Quantitative approach to early neonatal EEG visual analysis in hypoxic-ischemic encephalopathy severity: Bridging the gap between eyes and machine

Laure Lacan, Nacim Betrouni, M. D. Lamblin, L. Chaton, Arnaud Delval, J. L. Bourriez, Laurent Storme, Philippe Derambure, Sylvie Nguyen The Tich

Original Article

QUANTITATIVE APPROACH TO EARLY NEONATAL EEG VISUAL ANALYSIS IN HYPOXIC-ISCHEMIC ENCEPHALOPATHY SEVERITY: BRIDGING THE GAP BETWEEN EYES AND MACHINE

Short title: QUANTITATIVE EEG IN NEONATAL HYPOXIC-ISCHEMIC ENCEPHALOPATHY

Laure Lacan[a,b,], Nacim Betrouni[c,d], Marie-Dominique Lamblin[d], Laurence Chaton[c,d], Arnaud Delval[c,d], Jean-Louis Bourriez[c,d], Laurent Storme[a], Philippe Derambure[c,d], Sylvie NguyenThe Tich[a,b]*

[a] EA4489, Environnement périnatal et santé, Faculté de médecine, Université de Lille, 2 Avenue Eugène Avinée, 59120 Loos, France

[b] Service de Neurologie pédiatrique, Hôpital Roger Salengro, CHRU de Lille, Avenue du Professeur Emile Laine, 59037, France

[c] Inserm U 1172, Faculté de médecine, Université de Lille, 2 Avenue Eugène Avinée, 59120 Loos, France

[d] Service de Neurophysiologie Clinique, Hôpital Roger Salengro, CHRU de Lille, Avenue du Professeur Emile Laine, 59037, France

Corresponding author: Laure Lacan – laure.lacan@chru-lille.fr – +33(0)3 20 44 40 57 –
Service de neurologie pédiatrique, Hôpital Roger Salengro, Avenue du Professeur Emile Laine, CHRU, 59037 Lille, France

ABSTRACT

Objectives: To identify relevant quantitative parameters for early classification of neonatal hypoxic-ischemic encephalopathy (HIE) severity from conventional EEGs.

Methods: Ninety EEGs, recorded in full-term infants within 6 hours of life after perinatal hypoxia, were visually classified according to the French EEG classification into three groups of increasing HIE severity.
Physiologically significant EEG features (signal amplitude, continuity and frequency content) were automatically quantified using different parameters. The EEG parameters selection was based on their ability to reproduce the visual EEG classification. Post hoc analysis based on clinical outcome was performed.

Results: Six EEG parameters were selected, with overall EEG classification performances between 61% and 70%. All parameters differed significantly between group 3 (severe) and groups 1 (normal-mildly abnormal) and 2 (moderate) EEGs ($p<0.001$). Amplitude and discontinuity parameters were different between the 3 groups ($p<0.01$) and were also the best predictors of clinical outcome. Conversely, pH and lactate did not differ between groups.

Discussion: This study provides quantitative EEG parameters that are complementary to visual analysis as early markers of neonatal HIE severity. These parameters could be combined in a multiparametric algorithm to improve their classification performance. The absence of relationship between pH lactate and HIE severity reinforces the central role of early neonatal EEG.

Keywords: French classification; HIE; neonatal EEG; perinatal asphyxia; quantitative EEG

INTRODUCTION

With reported incidences of 3–5 per 1000 term live births, perinatal asphyxia remains a common cause of neonatal death and long-term disabilities [27,47]. Mild therapeutic hypothermia (TH) is now the standard of care for full-term neonates with moderate to severe encephalopathy [5, 37]. Whole body cooling to a temperature between 32–34°C for 72 hours has been shown to improve survival and neurodevelopmental outcome, especially when started within 6 hours after delivery [4,22,41]. Hence, early detection of moderate or severe neonatal hypoxic-ischemic encephalopathy (HIE) is essential for TH initiation.

The degree of cerebral injury after perinatal hypoxia is commonly evaluated by clinical assessment, using scores such as the Sarnat score, which includes EEG in its original description [38]. Numerous studies have demonstrated that conventional EEG (cEEG) provides an early and reliable evaluation of cerebral injury, including in newborns undergoing hypothermia [18,31,33,34]. Visual analysis of EEG background activity remains the gold standard method for grading HIE and several classification systems are proposed [48]. The French EEG classification (Table A.1), adapted from Scavone and Pezzani [33,39], considers four severity grades (normal, mild, moderate and severe abnormalities) that correlate with long-term prognosis [24,25,26].

However, visual analysis can sometimes be discordant between interpreters, is time-consuming and requires a qualified neurophysiologist available around the clock, which may be challenging in some neonatal intensive care units (NICU). Simplified EEG analysis tools such as amplitude-integrated EEG (aEEG) are commonly used. Nevertheless, visual interpretation of aEEG is also a highly skilled task and remains user-dependant [42]. Some studies have proposed algorithms for the automatic classification of HIE severity, based on the combination of different EEG quantitative features [2,13,29,45,46] but the clinical implementation of these methods is still difficult since they require specific software platforms to compute these features. Thus, there is a lack of a simple and reliable EEG tool capable of reproducing the HIE visual classification and usable as a diagnostic support in clinical practice.

The aim of this study was to identify quantitative EEG parameters that are relevant in reproducing the French visual EEG classification in early neonatal EEGs.

METHODS

Database

Inclusion

EEGs recorded between 2013 and 2017 during the first six hours of life after perinatal asphyxia in newborns admitted to our neonatal intensive care unit (NICU) were retrospectively analysed.

Inclusion criteria were as follows: gestational age ≥ 36 weeks; birth weight > 1800 g; post-natal age ≤ H6; clinical suspicion of HIE with at least one of the following criteria: pH ≤ 7 or base deficit ≥ 16 mmol/L or lactate ≥ 11 mmol/L before 1 hour of life (measured in umbilical cord, capillary, venous or arterial blood); or Apgar score ≤ 5 at 10 minutes of life, or ventilatory support (intubation or mask ventilation) needed at birth and continued at 10 min of life. If another cause of metabolic acidosis or encephalopathy was suspected, such as congenital heart disease or inborn errors of metabolism, the EEG was excluded, as were unreadable EEG traces. One EEG per infant was retained for analysis. The clinical status at the time of EEG was noted, including body temperature, use of sedative, analgesic or anticonvulsant drugs, intubation and mechanical ventilation.

EEGs that met these criteria (n=143) were visually classified into three groups according to their HIE severity grade (normal or mildly abnormal, moderate and severe EEGs), as explained below. The first 30 consecutive EEGs were retained in each group in order to obtain three groups of equal size (Fig 1), i.e., 90 EEGs.

Clinical follow-up was systematically proposed, and clinical data were collected from medical visits reports. Neurological assessment was graded at one and three years of age using a score adapted from the International Classification of Functioning, Disability and Health (ICF) classification (Table A.2). For the analyses, children were separated into two groups of good and poor outcomes, the latter being defined as significant sequelae (score ≥ 3) at age three years, or death.

Ethics

The study was declared to the National Commission of Information Technology and Liberties (agreement reference: DEC-20 138). The parents were informed by a written document of the anonymized use of data and non-opposition consent was obtained.

