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Original

Establishing reference values for amplitude-integrated electroencephalography in preterms below 35 weeks of gestational age: A prospective observational cohort study

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Characteristic patterns in amplitude-integrated electroencephalography (aEEG) develop with gestational age (GA), and so can be used to evaluate brain maturation in premature infants. Reference aEEG values in normal preterm infants have not been identified and data are scarce. We aimed to validate a currently available aEEG scoring system. We also investigated the development of aEEG activity during the first week after birth, determining reference values in preterm infants with no abnormal cranial ultrasound findings. We prospectively studied aEEG and cranial ultrasounds in infants with a GA of <35 weeks. We conducted aEEG at 12–14 hours, 46–48 hours, 70–72 hours, and 1 week after birth. The aEEG recordings were evaluated using Burdjalov criteria, scored by two independent neonatologists. Thirty-four infants were enrolled and completed the 1-week evaluation. GA ranged from 24 to 35 weeks and birth weights varied between 570 and 2,100 g. We analyzed 134 aEEG tracings, with a mean difference between raters of −0.05. Total scores, summed from scores for each assessed variable, increased gradually with advancing gestational and postnatal age. However, highest scores were not attained until 35 weeks' gestational age. There was high inter-rater agreement for aEEG scoring, and we could ascertain some approximate reference values for aEEG development in preterm infants at varying GA. To establish standardized aEEG reference criteria, further studies in larger cohorts of premature infants should be performed over longer periods.

Key words: amplitude-integrated electroencephalography, cerebral function monitor, preterm infant, reference values.

In a study by Stoll BJ et al.[1] the survival rate for very premature infants has improved following advances in neonatal intensive care during the past decade. However, very low birth weight infants are still at a high risk for various neurologic injuries, including periventricular leukomalacia (PVL), intraventricular hemorrhage (IVH), and hypoxic-ischemic encephalopathy (HIE), which occur during the neonatal period. Therefore, improved surveillance of cerebral function is required during this critical period in the neonatal intensive care unit (NICU).

Electroencephalography (EEG) is a sensitive technique to evaluate the degree of neurological insult in neonates. However, because it requires an EEG specialist, physicians often have difficulty interpreting neonatal EEG, and it is inconvenient to use. In contrast, amplitude-integrated EEG (aEEG) conducted using a cerebral function monitor (CFM) is comparable to electrocardiogram monitoring in the NICU. According to Azzopardi D[2], it is a readily available tool for the evaluation of EEG activity at the bedside, and it is easy for neonatologists to learn to use the technique. Since the first applications of aEEG to newborns in the late

1970s and early 1980s[3], it has been increasingly used in neonatal centers to evaluate hypoxic-ischemic brain injury[4], detect brain lesions earlier[5], monitor the influences of different interventions[6], and predict future outcome[7].

Despite several studies on the development of aEEG activity in preterm infants[8], aEEG data in Korean preterm infants remain scarce, with only two studies by Sohn JA et al. [5] and Lee HJ et al. [9] performed to date. Indeed, the use of aEEG has not yet become a standard of care for premature infants in NICU. It has mainly been used to monitor the effectiveness of treatment in infants receiving hypothermic treatment. Additionally, a major problem with the current use of aEEG is that the majority of researchers have used subjective visual assessment of patterns when assessing aEEG tracings. Therefore, there are difficulties when comparing different tracings, either from the same infant at different postnatal ages or from different infants. The aEEG evaluation criteria proposed by Burdjalov et al.[10] have been widely used to evaluate developmental aEEG patterns because they consider overall brain function[11]. However, no previous studies have independently validated the Burdjalov scoring system in a Korean sample. Initially, baseline aEEG patterns must be elucidated such that deviations can be accurately identified allowing early diagnosis of brain lesions and prediction of prognosis, and to inform treatment decisions. It is particularly important to consider that most preterm infants require a period of stabilization after birth.

