Appendix S2: SUPPLEMENTAL-MATERIAL FOR RESULTS

Spearman coefficient correlations between FC-scales and ES-scales for the seven time-periods (H6-12 to H72-78)

FC-scales from H6-12 to H72-78 and ES-scales from H6-12 to H72-78 were highly correlated. The highest correlations were found in both scales for the H30-36 / H42-48 and H42-48 / H54-60 periods (indicated in the table by bold typeface).

![Correlation table](image)

Characteristics of the population and pairwise comparisons for outcome (favorable / adverse) and seizure / seizure-free groups.

No pairwise comparisons between outcome groups or between seizure/seizure-free groups reached the statistical threshold of 0.05.

| Characteristics | All sample (N=95) | Favorable outcome (N=64) | Adverse outcome (N=31) | p-value | Seizure free (N=64) | Seizure (N=31) | p-value |
|-----------------|------------------|--------------------------|------------------------|---------|---------------------|----------------|---------|
| NVD / IVD / ECS |                  |                          |                        |         |                     |                |         |
| %               | 30 / 16 / 49     | 22 / 11 / 31             | 8 / 5 / 18              | 0.67[†] | 23 / 11 / 30        | 11 / 3 / 17   | 0.61[†] |
|                | 32% / 17% / 51%  | 35% / 17% / 52%          | 26% / 16% / 58%         |         | 30% / 17% / 47%     | 35% / 10% / 55%|         |
| Females / Males| 36 / 39          | 22 / 42                  | 14 / 17                | 0.37[†] | 23 / 41             | 13 / 18       | 0.66[†] |
| %              | 38% / 62%        | 34% / 66%                | 45% / 55%              |         | 36% / 64%           | 42% / 58%     |         |
| Sarnat-2 / Sarnat-3 | 51 / 34 | 47 / 17                  | 17 / 14                | 0.07[†] | 44 / 20             | 20 / 11       | 0.82[†] |
| %              | 64% / 36%        | 73% / 27%                | 52% / 48%              |         | 69% / 31%           | 65% / 35%     |         |
| Gestational age (week mean) (SD) | 39.3 (1.4) | 39.3 (1.4)               | 39.3 (1.5)             | 0.85[†] | 39.3 (1.4)          | 39.3 (1.4)    | 0.86[†] |
| Birth weight (g mean) (SD) | 3178 (580) | 3130 (613)               | 3236 (556)             | 0.37[†] | 3152 (621)          | 3189 (544)    | 0.73[†] |
| Apgar 5min (mean) (SD) | 5.4 (2.7) | 5.5 (2.5)                | 4.8 (3.0)              | 0.32[†] | 4.9 (2.6)           | 5.2 (2.8)     | 0.99[MW] |
| Blood gases (mean) (SD) | 6.5 (0.2) | 7.0 (0.2)                | 6.9 (0.2)              | 0.65[MW] | 7.0 (0.2)           | 7.0 (0.2)     | 0.16[MW] |
| Lactate (mmol/L mean) (SD) | 13.4 (4.4) | 13.0 (4.6)               | 14.5 (4.4)             | 0.49[MW] | 13.1 (4.9)          | 14.2 (3.8)    | 0.28[†] |

NVD: normal vaginal delivery; IVD: instrumental vaginal delivery; ECS: caesarean section; (F): Fischer-exact test; (S): Student-t test; (MW): Mann-Whitney-U test. Missing values: 31 for Apgar (5 mn), 16 for blood gases and 8 for lactates.
Graphical representation of temporal characteristics of seizure burden per neonate\textsuperscript{1,2}, seizure durations (max, min, mean and SD) for the seizure subgroup and according to the outcome (favorable / adverse), and comparison of Z-scores (CI-95\%) of ES-scale scores and seizure duration as function of periods from H6 to H78 (seizure subgroup, N=31).

Total time spent in seizures from 6 to 78h [right-panel] and time in seizures for each period from H6-12 to H72-78 [left-panel] for each neonate. The periods in grey were considered by the PCA. The period highlighted by a dotted line indicated the rewarm period. IS: Isolated seizures (i.e. seizures occurring during a period immediately preceding and followed by seizure-free periods, H72-78 period of rewarm excluded). In our population, the false negative risk to have IS during at least one H6-72 period was of 1\%. The maximum of the cumulative IS duration was 41.7 minutes (S86). The mean and longer durations of IS were 5.3 and 27 minutes, respectively).

Max, min, mean and standard deviation (SD) of the time spent in seizure (expressed in minutes) from H6-12 to H72-78 for the seizure subgroup, the Adverse and Favorable- outcome groups. na: non-applicable (seizure free).
Z-scores and CI-95% of ES-scale scores and the exact time spent in seizures expressed in minutes as function of H6 to H78 periods. The periods in grey were considered by the PCA. The period highlighted by a dotted line indicated the rewarmed period.