EEG recordings

EEGs were recorded using the Micromed EEG System® (Micromed, Mâcon, France). They were started as soon as possible after birth on the basis of a 24/7 availability. Eight scalp electrodes (F4, C4, T4, O2, F3, C3, T3, O1), one ground electrode and one reference electrode were positioned

according to the international 10:20 electrode placement system, modified for neonates. After scalp cleaning, Nicolet® cup electrodes (Natus, Paris, France) were fixed using adhesive paste and secured by a net. The electrode impedance was maintained below 5 kOhm. The EEG signal was digitised at a sampling rate of 256 Hz. They were stored on a computer hard drive and read on the System Plus Evolution Software® (Micromed). ECGs were concomitantly recorded and stored on the same disk.

Visual EEG classification

EEGs were classified on the basis of the initial neurophysiological report confirmed by independent analysis by the authors (LL, MDL) blinded to clinical data. Inter-observer agreement between the initial report and the second neurophysiological analysis was high (kappa = 0.9 [0.83–0.97]). In case of disagreement, a third neurophysiologist (LC) read the EEG blinded to clinical data and to the previous interpretations and assessed the final EEG grade.

The visual analysis was conducted on a bipolar display montage, with 0.53–70 Hz digital filters, an amplitude of 100 µV/cm and a display of 30 mm/sec. HIE severity was assessed using the French classification in grade from 0 to 4 (Table A.1). Grades 0 and 1 were both included in group 1 (normal or mildly abnormal EEGs). Groups 2 and 3 corresponded respectively to moderate (grade 2) and severe (grade 3) EEGs (Fig. 2). Spikes and seizures (defined as a sudden, repetitive, stereotyped discharge lasting at least 10 seconds [10]) were noted. For each EEG, artefact periods were marked and a set of "artefact-free" EEGs was constituted by removing these periods from the trace.

Quantitative EEG analysis

Six EEG features proposed in the program "EEG Analyser" (Micromed) and reflecting the amplitude, continuity and frequency content of the signal, were used for quantitative analysis:

- *Minimal and Maximal Amplitude Index (MinAI, MaxAI)* represent the minimal and maximal peak of amplitude during a time interval (µV). The amplitude was measured using CFM after filtering the signal at 2–20 Hz.
- *Absolute Total Power (ATP)* was calculated using a Fourier transform as the total spectral power over the range 0.5–19.5 Hz (μV^2).
- *Spectral Edge Frequency (SEF)* was defined as the frequency (Hz) below which 95% of the power in EEG exists [20].
- *Relative Low Frequency Power (RLFP)* was the ratio of the spectral power in low frequencies (0.5–5 Hz) on the absolute total power (%).
- *Burst Suppression Ratio (BSR)* was measured as the ratio of the Interburst Interval (IBI)'s length (in seconds) on the total length of the analysed interval (%). IBIs were detected for an amplitude of less than 5 µV during more than 500 ms.

These six features were measured on C3-C4 derivation every two seconds over the entire tracing. Mean, median, minimal and maximal values and variation coefficient were considered for each feature over the analysed period (i.e., 30 quantitative parameters in total).

Data analysis

Data descriptive analysis

Demographic, clinical and EEG characteristics were described by the mean and standard deviation or the median and interquartile range for quantitative variables, and by frequency and percentage for qualitative variables. Differences between the 3 groups were analysed by a Kruskal-Wallis test for quantitative variables and chi-squared for qualitative variables.

EEG parameters selection

Logistic regression analyses, adjusted for the significant covariates from the previous analyses, were first run as a parameter-selection strategy. A second selection round for the parameters with significant results from the regression analyses was applied by taking into account the performance of EEG classification (global performance and specifically for the critical grade 2) and importance of each parameter, tested using a random forest classifier with calculation of the precision decay when the variable is removed from the model.

Lastly and in order to consider the influence of artefacts, the same analyses were repeated in the second set of “artefact-free” EEGs after manual removal of artefact periods. Only parameters with robust performances on artefact and artefact-free traces were retained.

Post-hoc analysis

Differences between the three groups for each selected EEG parameter were then verified by post-hoc analysis. Kruskal-Wallis test followed by pairwise comparisons using Dunn tests were used.

The quantitative data were also analysed in relation to clinical outcome (good or poor) at three years of age. The performance of each parameter in predicting the clinical outcome was determined using ROC (receiver operating characteristic) curve analysis.

All the analyses were run using XlStat add-on for Microsoft Excel (AddInSoft, Paris, France). The significance level was set at p-value < 0.05. Corrections for multiple comparisons were done using Bonferroni correction.

RESULTS

Characteristics of the population

The demographic and perinatal characteristics of the 90 infants were not different (including for laboratory results), except for the Apgar score at 10 minutes, which was lower in group 3 (Table 1a).

EEGs were recorded soon after birth (3.6 ± 1.2 hours of life) for a mean duration of 50 minutes (50.8 min ± 19.2 [22–175]). Recording conditions differed for group 3 (most severe EEGs) with 90% of patient intubated and 86% in passive hypothermia at the time of EEG. There was no difference in the presence of sedative, anticonvulsant or analgesic drugs between the 3 groups (Table 1b).

Thirteen neonates died during the neonatal period. Among the 77 surviving infants, 71 (92%) were followed up at one year and 69 (90%) at three years. All the children in group 1 had a normal outcome at one and three years of age. Four infants in group 2 (15% of the available data) had a poor outcome at three years of age (death: n=2, major sequelae: n=2), as well as 14 (54%) infants in group 3 (death: n=11, major sequelae: n=3) (Table 1c).

EEG parameters selection

Logistic regression analysis was adjusted for body temperature at the time of EEG recording, as this covariate was significantly associated with EEG groups (Table 1a). Of the 30 quantitative parameters tested, 18 were significantly different between the 3 groups ($p_{corrected} = 0.0016$). Comparison between raw EEGs and "artefact-free" EEGs removed 7 parameters that appeared to be highly sensitive to artefacts. Among the 11 remaining parameters, four were retained that performed best in logistic regression and in the random forest model: the mean value of the Minimal Amplitude Index (MinAI [mean]), the minimal values of the Maximal Amplitude Index (MaxAI [min]) and of the Absolute Total Power (ATP [min]), the maximal value of the Burst Suppression Ratio (BSR [max]). Two variables were added to the selection because of their low correlation with the others: the variation coefficient of the Relative Low Frequency Power (RLFP [var]) and the maximal value of the Spectral Edge Frequency (SEF [max]). A total of six parameters were finally selected. For each parameter, the overall EEG classification performance (61-70%), the classification performance of EEG grades 2 (43-50%) in logistic regression analysis and precision decay when removed from the random forest model (1.4-9.1) are presented in Table 2.

Post-hoc analysis

In relation to HIE severity groups

The values of the six selected parameters are presented by group in the Table 3. The six parameters differed significantly between group 3 and groups 1 and 2 EEGs ($p<0.001$). The mean value of the Minimal Amplitude Index and the maximal value of the Burst Suppression Ratio were different between the three groups ($p<0.01$) (Fig.3).