In this study, we aimed to establish reference values for aEEG in preterm infants below 35 weeks' gestational age (GA) using the Burdjalov scoring system. We initially aimed to validate a currently available scoring system that has previously been used to interpret aEEG. This validation was conducted by investigating the level of agreement between two raters independently applying the scoring system. The ultimate aim of the study was to elucidate aEEG reference values for normal premature infants with a GA of <35 weeks. Therefore, we subsequently examined the longitudinal development of aEEG activity during the first week of life in preterm infants who had no abnormality of cranial ultrasounds. We then scored each aEEG tracing using previously described techniques for interpreting aEEG.

Material and Methods

Study Population

This is a prospective observational study of premature infants admitted to the NICU at Pusan National University Children's Hospital, Yangsan, Korea between April 2015 and March 2016. Infants were included if they were <35 weeks' GA at birth. Exclusion criteria for study participation were: 1) transferred after birth; 2) presence of known or suspected major congenital malformation and/or chromosomal abnormality; 3) perinatal asphyxia; 4) IVH or PVL on cranial ultrasounds; and 5) metabolic disorders or central nervous system infection.

Perinatal asphyxia was suspected if there was evidence of fetal distress (heart rate <100/min, late decelerations, variable heart rate, or meconium-stained amniotic fluid), and if at least one of the following were present: Apgar score <6 at 5 minutes, or a pH <7 or base deficit >12 mmol/L for cord blood or blood sample obtained within 60 minutes of birth. IVH was classified according to Papile et al.[12], determined by whether there was germinal matrix hemorrhage with/without ventricular dilatation and parenchymal hemorrhage. According to a study by de Vries LS et al.[13] PVL was diagnosed by the presence of localized or extensive echo-lucent areas in the periventricular area. We did not exclude infants who had received a central sedative before 12 hours of aEEG monitoring or arousing medication at any point.

This study was approved by the Institutional Review Board (IRB) of the Pusan National University Yangsan Hospital (IRB No. 05-2015-034). Written informed consent was obtained from the parents of each patient prior to study participation.

Data collection

We recorded demographic information including GA at birth, birth weight, Apgar score at 5 minutes, multiple pregnancies, sex, small for gestational age (SGA), postnatal age (PNA) at the time of aEEG recordings, and clinical condition of patients. GA was assessed from the last menstrual period and/or using prenatal ultrasonography. Clinical information was noted including the presence of patent ductus arteriosus, respiratory distress syndrome, use

of mechanical ventilation, medication history including use of inotropic medications or sedatives, and survival. If a large amount of left-to-right shunting over the ductus arteriosus was diagnosed, pharmacological treatment with ibuprofen (Pedea; Orphan Europe SARL, Puteaux, France) was prescribed or ligation was performed. Infants were classified as SGA at birth using revised Fenton growth charts from 2013[14], with SGA defined as having a birth weight lower than the 10th percentile for their sex and GA.

aEEG monitoring

The aEEG recordings were performed in each infant four times to evaluate brain function and maturation in the early period of life. According to our study protocol, aEEG tracings were performed at 12–14 hours (aEEG-1), 46–48 hours (aEEG-2), 70–72 hours (aEEG-3), and 1 week (aEEG-4) after birth. We used a cerebral function monitor (CFM; Olympic CFM 6000, Natus Medical Incorporated, Pleasanton, CA, United States) with disc electrodes prepared with Elefix® paste (Nihon Kohden Corporation, Tokyo, Japan) to achieve low impedance. Full details of the aEEG technique have been outlined in a study by Hellstrom-Westas L et al. [15]. In short, two electrodes attached to the infant's scalp receive the single-channel EEG signal (C3–C4), with a third electrode (reference electrode) placed toward the front of the head as a ground to suppress noise from other sources. The signal was filtered, rectified, smoothed and selectively amplified. The speed of recording was regulated at 6 cm/h. The quality of the aEEG trace was monitored using a simultaneous continuous impedance trace, and aEEG samples with an impedance of >20 kΩ were discarded. The CFM was calibrated prior to each recording. Each recording lasted at least 2 hours. Routine nursing care periods or handling were marked on the tracing. The quality of the recording was checked frequently by one of the investigators or by the nurse caring for the infant.

