and hemoglobin. A \( p \) value <.05 was considered statistically significant.

Results and Discussion

Results

The selected epochs had a mean duration of (mean ±SD) 50 ± 8 min, this was manually selected between 4 and 6 h after birth and the rScO2, cFTOE and aEEG/EEG variables were computed. Simultaneously measured arterial blood pressure was always within normal values (mean blood pressure during the selected period: 32 ± 6 mm Hg). Data about arterial hemoglobin and pCO2 during the study period were also collected. None of the infants had hemoglobin values lower that 7 mmol/l (maximum value 12.5 mmol/l) during the study period (Table 1). Minimum and maximum value of pCO2 were respectively 30 and 60 mmHg. Seven infants developed GMH-IVH at any point in time, but only two of the seven infants showed a GMH-IVH during the first ultrasound examination (within 6 hours after birth), showing a grade III (Table 1). None of the patients was reported to have an abnormal resistance index at cerebral ultrasound. None of the babies experienced arterial oxygen saturation values lower than 88%

Table 1. Baseline clinical characteristics [mean (SD), n (%) or median (IR)] of studied infants.

| Clinical characteristics of the population (n = 44) |  |
|------------------------------------------------|---|
| GA, mean (SD) wks | 26.4 (1.0) |
| BW, mean (SD) g | 892 (155) |
| Gender |  |
| Male n (%) | 21 (47.7) |
| Female n (%) | 23 (52.3) |
| Apgar score |  |
| 1 min, median (IR) | 5 (3–6) |
| 5 min, median (IR) | 8(7–8) |
| 10 min, median (IR) | 8 (8–9) |
| GMH-IVH at any point in time |  |
| None, n (%) | 37 (84.1) |
| Grade I-II, n (%) | 3 (6.8) |
| Grade III, n (%) | 4 (9.1) |
| GMH-IVH during the first day of life |  |
| None, n (%) | 42 (95.5) |
| Grade I-II, n (%) | 0 (0) |
| Grade III, n (%) | 2 (4.5) |
| Initial respiratory support |  |
| SIMV or HFO, n (%) | 23 (52.3) |
| CPAP, n (%) | 21 (47.7) |
| Surfactant at any point in time n (%) | 30 (68.2) |
| Blood pressure |  |
| Systolic, mean (SD) mmHg | 41(6) |
| Diastolic, mean (SD) mmHg | 25 (6) |
| Mean blood pressure, mean (SD) mmHg | 30 (2) |
| Arterial blood gas analysis in P1 |  |
| pH, mean (SD) | 7.31 (0.05) |
| pCO2, mean (SD) mmHg | 43.8 (6.6) |
| Hb, mean (SD) mmol/L | 9.5 (1.3) |

GMH-IVH, germinal matrix hemorrhage-intraventricular hemorrhage (according to Papile et al. Ped. 1978); SIMV, synchronized intermittent mandatory ventilation; HFO, high frequency oscillatory ventilation; CPAP, continuous positive airway pressure; all values are referred to P1: 0-6h after birth unless otherwise stated.

doi:10.1371/journal.pone.0124623.t001
or seizures during the selected period. None of the patients were small for GA or was intrauterine growth restricted (p < 0.3).

The relationship between NIRS monitored rScO2 and cFTOE and aEEG/EEG measurements. Brain activity significantly changed with GA, with higher SAT rate in infants with a higher GA (Table 2). The rScO2 did not change with GA and was negatively associated with SAT rate and min aEEG (p < 0.01 and p < 0.05 respectively) and positively with ISI (p < 0.01) (“Fig 2” panels A, E, and C respectively). cFTOE showed a significant positive association with SAT rate (p < 0.01) and min aEEG (p < 0.05) and a negative association with ISI (respectively: p < 0.01) (“Fig 2” panels B, F and D resp).