In relation to long-term prognosis

When EEGs were analyzed regarding the clinical outcome, independently from initial EEG group, it was observed that EEGs of children with a good outcome at three years of age were characterized by high amplitude and absolute total power values (MinAI, MaxAI, ATP). These children also had low BSR, RLFP and SEF values (Table 4). The areas under the ROC curves (AUC), reflecting the performance of each parameter in predicting clinical outcome, were all above 75% (Fig. 4).

DISCUSSION

This study provides quantitative data that is complementary to visual analysis in early neonatal EEGs. Using logistic regression and a random forest classifier, we selected six EEG parameters as the best reflectors of the French EEG classification for HIE; Minimal Amplitude Index (MinAI [mean]), Maximal Amplitude Index (MaxAI [min]) Absolute Total Power (ATP [min]), Burst Suppression Ratio (BSR [max]), Relative Low Frequency Power (RLFP [var]) and Spectral Edge Frequency (SEF [max]). These EEG parameters reflect EEG background characteristics known to be well associated with the severity of neonatal HIE: signal continuity, amplitude and frequency content [6,7,9,30,31,44].

The amplitude parameters (MinAI and MaxAI) decreased with cerebral injury severity. The mean value of Minimal AI decreased significantly from 5 µV, to 3 µV and 1.5 µV in group 1, 2 and 3, respectively. The lowest value of Maximal AI was not significantly different between group 1 (29 µV) and 2 (26.5 µV) but decreased significantly in group 3 EEGs (7 µV). These results are consistent with the values found in previous studies [11,23,42,43] and in particular in aEEG classifications of HIE severity proposed by al Naqeeb in 1999 and Hellström-Westas in 2006 [19,32] (Table A.3). Experimental studies have shown that severe cerebral anoxia was associated with an initial suppression of the EEG amplitude during the primary phase of energy failure and during the latent phase despite normalisation of energy mechanism [3,17,21].

Burst Suppression Ratio (BSR) increased with HIE severity grades (8%, 37% and 86% in groups 1, 2 and 3, respectively). As in previous studies, the results show that IBI length was directly

associated with long term prognosis [30]. Different methods have already been proposed for IBI detection [12,14,16]. The BSR appears to be a simple and reliable measure of discontinuity in this study. Similarly, the variation coefficient of the Relative Low Frequency Power (RLFP) appears to be a marker of discontinuity, sensitive to the alternation between burst and IBI. However, this parameter was discriminative only for very discontinuous tracings (group 3).

Regarding frequency content, Absolute Total Power (ATP) tended to decrease with HIE severity (50 μV^2, 37 μV^2 and 3 μV^2 in group 1, 2 and 3 respectively) but differed significantly only between group 1–2 and group 3. Between group 1 and 2 the decreasing reflected the slowing of the signal [7,49]. In group 3, the very low value corresponded to signal suppression in inactive tracings. We observed quite a similar pattern with SEF. SEF did not differ between group 1 and 2 but was high in very discontinuous traces: 26 Hz maximum in group 3. SEF is generally used to measure the depth of anaesthesia in adults and decrease from 16 to 12 Hz during effective anaesthesia [40]. It has been studied by Inder et al. in preterm born infants: SEF increased with gestational age (from 5 to 12 Hz between 30 and 40 SA), and a low value at term age was associated with white matter lesions and poor prognosis [8,20]. However, to date, no study had shown significant differences in SEF values between EEG HIE grades [13,23,49]. We interpret our results as an indirect reflection of discontinuity: to reach 95% of the signal, the algorithm needed to include nearly all the frequencies contained in the tracing, which explains this high value in group 3.

The six EEG quantitative parameters easily classified group 3 EEGs. Severe EEGs were characterized by high Burst Suppression Ratio (86% maximum), low amplitude (1.5 µV minimum), low total power (2.7 μV^2 minimum) and high SEF values (26 Hz maximum). Minimal Amplitude Index and Burst Suppression Ratio by themselves discriminated between the three EEG groups. This confirms the importance of these two features as markers of anoxia and the results of previous studies [6,23,30].

As visual discrimination between EEG grades 1 and 2 is the most difficult and represents a major therapeutic impact (indication for TH), the selection of our EEG parameters was particularly optimized for EEG grade 2 classification accuracy. BSR and Minimal Amplitude Index were significantly different between groups 1 and 2, Minimal Amplitude Index being the most accurate. When the group 2 EEGs (moderate abnormalities) misclassified by MinAI were checked, we observed that these were either discontinuous type A traces or slow EEG, with one hyperactive rapid trace (Fig. 5-A). All of these children had normal neurological status at age 3 years. It is important to note that in group 2, the French classification is based also on patterns less amenable to quantitative analysis (hyperactive rapid traces for example). We also wish to emphasize that our quantitative analysis was performed over the entire EEG trace and did not measure the temporal organization and lability of the EEG, which are major criteria in visual interpretation.

In a second step we analysed the prognostic value of quantitative parameters themselves, independently from EEG visual classification. We were able to confirm that high amplitude values and low discontinuity values in early neonatal EEGs were associated with a favourable clinical outcome at 3 years of age.

We also want to highlight that we did not observe any significant difference in birth condition and neonatal laboratory results between our three groups of increasing HIE severity, even though these parameters are still considered as hallmarks of HIE in routine care. Lactate and pH values were very close in our 3 groups. Previous studies have shown limitations of these biomarkers for brain damage detection and have searched for more sensitive biological parameters is an active field [1,15,36]. This is the first study to demonstrate the absence of relationship between pH, lactate and EEG grades. This again highlights the relevance of EEG as an early marker of anoxic brain injury and its central role in NICUs, ideally embedded in a Neonatal Neurocritical Care Program [35].

Our study was conducted in conditions as close as possible to daily practice. We used commercially available embedded tools and analyzed the entire EEG trace, using the raw signal. Results were adjusted for newborn body temperature at the time of EEG recording, as this parameter was significantly associated with HIE severity groups. As a consequence, we expect that these quantitative EEG values, especially for amplitude and discontinuity, can be used as references to facilitate visual classification.

This study has some limitations. Some unreadable EEGs were removed from analysis. We did not specifically deal with traces showing seizures, which could lead to underestimation of discontinuity or low amplitude signal (Fig. 5-B). However, seizures were not frequent in our tracings, as expected in early EEGs recorded here at H4 of life on average [28]. As well, the presence of artefacts could disrupt the quantitative analysis and we tried to minimize their impact by selecting the least sensitive EEG parameters.

In most studies including consecutive EEG recordings, the most severe ones are underrepresented, as this situation is less frequent. To achieve valid statistical analysis, we decided to constitute three groups of equal size.

Using these quantitative EEG parameters individually remains limited for intermediate EEG grading. A combining approach with a multiparametric algorithm may contribute to improve the EEG classification performances. Such an algorithm could be easily implemented in an EEG machine for early HIE automatic classification at the bedside in NICU, helping to determine the indication for TH when the neurophysiologist is not available to interpret the EEG within 6 hours after perinatal asphyxia. In this case, optimisation of grade 2 classification remains necessary.