Interpretation of aEEG tracings

The aEEG tracings were interpreted using criteria previously described by Burdjalov et al. Four components of the tracing were assessed: continuity, sleep–wake cycling, amplitude of the lower border, and bandwidth span. These components can be summated into a total score ranging from 0 to 13[10,16,17] (Table I). Each criterion is described more fully below: 1) Continuity: This was assessed by observing the overall density of the sample tracing. This refers to the appearance of frequent variations in the aEEG electrical activity response. High levels of continuity indicated constant and frequently alternating electrical activity (pen peaks and troughs), such that the recording appeared either very tightly compressed on the tracing or not. Low levels of continuity had much reduced electrical variation, with

Table I. Amplitude-integrated Electroencephalography (aEEG) Scoring System According to the Method Proposed by Burdjalov et al.[10]

Score	Continuity	Cycling	Amplitude of lower border	Bandwidth span and amplitude of lower border
0	Discontinuous	None	Severely depressed (<3 μV)	Very depressed low span (≤15 μV) and low voltage (5 μV)
1	Somewhat continuous	Waves first appear	Somewhat depressed (3–5 μV)	Very immature high (>20 μV) or moderate (15–20 μV) span and low voltage (5 μV)
2	Continuous	Not definite, somewhat cycling	Elevated (>5 μV)	Immature high span (>20 μV) and high voltage (>5 μV)
3		Definite cycling, but interrupted		Maturing moderate span (15–20 μV) and high voltage (>5 μV)
4		Definite cycling, non-interrupted		Mature low span (<15 μV) and high voltage (>5 μV)
5		Regular and mature cycling		

greater separation of the recording signal peaks and troughs. 2) Cycling: This refers to the emergence and progression of periods during the CFM epoch analyzed, where the peak-to-trough width of the recording would expand and subsequently contract. It was observed as variations in both amplitude and continuity of electrical activity on aEEG tracings. 3) Amplitude of the lower border (μV): The magnitude of the CFM tracing's lower border (voltage troughs) was estimated as the average lower microvolt level during the recording epoch. A line drawn though the lower margin of the aEEG band appeared with half of the microvolt troughs below the line and half above. With the emergence of cycling, the narrowest part of the recording was evaluated. 4) Bandwidth: This reflects a combination of the voltage span (peak-to-trough) of the tracing and the magnitude of the aEEG depression (amplitude of the lower border). The span was calculated as the difference between the upper and lower voltage margins of the tracing's narrowest part.

The individual component variable scores and total scores from all aEEG recordings were subsequently evaluated in relation to each infant's GA and PNA. Two independent neonatologists (Y.M.H and N.R.L) scored the aEEG and were blind to other information.

Cranial ultrasounds

Cranial ultrasound scans were conducted during the first week of life using a Voluson™ *i* (GE Healthcare, USA) with an RNA5-9 RS transducer. Depending on initial ultrasound findings and clinical changes, subsequent cranial ultrasound evaluations were prescribed and conducted once a week until discharge. Ultrasound scans were checked and assessed by one radiologist. IVH and PVL were classified according to Papile et al.[12] and de Vries et al.[13], respectively.

Statistical analysis

Bland–Altman analyses were performed to determine the extent of agreement between the aEEG interpretations of the two raters. To investigate the influence of GA and PNA on aEEG activity, participants were divided into four groups: Group 1, GA 24–26 weeks; Group 2, GA 27–29 weeks; Group 3, GA 30–32 weeks; and Group 4, GA 33–35 weeks. All

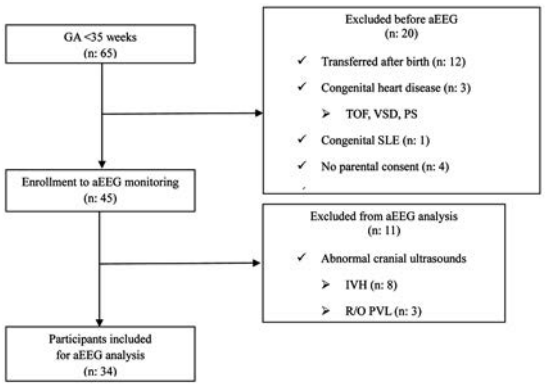

Fig. 1. Overview of included and excluded participants
Thirty-four clinically stable preterm infants with normal cranial ultrasounds were included. aEEG: amplitude-integrated electroencephalography; GA: gestational age; IVH: intraventricular hemorrhage; NICU: neonatal intensive care unit; PS: pulmonic stenosis; PVL: periventricular leukomalacia; TOF: tetralogy of Fallot; VSD: ventricular septal defect;