In the multivariable analysis, correcting for GA, arterial pCO2 and hemoglobin the rScO2 and cFTOE were independently related to SAT rate and ISI. In particular rScO2 was negatively associated with SAT rate ($\beta = -3.45$ [CI = -5.76 - -1.15], p = 0.004) and positively related to ISI ($\beta = 1.45$ [CI = 0.44 - 2.45], p = 0.006). In addition cFTOE was found to be positively associated with SAT rate ($\beta = 0.034$ [CI = 0.009 - 0.059], p = 0.008) and negatively associated with ISI ($\beta = -0.015$ [CI = -0.026 - -0.004], p = 0.007) (Table 3). When repeating the analysis excluding the 7 infants who showed a GMH-IVH at any point in time (excluding also the two babies who showed a GMH-IVH within 6 hours after birth) the results did not change. Hemoglobin and pCO2 were not significantly associated to NIRS variables.

Discussion

Our study suggests a higher metabolism, as indicated by lower rScO2 and increased cFTOE, during increased brain activity, as indicated by SAT rate independently of GA, hemoglobin and pCO2 values. Consistently with these findings, O2 delivery (rScO2) was higher and O2 extraction (cFTOE) was lower during decreased brain activity (ISI). These results are in agreement with previous studies showing an increased cFTOE with more mature electro-cortical activity [14]. However, our results extend previous findings because we used a new objective quantitative digital measurement of early brain activity based on automatic detection of SAT rate and ISI, overcoming all the subjective aEEG/EEG measurements [5]. To our knowledge, this is the first study focusing on the relation between NIRS and quantitative digital aEEG measurements in the first 6 hours after birth. During this transitional period, brain perfusion and metabolism undergo large changes to adapt to extra-uterine life [18]. Ter Horst and colleagues speculated that the higher cFTOE reflects higher cerebral oxygen extraction and consumption [14]. However, the increase in cFTOE can also be related to impaired cerebral blood flow (CBF) and decreased oxygen delivery but in the latter case we would have likely observed suppression of brain electrical activity.

In contrast to our findings, ter Horst and colleagues did not find any relationship between aEEG/EEG and rScO2. We speculate that the negative association between rScO2 and increased

| Table 2. Mean NIRS and aEEG/EEG values during the study period. |
|---------------------------------------------------------------|
| NIRS and aEEG/EEG variables                                   | Total population |
|---------------------------------------------------------------|
| rScO2, mean (SD) %                                            | 65(9)            |
| cFTOE, mean (SD)                                              | 0.31(0.09)       |
| SAT rate, mean (SD) min                                       | 5.5 (1.1)*       |
| ISI, mean (SD) sec                                            | 5.1 (2.6)        |
| Min aEEG, mean (SD) μV                                        | 4 (1)            |

*SAT rate was significantly related to GA (r = 0.31, p < 0.05 in relation to GA)

doi:10.1371/journal.pone.0124623.t002
brain activity (SAT rate) was due to a higher oxygen use caused by an increased metabolism demonstrated also by the increased cFTOE and the related higher electro-cerebral activity. Yoxall and colleagues reported that increased metabolism is accompanied by an increase in cerebral oxygen consumption and consequently by an increase in CBF as part of the so called
neurovascular coupling [24]. Recently, a significant association between superior vena cava flow and aEEG at 12 h after birth was reported. Interestingly, infants with low superior vena cava flow had significantly lower min aEEG at 12 h as compared with those with normal flow [25]. Thus, hemodynamic changes and especially changes in CBF that occur immediately after birth may affect cerebral circulation and also neuronal activity as shown in our results.

Kissack and colleagues demonstrated that hemodynamic responses to neuronal activation are not fully developed in the neonatal brain, compared with the adult brain. In extremely preterm infants there is no correlation between CBF and spontaneous changes in the cerebral metabolic rate of oxygen during the first 48 h after birth; instead, cFTOE changes rather than CBF to meet changes in oxygen requirement [26, 27]. On the other hand, some old studies demonstrated that basically the decrease in rScO2 and the related increase in O2 extraction (cFTOE) means a higher metabolism, which is reported to happen with increased fetal age, straight forward physiology [28]. Another study from Arichi T et al. comparing the BOLD signal response in preterm infants, term infants, and healthy adults showed decreased response time and increased signal amplitude with increasing postnatal age, suggesting that in young infants the increase in cerebral oxygen consumption may be relatively greater than the corresponding increase in CBF during functional activation [29].