CONCLUSION

Our study proposes six relevant quantitative EEG parameters as early markers of neonatal HIE severity, selected for their capacity to replicate neonatal HIE visual classification. These parameters also demonstrated their prognostic value for clinical outcome independently from visual EEG grading mainly by detecting traces that were very severely abnormal. Their usefulness on an individual basis remains limited for intermediate traces. Thus, they should be considered as complementary to visual analysis. We plan to combine them in a multiparametric algorithm to improve their performance.

Declarations of interest: none. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

[1] Ahearne CE, Boylan GB, Murray DM. Short and long term prognosis in perinatal asphyxia: An update. World J Clin Pediatr 2016;5:67–74.

[2] Ahmed R, Temko A, Marnane WP, Boylan G, Lightbody G. Classification of hypoxic-ischemic encephalopathy using long term heart rate variability based features. Conf Proc IEEE Eng Med Biol Soc 2015:2355–8.

[3] Azzopardi D, Wyatt JS, Cady EB, Delpy DT, Baudin J, Stewart AL, et al. Prognosis of newborn infants with hypoxic-ischemic brain injury assessed by phosphorus magnetic resonance spectroscopy. Pediatr Res 1989;25:445–51.

[4] Azzopardi D, Brocklehurst P, Edwards D, Halliday H, Levene M, Thoresen M, et al. The TOBY Study. Whole body hypothermia for the treatment of perinatal asphyxial encephalopathy: a randomised controlled trial. BMC Pediatr 2008;8:17.

[5] Azzopardi DV, Strohm B, Edwards AD, Dyet L, Halliday HL, Juszczak E, et al. Moderate hypothermia to treat perinatal asphyxial encephalopathy. N Engl J Med 2009;361:1349–58.

[6] Azzopardi D, TOBY study group. Predictive value of the amplitude integrated EEG in infants with hypoxic ischaemic encephalopathy: data from a randomised trial of therapeutic hypothermia. Arch Dis Child 2014;99:F80-2.

[7] Bell A, G McClure B, M Hicks E. Power spectral analysis of the EEG of term infants following birth asphyxia. Dev Med Child Neurol 1990;32:990–8.

[8] Bell AH, McClure BG, McCullagh PJ, McClelland RJ. Spectral edge frequency of the EEG in healthy neonates and variation with behavioural state. Biol Neonate 1991;60:69–74.

[9] Chandrasekaran M, Chaban B, Montaldo P, Thayyil S. Predictive value of amplitude-integrated EEG (aEEG) after rescue hypothermic neuroprotection for hypoxic ischemic encephalopathy: a meta-analysis. J Perinatol 2017;37:684–9.

[10] Clancy RR, Legido A. The exact ictal and interictal duration of electroencephalographic neonatal seizures. Epilepsia 1987;28:537–41.

[11] Csekő AJ, Bangó M, Lakatos P, Kárdási J, Pusztai L, Szabó M. Accuracy of amplitude-integrated electroencephalography in the prediction of neurodevelopmental outcome in asphyxiated infants receiving hypothermia treatment. Acta Paediatr 2013;102:707–11.

[12] Dereymaeker A, Matic V, Vervisch J, Cherian PJ, Ansari AH, De Wel O, et al. Automated EEG background analysis to identify neonates with hypoxic-ischemic encephalopathy treated with hypothermia at risk for adverse outcome: A pilot study. Pediatr Neonatol 2019;60:50–8.

[13] Doyle OM, Temko A, Murray DM, Lightbody G, Marnane W, Boylan GB. Predicting the neurodevelopmental outcome in newborns with hypoxic-ischaemic injury. Annu Int Conf IEEE Eng Med Biol Soc 2010:1370–3.

[14] Dunne JM, Wertheim D, Clarke P, Kapellou O, Chisholm P, Boardman JP, et al. Automated electroencephalographic discontinuity in cooled newborns predicts cerebral MRI and neurodevelopmental outcome. Arch Dis Child 2017;102:F58–64.

[15] East CE, Leader LR, Sheehan P, Henshall NE, Colditz PB, Lau R. Intrapartum fetal scalp lactate sampling for fetal assessment in the presence of a non-reassuring fetal heart rate trace. Cochrane Database Syst Rev 2015;(5):CD006174.

[16] Flisberg A, Kjellmer I, Löfhede J, Lindecrantz K, Thordstein M. Prognostic capacity of automated quantification of suppression time in the EEG of post-asphyctic full-term neonates. Acta Paediatr 2011;100:1338–43.

[17] Gunn AJ, Gunn TR, de Haan HH, Williams CE, Gluckman PD. Dramatic neuronal rescue with prolonged selective head cooling after ischemia in fetal lambs. J Clin Invest 1997;99:248–56.

[18]Hamelin S, Delnard N, Cneude F, Debillon T, Vercueil L. Influence of hypothermia on the prognostic value of early EEG in full-term neonates with hypoxic ischemic encephalopathy. Neurophysiol Clin 2011;41:19–27.

[19]Hellström-Westas L, Rosén I, Vries LS de, Greisen G. Amplitude-integrated EEG classification and interpretation in preterm and term infants. NeoReviews 2006;7:e76–87.

[20]Inder TE, Buckland L, Williams CE, Spencer C, Gunning MI, Darlow BA, et al. Lowered electroencephalographic spectral edge frequency predicts the presence of cerebral white matter injury in premature infants. Pediatrics 2003;111:27–33.

[21]Iwata O, Iwata S, Bainbridge A, De Vita E, Matsuishi T, Cady EB, et al. Supra- and sub-baseline phosphocreatine recovery in developing brain after transient hypoxia-ischaemia: relation to baseline energetics, insult severity and outcome. Brain 2008;131:2220–6.

[22]Jacobs SE, Berg M, Hunt R, Tarnow-Mordi WO, Inder TE, Davis PG. Cooling for newborns with hypoxic ischaemic encephalopathy. Cochrane Database Syst Rev 2013;(1):CD003311.

[23]Korotchikova I, Stevenson NJ, Walsh BH, Murray DM, Boylan GB. Quantitative EEG analysis in neonatal hypoxic ischaemic encephalopathy. Clin Neurophysiol 2011;122:1671–8.

[24]Lamblin M, Racoussot S, Pierrat V, Duquennoy C, Ouahsine T, Lequien P, et al. Encéphalopathie anoxo-ischémique du nouveau-né à terme. Apport de l'électroencéphalogramme et de l'échographie transfontanellaire à l'évaluation pronostique. À propos de 29 observations. Neurophysiol Clin 1996;26:369–78.

[25]Lamblin MD, André M, Challamel MJ, Curzi-Dascalova L, d'Allest AM, Giovanni ED, et al. EEG in premature and full-term newborns. Maturation and glossary. Neurophysiol Clin 1999;29:123–219.

[26]Lamblin MD, Walls EE, André M. The electroencephalogram of the full-term newborn: review of normal features and hypoxic-ischemic encephalopathy patterns. Neurophysiol Clin 2013;43:267–87.

[27]Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: When? Where? Why? Lancet 2005;365:891–900.

[28]Lynch NE, Stevenson NJ, Livingstone V, Mathieson S, Murphy BP, Rennie JM, et al. The temporal characteristics of seizures in neonatal hypoxic ischemic encephalopathy treated with hypothermia. Seizure 2015;33:60–5.