The profiles of the curves for ES-scale scores and the time spent in seizures were consistent with each other. Both were characterized by an increase in the seizure burden from H6 to H30, followed by a decrease until H72 before increasing during warm-up. The evolution of the Z-score between two 6h-periods was progressive without drastic change specific of a time window. The range between max and min values was slightly greater for the time spent in seizures than for the ES-scale scores. Nevertheless, the difference was moderate as showed by CI-95%. The overlap of the CI-95% for the two curves indicated that (1) the difference in sensitivity is moderate between the seizure burden evaluations, and (2) that the sampling by 6h-periods every 12 hours accurately reflected the seizure burden as assumed previously.
Antiepileptic drug management in neonates with at least one epileptic seizure during TH (N=31)

Antiepileptic drug (AED) management between outcome groups was compared to ensure that both the higher and longer ES burden in the adverse-outcome group relative to the favorable-outcome group was not the result of the use of AEDs.

[A] Means and 95% confidence intervals for ES- and [B] FC-graded scales during TH in neonates with at least one episode of epileptic seizures as a function of their outcome (adverse / favorable). Percentage of seizure onset in each outcome group as a function of the TH period.

[C] Means for the ES- and [D] FC-scales during HT in neonates (adverse / favorable) according to whether they were treated with AEDs or not (no AED).

[E] Percentage of AEDs used in the first, second, and third lines (L1, L2 and L3) during TH as a function of outcome (adverse / favorable).

[F] Number of neonates treated in line (L)1, L2, and L3 by phenobarbital, fosphenytoin, or clonazepam.

| AED          | L1 | L2 | L3 |
|--------------|----|----|----|
| Phenobarbital | 20 |    |    |
| Fosphenytoin | 5  | 2  |    |
| Clonazepam   | 3  | 2  | 2  |
| Total        | 23 | 7  | 4  |
| no AED       | 8  | 24 | 25 |
In the favorable-outcome group, 10 neonates had at least one epileptic seizure. Among them, all began having epileptic seizures within the first day and 7/10 (30.0%) were treated with at least one line of AEDs. Both ES burden [C] and EEG-background [D] grades were slightly higher in neonates who were treated than in those who were not [C]. Among the neonates treated with AEDs, 70% were treated with one line of AEDs (L1), 30% with two lines (L2), and none with three lines (L3) [E].

In the adverse-outcome group, 21 neonates had at least one epileptic seizure. Among them, 15/21 (71%) began having epileptic seizures within the first day, 5/21 (24%) began within the second day, and 20/21 (95.2%) were treated with AEDs: 40% with one line of AEDs [E], 40% with two lines, and 20% with three lines. The proportion of neonates treated with AEDs increased from H6-12 to H42-48 upon seizure onset. Only one neonate of the adverse-outcome group was not treated with AEDs. Seizures were initially considered as respiratory artefacts on an inactive EEG-background [D] and were therefore not treated. The second examination of the EEG later revealed the epileptic nature of the pattern.

**Principal Component Analysis (PCA) of cEEG monitoring and promax rotation performed on PCA coordinates**

Graph of the correlations between the FC- and ES-scales for H6-78 for PC1-PC2, and PC1-PC3 with the supplemental variable ICF-CY represented in blue [A]. Contributions of the FC- and ES-scales to H6-78 for PC1, PC2, and PC3 [B]. Bivariate plots of the scores for PC1-PC2 and PC1-PC3 [C]. Black arrow: outlier who died before discharge.
Correlation graphs between FC- and ES-scales from H6-12 to H72-78 and bivariate plots of scores for promax PC1-PC2 [A] and promax PC1-PC3 [B]. The promax rotation, in modifying the components such that the axes are orthogonally superimposed on the original EEG-plot data set, facilitates reading of the PCA graphs. Promax rotation was performed using “psych” and “GPArotation” functions (R Version 3.6.2).

Examination of promax-PC1, formed mainly by the H6-12 to H72-78 FC-scales, shows that the vast majority of neonates with a favorable outcome (normal development and moderate sequelae) showed better EEG-background activity than neonates with an adverse outcome (major sequelae or death before discharge).

Promax-PC2, formed mainly by the H30-36 to H72-78 ES-scales, shows that nearly all neonates that had epileptic seizures after 24 h of TH had an adverse outcome.

Promax-PC3, formed mainly by the H6-12 and H12-18 ES-scales, shows that both neonates with favorable and adverse outcomes can experience epileptic seizures during the first 24 h of TH.
Principal Component Analysis results as function of hospital

Bivariate plot of scores for PC1-PC2 and PC1-PC3 in neonates of the Amiens-Picardie University-Hospital (Center 1) [A] and the Lille Regional University-Hospital (Center 2) [B]. A jitter function was applied to distinguish individuals with the same X, Y, and Z coordinates. Three-dimensional graphs were created using “rgl” functions (R Version 3.6.2.).