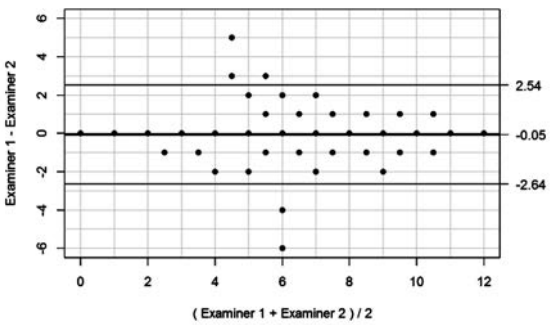

Fig. 2. Bland–Altman analysis
Differences in the aEEG total scores between rater 1 and 2 are plotted against the averages of the two raters' scores for 134 paired measurements. The mean of the raters' scores is shown on the x-axis. Differences in aEEG total scores are shown on the y-axis. The solid line indicates the mean differences between the raters' scores, and the dashed lines indicate the 95 % confidence intervals of these differences.

statistical calculations were conducted in SPSS version 20.0 (IBM Corp., Chicago, IL, USA) and parametric statistics were applied. To compare group means, analysis of variances (ANOVA) was applied. Chi-square was conducted to compare categorical variables. Fisher's exact test was applied to assess changes in aEEG measures (four components of the tracing, and total score) over time for each group. Data were found to be normally distributed so are presented as mean and standard deviation. *P* values less than 0.05 were considered statistically significant.