[29]Matic V, Cherian PJ, Koolen N, Naulaers G, Swarte RM, Govaert P, et al. Holistic approach for automated background EEG assessment in asphyxiated full-term infants. J Neural Eng 2014;11:066007.

[30]Menache CC, Bourgeois BFD, Volpe JJ. Prognostic value of neonatal discontinuous EEG. Pediatr Neurol 2002;27:93–101.

[31]Murray DM, Boylan GB, Ryan CA, Connolly S. Early EEG findings in hypoxic-ischemic encephalopathy predict outcomes at 2 years. Pediatrics 2009;124:e459-67.

[32]al Naqeeb N, Edwards AD, Cowan FM, Azzopardi D. Assessment of neonatal encephalopathy by amplitude-integrated electroencephalography. Pediatrics 1999;103:1263–71.

[33]Pezzani C, Radvanyi-Bouvet MF, Relier JP, Monod N. Neonatal electroencephalography during the first twenty-four hours of life in full-term newborn infants. Neuropediatrics 1986;17:11–8.

[34]Pressler RM, Boylan GB, Morton M, Binnie CD, Rennie JM. Early serial EEG in hypoxic ischaemic encephalopathy. Clin Neurophysiol 2001;112:31–7.

[35]Roychoudhury S, Esser MJ, Buchhalter J, Bello-Espinosa L, Zein H, Howlett A, et al. Implementation of neonatal neurocritical care program improved short-term outcomes in neonates with moderate-to-severe hypoxic ischemic encephalopathy. Pediatr Neurol 2019;101:64-70.

[36]Ruth VJ, Raivio KO. Perinatal brain damage: predictive value of metabolic acidosis and the Apgar score. Br Med J 1988;297:24–7.

[37]Saliba E, Debillon T. Neuroprotection par hypothermie contrôlée dans l'encéphalopathie hypoxique-ischémique du nouveau-né à terme. Arch Pediatr 2010;17:S67-77

[38]Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. Arch Neurol 1976;33:696–705.

[39]Scavone C, Radvanyi-Bouvet MF, Morel-Kahn F, Dreyfus-Brisac C. Coma apres souffrance foetale aigue chez le nouveau-ne a terme: Evolution electro-clinique. Neurophysiol Clin 1985;15:279–88.

[40]Schwender D, Daunderer M, Mulzer S, Klasing S, Finsterer U, Peter K. Spectral edge frequency of the electroencephalogram to monitor “depth” of anaesthesia with isoflurane or propofol. Br J Anaesth 1996;77:179–84.

[41]Shankaran S, Pappas A, McDonald SA, Vohr BR, Hintz SR, Yolton K, et al. Childhood Outcomes after Hypothermia for Neonatal Encephalopathy. N Engl J Med 2012;366:2085–92.

[42]Shellhaas RA, Gallagher PR, Clancy RR. Assessment of neonatal electroencephalography (EEG) background by conventional and two amplitude-integrated EEG classification systems. J Pediatr 2008;153:369–74.

[43]Shellhaas RA. Continuous long-term electroencephalography: the gold standard for neonatal seizure diagnosis. Semin Fetal Neonatal Med 2015;20:149–53.

[44]Sinclair DB, Campbell M, Byrne P, Prasertsom W, Robertson CM. EEG and long-term outcome of term infants with neonatal hypoxic-ischemic encephalopathy. Clin Neurophysiol 1999;110:655–9.

[45]Stevenson NJ, Korotchikova I, Temko A, Lightbody G, Marnane WP, Boylan GB. An automated system for grading EEG abnormality in term neonates with hypoxic-ischaemic encephalopathy. Ann Biomed Eng 2013;41:775–85.

[46]Temko A, Doyle O, Murray D, Lightbody G, Boylan G, Marnane W. Multimodal predictor of neurodevelopmental outcome in newborns with hypoxic-ischaemic encephalopathy. Comput Biol Med 2015;63:169–77.

[47]Volpe JJ. Neurology of the Newborn E-Book. Amsterdam: Elsevier; 2008.

[48]Walsh BH, Murray DM, Boylan GB. The use of conventional EEG for the assessment of hypoxic ischaemic encephalopathy in the newborn: a review. Clin Neurophysiol 2011;122:1284-94.

[49]Wong FY, Barfield CP, Walker AM. Power spectral analysis of two-channel EEG in hypoxic-ischaemic encephalopathy. Early Hum Dev 2007;83:379–83.

Figure legends

Fig. 1: Flow chart of the neonatal EEGs included in the study

Fig. 2. Examples of EEG traces with different HIE severity grades according to visual analysis
1 – Mild abnormalities: grade 1; **2B** – Moderate abnormalities: grade 2 (discontinuous type B); **3P** – Severe abnormalities: grade 3 (paroxysmal tracing); **3N** – Severe abnormalities: grade 3 (inactive tracing)

Fig. 3. Distribution of the six selected EEG parameters in relation to the three HIE severity groups.

Fig. 4. ROC curves of the six selected EEG parameters for outcome prediction at age three.

Fig. 5. Examples of misclassified EEGs
A - Visual classification: grade 2 (slow EEG) / MinAI classification: grade 1 (normal amplitude)
B - Visual classification: grade 3 (paroxysmal tracing) / MinAI classification: grade 2 (falsely higher amplitude due to a permanent artefact on C4-O2).

Tables

Table 1a: Demographic and perinatal characteristics of the population.

	N=90	Group 1 (n=30)	Group 2 (n=30)	Group 3 (n=30)	p-value
Gender *(M/F)*	62/28	22/8	20/10	20/10	-
Gestational age at birth (weeks) *mean ± sd*	39.4 ± 1.5	39.60 ± 1.63	39.27 ± 1.30	39.31 ± 1.43	0.42
Birth weight (g) *mean ± sd*	3161 ± 629	3259 ± 539	2977 ± 739	3239 ± 577	0.15
Progress of pregnancy: *N(%)*					
Normal	53 (58.9%)	21 (70%)	13 (43.3%)	19 (63.3%)	-
Maternal complication[1]	22 (24.4%)	6 (20%)	10 (33.3%)	6 (20%)	
Fetal complication[2]	15 (16.7%)	3 (10%)	7 (23.3%)	5 (16.7%)	
Delivery: *N(%)*					
Emergency caesarean section	48 (53.3%)	19 (63.3%)	17 (56.7%)	12 (40%)	-
Physiological vaginal delivery	24 (26.7%)	4 (13.3%)	7 (23.3%)	13 (43.3%)	
Instrumental delivery	17 (18.9%)	7 (23.3%)	5 (16.7%)	5 (16.7%)	
Scheduled caesarean section	1 (1.1%)	0	1 (3.3%)	0	
Perinatal asphyxia criteria	*(mean ±sd)*				
pH before H1	6.95 ± 0.2	6.93 ± 0.10	6.98 ± 0.24	6.94 ± 0.18	0.78
Lactate before H1 (mmol/L)	12.6 ± 4.0	12.5 ± 4.1	12.1 ± 4.2	13.3 ± 3.9	0.41
Apgar score at 10 min	5.5 ± 5.0	5.7 ± 3.0	6.3 ± 2.4	3.9 ± 2.6	**0.004**
Ventilatory support at 10 min	68 (76.7%)	17 (56.7%)	24 (80%)	28 (93.3%)	-
Sarnat score: *N(%)*					
1	21 (23.3%)	16 (53.3%)	4 (13.3%)	1 (3.3%)	-
2	52 (57.8%)	14 (46.7%)	21 (70%)	17 (56.7%)	
3	17 (18.9%)	0	5 (16.7%)	12 (40%)	

[1] Maternal complication: e.g., diabetes during pregnancy, maternal pathology, treatment, toxic substances, etc.