No comparisons reached the statistical threshold of 0.05 for PC1, PC2, or PC3 (the p values were not corrected for multiple comparisons and were consequently very lax). These results show that confounding factors between hospitals, especially different protocols of sedation or antiepileptic drugs used, did not affect the EEG-plot features or their predictive value.
Hierarchical clustering on principal components (HCPC) and K-means clusters - Comparison of HCPC performed on linear versus non-linear PCA

Bivariate plot of scores for PC1 and PC2 of Cluster 1 (in blue) and Cluster 2 (in brown) determined by HCPC [A] and by K-means [B] clustering.

Cluster 1 corresponds to the “better” EEG-features, whereas Cluster 2 corresponds to the “worse” EEG-features. Neonates with a favorable outcome at two years were labelled from 1 to 32 (Center-1) and 46 to 76 (Center-2). Neonates with an adverse outcome were labelled from 33 to 45 (Center-1) and N° 77 to 95 (Center-2). The clusters diverged according to the clustering method for neonate 62, who was classified in Cluster-1 by HCPC and in Cluster-2 by K-means clustering. Brown circles indicate the neonates with an adverse outcome included in Cluster 1 (“better” EEG-features) and the blue circle indicates the child with a favorable outcome classified in Cluster 2 (“worse” EEG-features).

Comparison of HCPC performed on linear versus non-linear PCA. Non-linear PCAs (or Multiple Correspondence Analysis (MCA)) might be more appropriate for treating ordinal data. Nevertheless, nonlinear PCAs data are often instable for sample inferior to 100 individuals like in our study– with the consequence that non-linear PCAs on categorical data can underperform compared to linear PCAs on the same data but treated as ordinal data (on this point see:7-8).
A caution for the proper use of a linear PCA on ordinal data is to ensure that its results do not diverge from those of non-linear PCA (the data are coded in a categorical way and performed by a MCA). The HCPCs performed on the three first PCs resulting from the linear PCA (data treated as ordinal) or the non-linear PCA (data treated as categorical) diverged only for 3 neonates. The coincidence between the two classifications reached a very satisfactory level of 97%.

The prognostic performances of the HCPC performed with the non-linear PCA gave an efficiency of 93% [CI-95%: 85-97%] which was slightly inferior to those obtained with the HCPC performed with the linear PCA 96% [CI-95%: 90-99%] which (Indicators performances are detailed in the Table above).

| HPCP on       | EFFICIENCY       | PPV          | NPV           | SPECIFICITY     | SENSITIVITY    |
|---------------|------------------|--------------|---------------|-----------------|----------------|
| Linear PCA    | 96% [90-99%]     | 100% [87-100%] | 94% [86-98%]  | 100% [94-100%]  | 87% [70-96%]  |
| Non-linear PCA | 93% [85-97%]     | 100% [86-100%] | 90% [81-96%]  | 100% [94-100%]  | 77% [59-90%]  |

Prognostic performances of Support-Vector-Machine (SVM)

SVMs have been developed for binary classification\(^9\). This tool determines the optimal separation hyperplane between the two classes by maximizing the margin between the classes’ closest points. SVMs were performed using the “e1071” package\(^10\) (R Version 3.6.2).

SVM classifications were performed on the original data set: FC grade scale and ES grade for the seven periods of time. Performance indicators based on the clusters defined by SVM clustering for a favorable versus adverse outcome and were calculated for the entire sample (N=95) and after the exclusion of neonates who died before discharge (N=73) (Performance indicators of HCPC and K-means are also reported for comparison with SVM results).

|                | EFFICIENCY       | POSITIVE PREDICTIVE VALUE | NEGATIVE PREDICTIVE VALUE | SPECIFICITY | SENSITIVITY |
|----------------|------------------|---------------------------|---------------------------|-------------|-------------|
| [A] Total sample (N= 95) |                |                           |                           |             |             |
| SVM [CI-95%]   | 97% [91-99%]     | 100% [88-100%]            | 96% [87-99%]              | 100% [94-100%] | 90% [74-98%] |
| HCPC [CI-95%]  | 96% [90-99%]     | 100% [87-100%]            | 94% [86-98%]              | 100% [94-100%] | 87% [70-96%] |
| K-means [CI-95%]| 95% [88-98%]     | 96% [82-100%]             | 94% [85-98%]              | 98% [92-100%] | 87% [70-96%] |
| [B] Death before discharged excluded (N= 73) |                |                           |                           |             |             |
| SVM linear [CI-95%] | 97% [80-100%] | 100% [59-100%]          | 97% [89-100%]            | 100% [94-100%] | 78% [40-97%] |
| HCPC [CI-95%]  | 96% [88-99%]     | 100% [54-100%]            | 96% [87-99%]              | 100% [94-100%] | 67% [30-93%] |
| K-means [CI-95%]| 95% [87-98%]     | 86% [42-100%]             | 96% [87-99%]              | 98% [92-100%] | 67% [30-93%] |

Performance indicators obtained by SVM classifications were highly convergent with those resulting from HCPC and K-means classifications (for details, see: Table-2). The high convergence of the results obtained by classification methods based on very different mathematical tools suggests that these results have a satisfactory robustness. These results should nevertheless be confirmed in a new population (validation with a different sample from the development set is advised, ideally with a population outside the original cohort\(^11\)).
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