Results

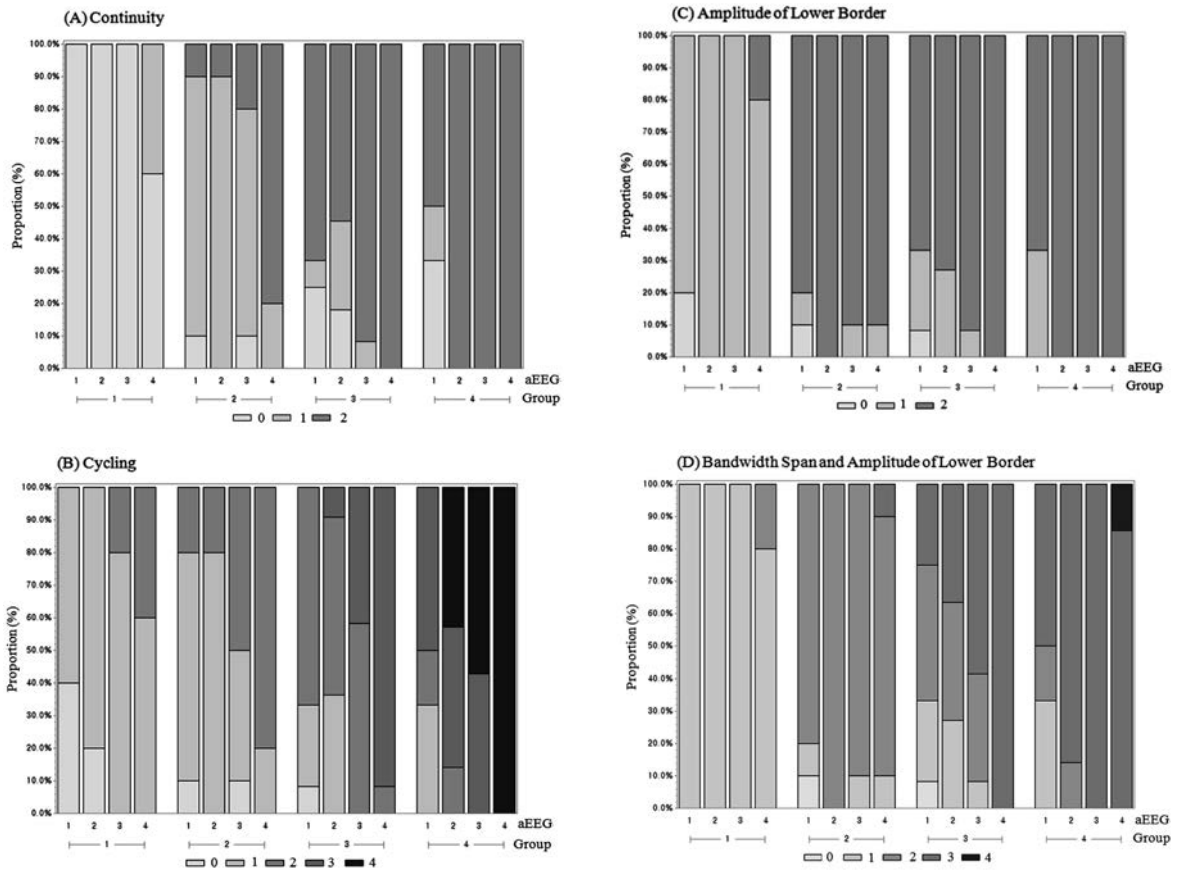

Fig. 3. Distributions of individual Burdjalov scores for each monitoring period
Each figure panel is divided into four parts according to the different gestational groups (Group 1, gestational age 24–26 weeks [n: 5]; Group 2, gestational age 27–29 weeks [n: 10]; Group 3, gestational age 30–32 weeks [n: 12]; Group 4, gestational age 33–35 weeks [n: 7]). The different time points of aEEG evaluation are shown as marked on the x-axis. The score of aEEG for each parameter is colored. The greater the gestational age, the more mature the aEEG tracing is at birth, and with increasing postnatal age, a progressive maturation of the aEEG pattern becomes apparent.

Study population

During the study period, 65 preterm infants with a GA <35 weeks at birth were eligible for inclusion. Finally, 34 infants were enrolled in the study, all of whom completed the 1-week aEEG evaluation (Fig. 1).

After excluding two aEEGs with an impedance of >20 kΩ, 134 aEEGs were analyzed: 33 at 12–14 hours, 33 at 46–48 hours, 34 at 70–72 hours, and 34 at 1 week of life. To evaluate the influence of GA on aEEG activity, participants were divided into four groups: Group 1, GA 24–26 weeks (n: 5); Group 2, GA 27–29 weeks (n: 10); Group 3, GA 30–32 weeks (n: 12); Group 4, GA 33–35 weeks (n: 7). There was no significant difference in birth weights between Group 2 and Group 4 because of a large proportion of SGA in Group 4. The demographic and clinical characteristics of the infants are shown in Table II.

Inter-rater agreement for aEEG total scores

Figure 2 shows Bland–Altman plots and 95% confidence intervals (CI) for the agreement between the two raters for aEEG total score for each aEEG recording and their average. The mean difference between raters at each time point was 0.15 (95% CI −1.44 to 1.74) at the first aEEG, 0.09 (95% CI −2.43 to 2.61) at the second aEEG, 0.12 (95% CI −2.13 to 2.36) at the third aEEG, and 0.15 (95% CI −1.77 to 2.06) at the last aEEG. The mean difference for the sum of these was −0.05 (95% CI −2.64 to 2.54). The Bland–Altman plots show that most of the differences were within ± 1.96 SD, suggesting that the differences are not significant. The correlation coefficient between scores made by the two raters was 0.899 (range: 0.861–0.927). Among the four components of

this proposed scoring system, cycling has the largest impact on the differences between the raters owing to its inherent subjectivity.

aEEG and GA

We found a significant positive effect of GA on all four components of the aEEG tracings. With increased GA, the aEEG becomes more continuous, displays definite sleep–wake cycles, the lower border amplitude score increases, and its bandwidth acquires a mature pattern. This was true at all assessed time points across the first week after birth.