We did not find any association between pCO2 or hemoglobin and NIRS variables. This suggests that within normal ranges, they do not highly influence oxygen delivery and extraction in preterm infants.

We found that SAT rate increased with increasing GA already within a few hours of extrauterine life followed by a simultaneous increase in FTOE and a decrease in rScO2. Previous studies, mainly focusing on weekly aEEG recordings, have already shown the maturational effect of GA on brain activity [30,31, 32].

An example of how this combined monitoring of NIRS and aEEG/EEG can be a useful indication for clinicians to focus on preserving oxygen supply is represented by GMH-IVH in preterm infants. In the presence of a GMH-IVH the background activity of the EEG/aEEG is depressed during the first days after birth, and the extent of the depression correlates with the degree of GMH-IVH [33, 34]. Recently Alderliesten et al demonstrated that higher rScO2 and lower cFTOE values were observed before brain injury became apparent and these changes were highly indicative for subsequent development of a severe GMH-IVH [35, 36]. Thus, the combined monitoring of cerebral oxygen delivery/utilization and brain activity can be useful for early identification of infants at risk of developing a GMH-IVH and these changes in continuous monitoring are seen before the injury became visible on ultrasound examination [35, 37]. In our study only 7 babies developed a GMH-IVH and none of them showed parenchymal involvement, thus we could not perform a separate analysis for this group of patients, but the relation between NIRS and aEEG was still present also after excluding those patients.

A possible limitation of the study is that NIRS signal was recorded in the fronto-parietal area while aEEG was recorded between P3-P4 electrodes. These areas are separated by 2 to 3

| Table 3. Multivariable linear regression analysis. |
|--------------------------------------------------|
| rScO2 | cFTOE | GA | pCO2 | Hb |
|       | B     | CI       | B   | CI       | B     | CI       | B   | CI       | B   | CI       |
| SAT rate | -3.45* | [-5.76;-1.15] | 0.034* | [0.009;0.059] | 88 | [-1.90;3.67] | -0.24 | [-0.635;1.153] | 1.34 | [-0.647;3.331] |
| ISI | 1.71* | [0.70;2.72] | -0.018* | [-0.028;-0.007] | -15 | [-2.91;2.59] | -0.39 | [-0.802;0.005] | 1.49 | [-0.429;3.421] |
| Min aEEG | -1.97 | [-4.85;0.91] | 0.02 | [0.008;0.052] | -17 | [-3.15;2.80] | 0.28 | [-0.724;0.149] | 1.42 | [-0.834;3.679] |

*p<0.01 adjusted for GA, pCO2, hemoglobin (Hb)

doi:10.1371/journal.pone.0124623.t003
The aim of the current study was to compare a measure of global brain activity to a measure of global brain perfusion/oxygenation. The P3-P4 (a)EEG electrodes positions are known to be representative for measuring global brain activity and no major differences were found between seizures detection with cross-sectional P3-P4 electrodes and two-channel aEEG [38, 39]. Furthermore frontal-parietal NIRS has been shown to correlate with measures of global cerebral perfusion [40]. Moreover, indices of cerebral oxygenation as measured by NIRS in preterm infants are quite comparable between regions [41].

Furthermore NIRS is known to be influenced by different compartments (arterial, capillary, venous), which can be affected by declivity. In our NICU incubators are set at similar declivity for every patient. Regarding head position in particular, the NIRS sensor was always placed on the front parietal side on which the infant was not lying at the moment. So the placement in terms declivity/head position has been very uniform among the infants in this study. Furthermore in 2010 Ancora and colleagues showed that hemodynamic changes after posture variations depend on GA and no statistically significant differences were found in CFTOE and rScO₂ in hemodinamically stable extremely preterm newborns with different postures [42].

Finally we found that oxygen consumption increases considerably with increasing activity anyway we would not suggest to sedate those babies more, since that would decrease brain activity, which is essential for brain development [43].