[2] Fetal complication: e.g., multiple pregnancies, intrauterine growth restriction, ultrasound abnormality, etc.

Table 1b: EEG data.

Recording conditions: *N(%)*	N=90	Group 1 (n=30)	Group 2 (n=30)	Group 3 (n=30)	p-value
Sedation or analgesia[3]	34 (37.8%)	6 (20%)	12 (40%)	16 (53.3%)	0.12
Intubation/ventilation	62 (68.9%)	13 (43.3%)	22 (73.3%)	27 (90%)	-
Hypothermia (T°<34°C)	47 (52.2%)	6 (20%)	15 (50%)	26 (86.7%)	-

Body temperature (°C)	34.2 ± 1.7	35.3 ± 1.4	34.2 ± 1.6	33.1 ± 1.4	**<0.001**
Temporal data *(mean ± sd)*					
Post-natal delay (hours)	3.6 ± 1.2	3.1 ± 1.2	3.8 ± 1.1	3.8 ± 1.3	0.056
EEG duration (minutes)	50.8 ± 19.2	57.8 ± 10.4	48.9 ± 14.0	45.6 ± 27.3	**<0.001**
Seizures/paroxysmal discharges: *N(%)*					
None	78 (86.7%)	30 (100%)	26 (86.7%)	22 (73.3%)	
< 5 per hour	8 (8.9%)	0	4 (13.3%)	4 (13.3%)	-
> 5 per hour	4 (4.4%)	0	0	4 (13.3%)	
Detailed EEG visual grades[4]		Grade 0 / 1	Grade 2a / 2b	Grade 3p / 3n	
		11 / 19	18 / 12	17 / 13	

[3] Presence of sedative, anticonvulsant or analgesic drugs: phenobarbital, clonazepam, morphine or sufentanyl.

[4] Grade 0 (normal); Grade 1 (mild abnormalities); Grade 2a: discontinuous trace type A; Grade 2b: discontinuous trace type B; Grade 3p: paroxysmal trace; Grade 3n: inactive trace (table A.1).

Table 1c: Clinical outcome of the children at 1 and 3 years of age.

Neonatal death	N=90	Group 1 (n=30)	Group 2 (n=30)	Group 3 (n=30)
	13 (14.4%)	0	2 (6.7%)	11 (36.7%)
One year				
Good outcome	68 (75.6%)	30 (100%)	24 (80%)	14 (46.7%)
Poor outcome	3 (3.3%)	0	0	3 (10%)
Missing data	6 (6.7%)	0	4 (13.3%)	2 (6.7%)
Three years				
Good outcome	64 (71.1%)	30 (100%)	22 (73.3%)	12 (40%)
Poor outcome	5 (5.6%)	0	2 (6.7%)	3 (10%)
Missing data	8 (8.9%)	0	4 (13.3%)	4 (13.3%)

Table 2: Selection of six EEG quantitative parameters considering their performance in logistic regression analysis (p-values and EEG classification performances) and their importance in the random forest model (precision decay when the variable is removed from the model).

Quantitative parameters	**p-value**	**Overall EEG classification performance**	**Grades 2 EEG classification performance**	**Precision decay**
MinAI [mean]	<0.0001	70%	50%	7.8

MaxAI [min]	<0.0001	66%	47%	9.1
ATP [min]	0.0011	62%	47%	6.2
RLFP [var]	<0.0001	61%	43%	3.4
SEF [max]	0.0003	61%	43%	1.4
BSR [max]	<0.0001	69%	47%	5.1

MinAI [mean]: mean value of the Minimal Amplitude Index; MaxAI [min]: minimal value of the Maximal Amplitude Index; ATP [min]: minimal value of the Absolute Total Power; RLFP [var]: variation coefficient of the Relative Low Frequency Power; SEF [max]: maximal value of the Spectral Ede Frequency; BSR [max]: maximal value of the Burst Suppression Ratio

Table 3: Values of the six EEG parameters in the three HIE severity groups.

EEG parameters *[mean ± sd (min-max)]*	**Group 1**	**Group 2**	**Group 3**
Mean value of MinAI (µV)	4.6 ± 1.3 (2.8–8.0)	3.0 ± 1.6 (1.2–7.5)	1.5 ± 0.7 (0.6–3.3)
Minimal value of MaxAI (µV)	28.7 ± 8.1 (13.7–45.4)	26.5 ± 14.5 (5.6–63.8)	6.9 ± 4.5 (2.4–24.5)
Minimal value of ATP (μV^2)	50.5 ± 36.9 (9.9–176.1)	36.7 ± 42.4 (1.5–209.1)	2.7 ± 4.1 (0.2–22.0)
Variation coefficient of RLFP (%)	6.1 ± 2.4 (2.0–12.2)	6.5 ± 3.4 (1.4–17.9)	13.2 ± 7.5 (5.1–42.2)
Maximal value of SEF (Hz)	20.7 ± 5.8 (10.2–27.7)	21.1 ± 5.6 (7.1–29.4)	25.9 ± 2.1 (22.1–29.7)
Maximal value of BSR (%)	7.8 ± 7.7 (0.0–28.0)	36.7 ±32.9 (0.0–96.7)	86.2 ± 20.8 (25.6–100.0)

MinAI: Minimal Amplitude Index; MaxAI: Maximal Amplitude Index; ATP: Absolute Total Power; RLFP: Relative Low Frequency Power; SEF: Spectral Ede Frequency; BSR: Burst Suppression Ratio

Table 4: Values of the six EEG parameters in relation to the outcome at three years of age.