For instance, at aEEG-1, Group 1 showed a relative duration of continuous cerebral activity of 0 %, Group 2 of 10 %, Group 3 of 66 %, and Group 4 of 50 % ($P = 0.012$). Forty percent of Group 1 had no sleep–wake cycling, 70 % of Group 2 showed some cycling, 66 % of Group 3 showed definite cycling with interruption, and 50 % of Group 4 showed cycling without interruption ($P = 0.013$). Severely depressed traces were detected in 20 % of Group 1, and 10 % of both Group 2 and 3. Meanwhile, 30 % of Group 4 showed a somewhat elevated amplitude of the lower border (p=0.003). Regarding bandwidth and amplitude of lower border, 100 % of Group 1 had high (>20 μV) or moderate (15–20 μV) span and low voltage (5 μV), 80 % of Group 2 had high span (>20 μV) and high voltage (>5 μV), and 25 % of Group 3 and 50 % of Group 4 had moderate span (15–20 μV) and high voltage (>5 μV) (p=0.002) (Fig. 3).

aEEG and PNA

A positive significant interaction was found in all groups between PNA and continuity, sleep–wake cycling, the amplitude of lower border, and bandwidth ($p < 0.05$). There was an increase in the duration of continuity, and a decrease in the duration of total discontinuous pattern as PNA increased in all groups.

At aEEG-4, 40 % of Group 1 showed somewhat continuous cerebral activity, while 80 % of Group 2 and all of Groups 3 and 4 showed continuous cerebral activity ($p < 0.001$). At this time, 100 % of Group 1 had first waves or somewhat cycling, 80 % of Group 2 had somewhat cycling, 90 % of Group 3 showed definite cycling with interruption, and 100 % of Group 4 showed definite cycling without interruption ($p < 0.001$). Eighty percent of Group 1 showed a relatively elevated amplitude of lower border, 90 % of Group 2, and 100 % of Groups 3 and 4 showed an elevated amplitude of lower border (p=0.005). Assessment of bandwidth showed 80 % of Group 1 had a score of 1, 80 % of Group 2 had a score of