Conclusions
In conclusion, our study shows how oxygen utilization, as indicated by rScO₂ and cFTOE, is directly related to parameters quantifying brain activity, as indicated by SAT rate and ISI in the immediate neonatal period. This combination of NIRS and aEEG simultaneous monitoring and consequently of rScO₂/cFTOE and electrocerebral activity may be a noninvasive useful biomarker of brain function in high-risk, hemodynamically stable infants and could therefore yield insight in brain metabolism and detect potentially vulnerable conditions.

Acknowledgments
Dr. Manon Benders and prof. Frank van Bel had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Author Contributions
Conceived and designed the experiments: MLT FvB MJB. Performed the experiments: MLT TA. Analyzed the data: MLT LdV FG PL TA MT. Contributed reagents/materials/analysis tools: MLT RvdV FG. Wrote the paper: MLT MJB FvB LdV.

References
1. Toet MC, Lemmers PM (2009) Brain monitoring in neonates. Early Hum Dev 85(2):77–84. doi: 10.1016/j.earlhumdev.2008.11.007 PMID: 19150756
2. Van Bel F, Lemmers PM, Naulaers G (2008) Monitoring neonatal cerebral oxygen saturation in clinical practice: value and pitfalls. Neonatology 94:237–244. doi:10.1159/000151642 PMID: 18784420
3. Naulaers G, Meyns B, Miserez M, Leunens V, Van Huffel S, Casaer P et al.(2007) Use of tissue oxygenation index and fractional tissue oxygen extraction as non-invasive parameters for cerebral oxygenation. A validation study in piglets. Neonatology 92:120–126. PMID: 17377413
4. Hellström-Westas L, Rosén I, de Vries LS, Greisen G (2006) Amplitude-integrated EEG. Classification and interpretation in preterm and term infants. NeoReviews 7:e76–e87.
5. Palmu K, Stevenson N, Wikström S, Hellström-Westas L, Vanhatalo S, Palva JM (2010) Optimization of an NLEO-based algorithm for automated detection of spontaneous activity transients in early preterm EEG. Physiol Meas 31:85–93.
6. Vanhatalo S, Kaila K (2006) Development of neonatal EEG activity: from phenomenology to physiology. Semin Fetal Neonatal Med 11:471–478. PMID: 17018268
7. Vanhatalo S, Kaila K (2009) Spontaneous and evoked activity in the early human brain. The Newborn Brain: Neuroscience & Clinical Applications 2nd edition, Cambridge University Press.
8. Wikström S, Pupp IH, Rosén I, Normal E, Fellman V, Ley D et al. (2012) Early single-channel aEEG/EEG predicts outcome in very preterm infants. Acta Paediatr 101(7):719–726. doi: 10.1111/j.1651-2227.2012.02677.x PMID: 22530996
9. Klebmerass K, Olsch M, Waldrohfer T, Fuiko R, Pollak A, Weninger M (2011) Amplitude-integrated EEG pattern predicts further outcome in preterm infants. Pediatr Res 70:102–108. doi: 10.1038/pr.2011.327 PMID: 21436758
10. Wikström S, Ley D, Hansen-Pupp I, Rosén I, Hellström-Westas L (2008) Early amplitude-integrated EEG correlates with cord TNF-alpha and brain injury in very preterm infants. Acta Paediatr 97:915–919. doi: 10.1111/j.1651-2227.2008.00787.x PMID: 18462469
11. Kidokoro H, Okumura A, Hayakawa F, Kato T, Maruyama K, Kubota T et al. (2009) Chronologic changes in neonatal EEG findings in periventricular leukomalacia. Pediatrics 124:e468–e475. doi: 10.1542/peds.2008-2967 PMID: 19706584
12. Hellström-Westas L, Rosén I (2006) Continuous brain-monitoring: state of the art in clinical practice. Semin Fetal Neonatal Med 11:503–511. PMID: 17067863