EEG parameters *[mean ± sd (min-max)]*	**Good outcome**	**Poor outcome**
Mean value of MinAI (µV)	3.5 ± 1.7 (0.6–8.0)	1.6 ± 0.6 (0.7–2.9)
Minimal value of MaxAI (µV)	24.1 ± 12.7 (2.4–56.6)	11.6 ± 14.4 (2.5–63.8)
Minimal value of ATP (μV^2)	36.1 ± 35.3 (0.2–176.1)	16.3 ± 48.5 (0.3–209.1)
Variation coefficient of RLFP (%)	7.1 ± 3.6 (2.0–17.9)	13.1 ± 9.1 (1.4–44.2)
Maximal value of SEF (Hz)	21.6 ± 5.2 (10.2–29.4)	25.1 ± 4.9 (7.1–29.7)
Maximal value of BSR (%)	31.4 ± 36.2 (0.0–100.0)	76.0 ± 29.5 (0.0–100.0)

MinAI: Minimal Amplitude Index; MaxAI: Maximal Amplitude Index; ATP: Absolute Total Power; RLFP: Relative Low Frequency Power; SEF: Spectral Ede Frequency; BSR: Burst Suppression Ratio

Fig. 1: Flow Chart of the neonatal EEGs included in the study

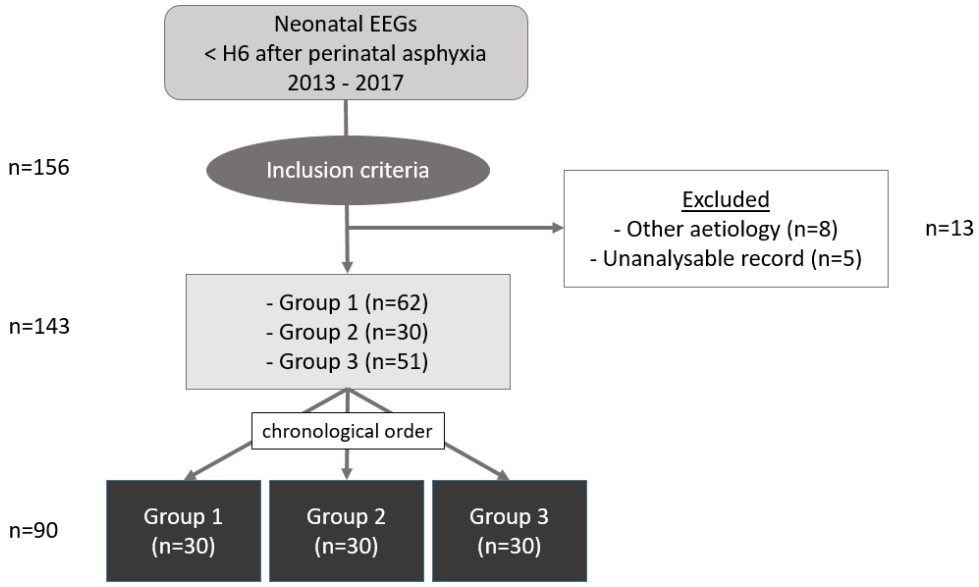


Fig. 2. Examples of EEG tracings with different HIE severity grades according to visual analysis **1** – Subnormal: grade 1; **2B** – Moderate abnormalities: grade 2 (discontinuous type B); **3P** – Severe abnormalities: grade 3 (paroxysmal tracing); **3N** – Severe abnormalities: grade 3 (inactive tracing)

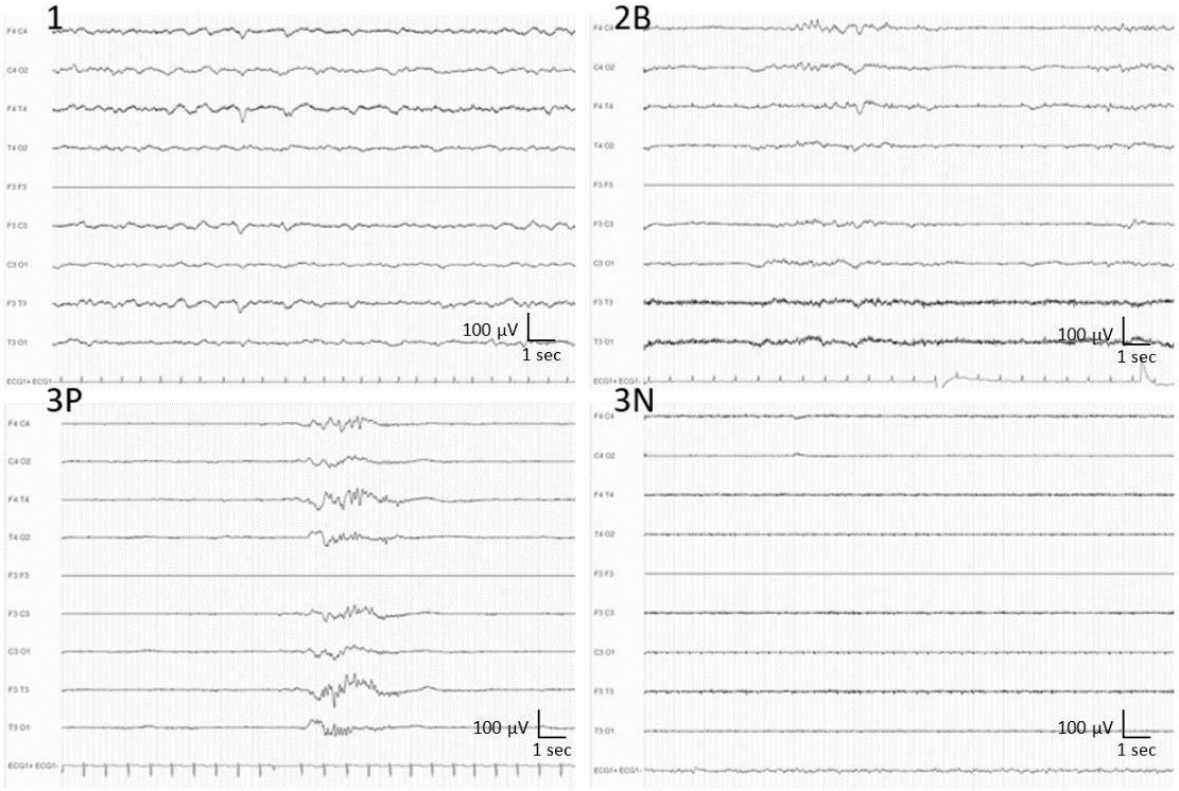
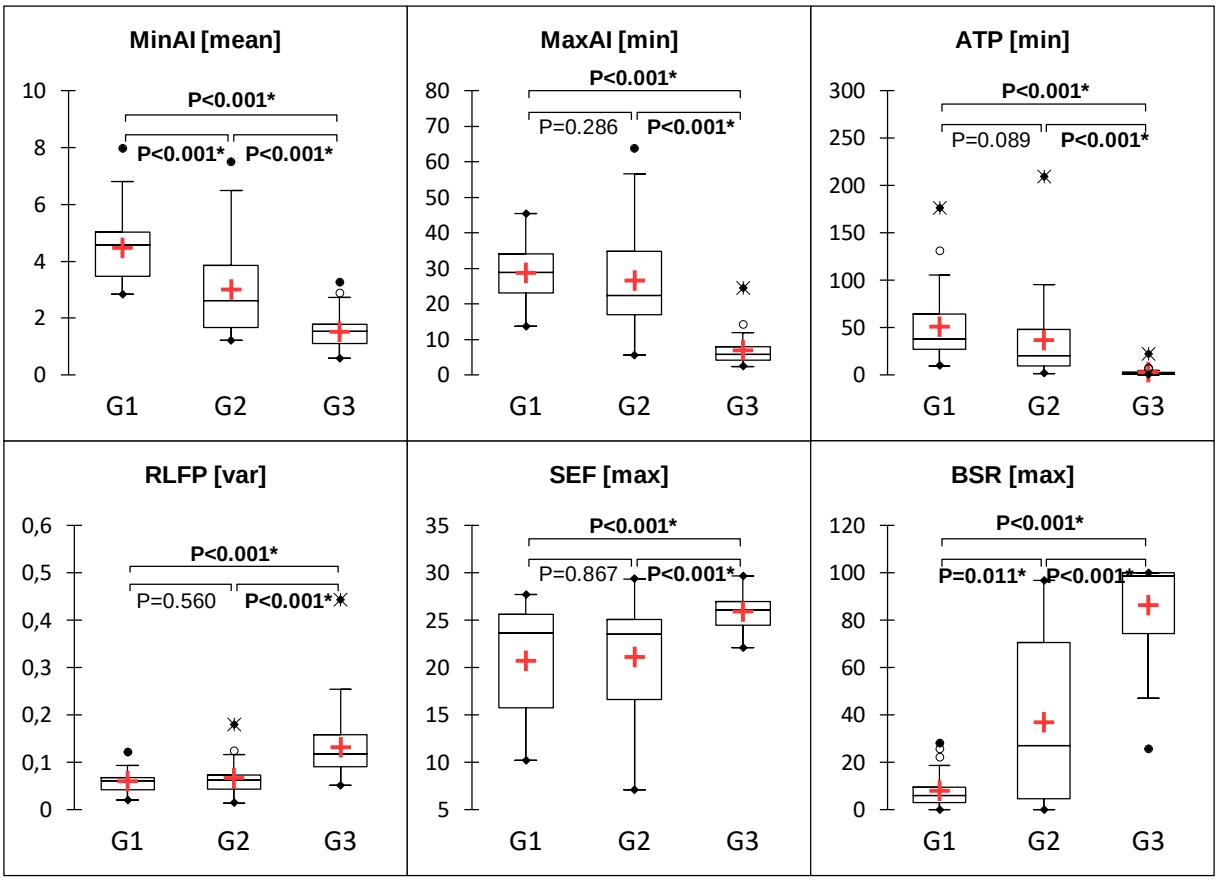