13. West CR, Harding JE, Williams CE, Nolan M, Battin MR (2011) Cot-side electroencephalography for outcome prediction in preterm infants: observational study. Arch Dis Child Fetal Neonatal Ed 96:F108–F113.
14. Ter Horst HJ, Verhagen EA, Keating P, Bos AF (2011) The relationship between electrocerebral activity and cerebral fractional tissue oxygen extraction in preterm infants. Pediatr Res 70:384–388. doi: 10.1038/pr.2011.609 PMID: 21691247
15. Norman E, Wikström S, Rosén I, Fellman V, Hellström-Westas L (2013) Premedication for intubation with morphine causes prolonged depression of electrocortical background activity in preterm infants. Pediatr Res 73:87–94. doi: 10.1038/pr.2012.153 PMID: 23128421
16. Van den Berg E, Lemmers PM, Toet MC, Klaessens JH, van Bel F (2010) Effect of the InSure procedure on cerebral oxygenation and electrical brain activity of the preterm infant. Arch Dis Child Fetal Neonatal Ed 95:F53–58. doi: 10.1136/adc.2008.156414 PMID: 19679893
17. Hahn GH, Hyttel-Sorensen S, Petersen SM, Pryds O, Greisen G (2013) Cerebral effects of commonly used vasopressor-inotropes: a study in newborn piglets. PLoS One 8:e63069. doi: 10.1371/journal.pone.0063069 PMID: 23700412
18. Takami T, Sunohara D, Kondo A, Mizukaki N, Suganami Y, Takey Y et al. (2010) Changes in cerebral perfusion in extremely LBW infants during the first 72h after birth. Pediatr Res 68:435–439. doi: 10.1203/PDR.0b013e3181f2bd4d PMID: 20657347
19. Papile LA, Burstein J, Burstein R, Koffler H (1978) Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1500gm. J Pediatr 92:529–534.
20. Vanhatalo S, Palva JM, Andersson S, Rivera C, Voipio J, Kaila K (2005) Slow endogenous activity tran- sient and developmental expression of K = -Cl- costransporter 2 in the immature human cortex. Eur J Neurosci 22:2799–2804. PMID: 16324114
21. Edwards AD, Wyatt JS, Richardson C, Delpy DT, Cope M, Reynolds EO (1988) Cot-side measurement of cerebral blood flow in ill newborn infants by near infrared spectroscopy. Lancet 2:770–771.
22. Vanhatalo S, Palva JM, Andersson S, Rivera C, Voipio J, Kaila K (2005) Slow endogenous activity tran- sient and developmental expression of K = -Cl- costransporter 2 in the immature human cortex. Eur J Neurosci 22:2799–2804. PMID: 16324114
23.Hellström-Westas L, Bell AH, Skov L, Greisen G, Svenningsen NW (1992) Cerebroelectrical depression following surfactant treatment in preterm neonates. Pediatrics 89:643–647. PMID: 1557244
24. Yoxall CW, Weindling AM (1998) Measurement of cerebral oxygen consumption in the human neonate using near infrared spectroscopy: cerebral oxygen consumption increases with advancing gestational age. Pediatr Res 44:293–290. PMID: 9727702
25. Shah D, Paradisi M, Bowen JR (2013) Relationship between systemic blood flow, blood pressure, inotropes, and aEEG in the first 48h of life in extremely preterm infants. Pediatr Res 74:314–320.
26. Kissack CM, Garr R, Wardle SP, Weindling AM (2004) Postnatal changes in cerebral oxygen extraction in the preterm infant are associated with intraventricular hemorrhage and hemorrhagic parenchymal in- farction but not periventricular leukomalacia. Pediatr Res 56: 111–116. PMID: 15152052
27. Brew N, Walker D, Wong FY (2014) Am J Physiol Regul Integr Comp Physiol 306(11):R773–786. doi: 10.1152/ajpregu.00487.2013 PMID: 24647591
28. Szymonowicz W, Walker AM, Cussen L, Cannata J, Yu VY (1988) Developmental changes in regional cerebral blood flow in fetal and newborn lamb. *Am J Physiol.* 254(1Pt2):h25–28. PMID: 2827524