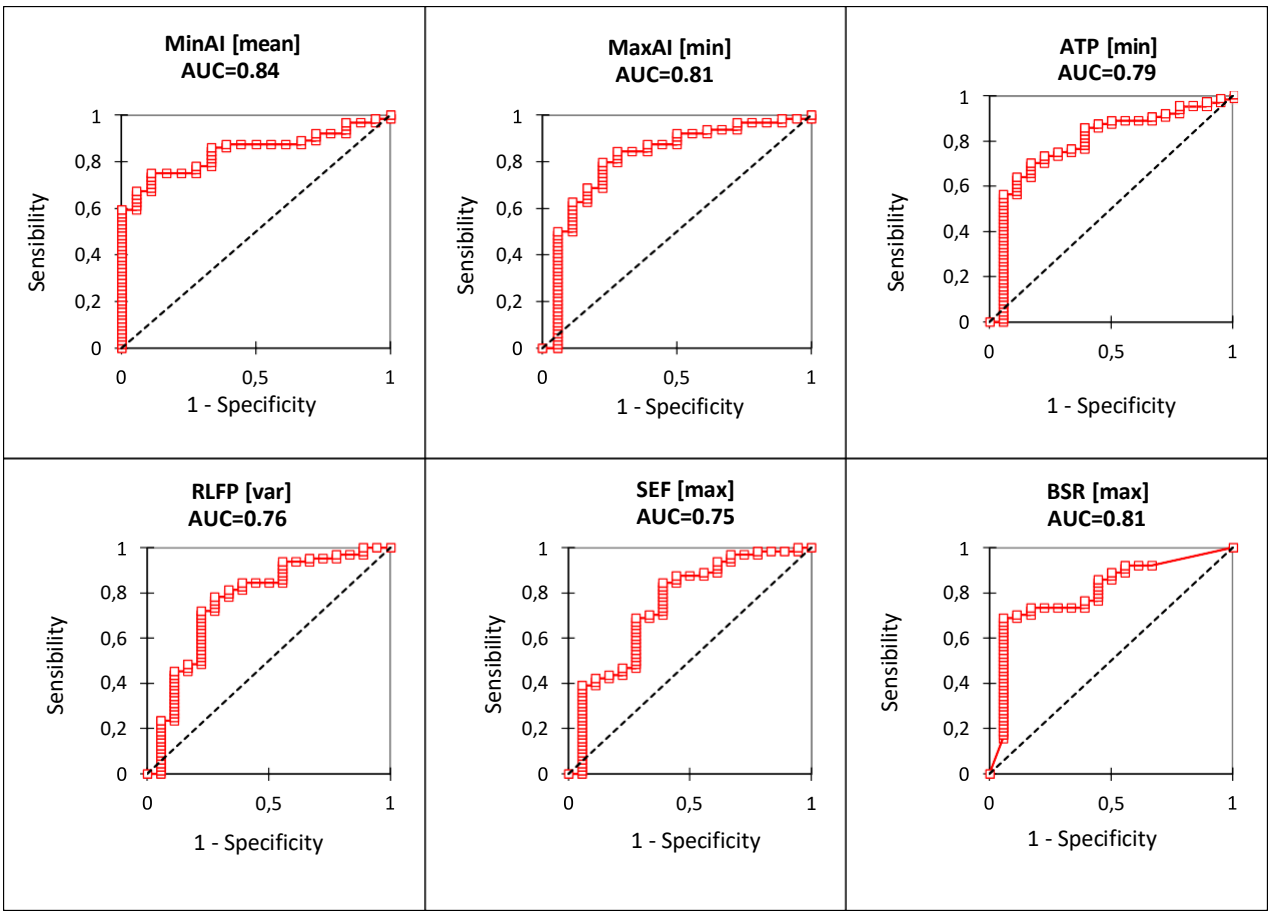
Fig. 3. Distribution of the six selected EEG parameters in relation to the three HIE severity grades



***Significant : p < 0.016**

MinAI [mean]: mean value of the Minimal Amplitude Index; MaxAI [min]: minimal value of the Maximal Amplitude Index; ATP [min]: minimal value of the Absolute Total Power; RLFP [var]: variation coefficient of the Relative Low Frequency Power; SEF [max]: maximal value of the Spectral Ede Frequency; BSR [max]: maximal value of the Burst Suppression Ratio
G1, G2, G3: visual EEG grades 1, 2 and 3

Fig. 4. ROC curves of the six selected EEG parameters for outcome prediction at age three



MinAI [mean]: mean value of the Minimal Amplitude Index; MaxAI [min]: minimal value of the Maximal Amplitude Index; ATP [min]: minimal value of the Absolute Total Power; RLFP [var]: variation coefficient of the Relative Low Frequency Power; SEF [max]: maximal value of the Spectral Edge Frequency; BSR [max]: maximal value of the Burst Suppression Ratio AUC: Area Under the Curve

Fig. 5. Examples of misclassified EEGs
A - Visual classification: grade 2 (slow EEG) / MinAI classification: grade 1 (normal amplitude)
B - Visual classification: grade 3 (paroxysmal tracing) / MinAI classification: grade 2 (falsely higher amplitude due to a permanent artefact on C4-O2).