29. Arichi T, Fagiol G, Varela M, Melendez-Calderon A, Alliavi A, Merchant N et al. (2012) Development of BOLD signal hemodynamic responses in the human brain. *NeuroImage* 63: 663–673. doi: 10.1016/j.neuroimage.2012.06.054 PMID: 22776460

30. Sisman J, Campbell DE, Brion LP (2005) Amplitude-integrated EEG in preterm infants: maturation of background pattern and amplitude voltage with postmenstrual age and gestational age. *J Perinatol* 25:391–396. PMID: 15815708

31. Klebermass K, Kuhle S, Olischar M, Rücklinger E, Pollak A (2006) Intra-and extraterine maturation of amplitude-integrated electroencephalographic activity in preterm infants younger than 30 weeks of gestation. *Biol Neonate* 89:120–125. PMID: 16219998

32. Herbert S, Pulzer F, Gebauer C, Panhofer M, Robel-Tillig E, Knüpfer M (2006) The effect of maturation and sedation on amplitude-integrated electroencephalogram of the preterm neonate: results of a prospective study. *Acta Paediatr* 95:1394–1399. PMID: 17062466

33. Hellström-Westas L, Klette H, Thorngren-Jerneck K, Rosén I (2001) Early prediction of outcome with aEEG in preterm infants with large intraventricular hemorrhages. *Neuropediatrics* 32:319–324. PMID: 11870588

34. Olischar M, Klebermass K, Waldoer T, Pollak A, Weninger M (2007) Background patterns and sleep-wake cycles on amplitude-integrated electroencephalography in preterm younger than 30 weeks gestational age with peri-/intraventricular haemorrhage. *Acta Paediatr* 96:1743–1750. PMID: 17971193

35. Alderliesten T, Lemmers PM, Smarius JJ, van de Vosse RE, Baerts W, van Bel F (2013) Cerebral oxygenation, extraction, and autoregulation in very preterm infants who develop per-intraventricular hemorrhage. *J Pediatr* 162:698–704.

36. Connell J, de Vries LS, Oozeer R, Regev R, Dubowitz LM, Dubowitz V (1988) Predictive value of early continuous electroencephalogram monitoring in ventilated preterm infants with intraventricular hemorrhage. *Pediatrics* 82:337–343.

37. Noori S, McCoy M, Anderson MP, Ramji F, Seri I (2014) Changes in cardiac function and cerebral blood flow in relation to per/intraventricular hemorrhage in extremely preterm infants. *J Pediatr* 164:264–270. doi: 10.1016/j.jpeds.2013.09.045 PMID: 24183212

38. Shah NA, Wusthoff CJ (2014) How to use: amplitude-integrated EEG (aEEG). *Arch Dis Child Educ Pract Ed* pii: edpract-2013-305676.

39. van Rooj LG (2010) Additional value of two-channel amplitude integrated EEG recording in full-term infants with unilateral brain injury *Arch Dis Fetal Neonatal Ed* 95(3):F160–168 doi: 10.1136/adc.2008.156711 PMID: 19815938

40. Wintermark P, Hansen A, Warfield SK, Dukhovny D, Soul JS (2014) Near-infrared spectroscopy versus magnetic resonance imaging to study brain perfusion in newborns with hypoxic-ischemic encephalopathy treated with hypothermia. *Neuroimage* 85 Pt 1:287–293. doi: 10.1016/j.neuroimage.2013.04.072 PMID: 23631990

41. Wijbenga RG, Lemmers PM, van Bel F (2011) Cerebral oxygenation during the first days of life in preterm and term neonates: differences between different brain regions. *Ped Res* 70(4):389–394.

42. Ancora G, Maranella E, Aceri A, Pierantoni L, Grandi S, Corvaglia L et al. (2010) Effect of posture on brain hemodynamics in preterm newborns not mechanically ventilated. *Neonatology* 97(3):212–217 doi: 10.1159/000253149 PMID: 19887848

43. Benders MJ, Palmu K, Menache C, Borradoti-Tolsa C, Laheyras F, Sizonenko S et al. (2014) Early Brain Activity Relates to Subsequent Brain Growth in Premature Infants. *Cereb Cortex* pii: bhu097.