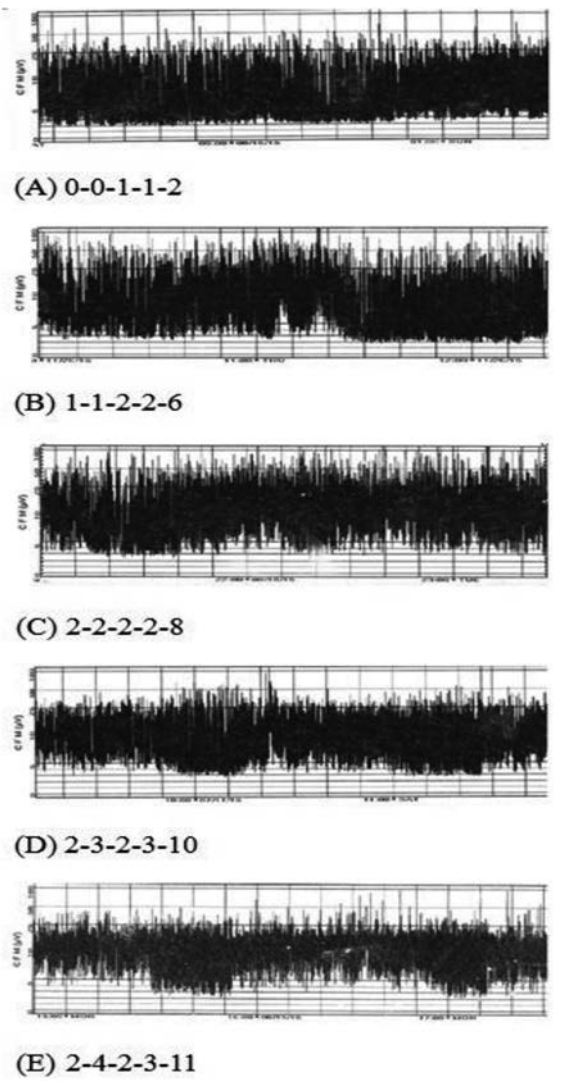

Fig. 4. Examples of the tracing analysis of aEEG
(A) Discontinuous aEEG background and no sleep-wake cycles at 25 weeks of gestation; **(B)** Continuous and discontinuous aEEG background with the beginning of sleep-wake cycles at 27 weeks of gestation; **(C)** Predominantly continuous aEEG background with some cycling at 29 weeks of gestation; **(D)** Continuous background with immature sleep-wake cycling at 31 weeks of gestation; **(E)** Continuous background with cyclical sinusoidal variations describing sleep-wake cycles at 34 weeks of gestation. The bottom numbers show the component score values for the respective chart. From the left number, these are 'continuity-cycling-amplitude of lower border-bandwidth span and amplitude of lower border-total score'. There is maturation of tracings from A to E.

Table II. Demographic and Clinical Characteristics of the Gestational Age Groups

Parameters	Group 1 (GA: 24–26) (n: 5)	Group 2 (GA: 27–29) (n: 10)	Group 3 (GA: 30–32) (n: 12)	Group 4 (GA: 33–35) (n: 7)	p*
Gestational age (weeks)	24.6 ± 0.5	28.1 ± 0.7	30.9 ± 0.8	33.9 ± 0.9	<0.001
Birth weight (g)	760.0 ± 47.9	1,030.0 ± 282.8†	1,652.5 ± 323.1	1,268.6 ± 167.0†	<0.001
Apgar score at 5 min	6.0 ± 0	6.3 ± 0.7	6.8 ± 0.7	7.4 ± 0.8	0.003
Multiple pregnancy, n (%)	4 (80.0)	1 (10.0)	2 (16.7)	3 (42.9)	0.023
Male, n (%)	3 (60.0)	4 (40.0)	7 (58.3)	1 (14.3)	NS
Small for gestational age, n (%)	0 (0)	2 (20.0)	1 (8.3)	7 (100)	<0.001
Patent ductus arteriosus, n (%)	2 (40.0)	2 (20.0)	4 (33.3)	1 (14.3)	NS
Respiratory distress syndrome, n (%)	5 (100)	9 (90.0)	10 (83.3)	1 (14.3)	0.001
Mechanical ventilation, n (%)	5 (100)	10 (100)	12 (100)	3 (42.9)	0.001
Inotropic medication, n (%)	1 (20.0)	3 (30.0)	1 (8.3)	0 (0)	NS
Sedative medication, n (%)	0 (0)	2 (20.0)	0 (0)	0 (0)	NS
Survival, n (%)	3 (60.0)	8 (80.0)	12 (100)	7 (100)	NS

Values are mean ± standard deviation unless otherwise stated. GA: gestational age (in weeks); NS: not significant.
* For any difference between the four groups.
†P >0.05 by analysis of variances.

2, 100 % of Group 3 had a score of 3, and 100 % of Group 4 had scores of 3 and 4 (p <0.001). With advancing PNA, scores for each variable, as well as the total score, progressively increased with CNS maturation (Fig.3).

Reference values for aEEG according to GA in the first week of life

Examples of the tracing analysis are shown in Fig. 4 and rounded averages of our study findings are displayed in Table III. These show some approximate reference values for the aEEG development in preterm infants with respect to varying GA and PNA. We observed increases in scores for the four components represented by changes in the aEEG visual patterns for each GA group. The total score calculated for each recording gradually increased with GA and PNA.

Discussion

In this study, we assessed serial aEEGs during the first week of life using an objective scoring system to evaluate the development of neurologically normal premature infants. The components of the aEEG tracings that we scored included continuity, presence and stage of sleep-wake cycling, the degree of voltage amplitude depression, and bandwidth span, all of which have been previously verified[10]. We could confirm that inter-rater agreement was high for most components of the tracing. There were progressive increases with advancing GA and PNA in all four component scores and the total score derived from these. We validated the values representative of aEEG patterns in a preterm infant, which correlated with maturation of these infants. Our findings are in agreement with results from previous aEEG studies by Soubasi V et al. [18].

One of the benefits of aEEG is the possibility of quick interpretation. However, there is still no consistent agreement regarding the assessment of aEEG recordings that change with increasing GA[8]. There have been several studies on the interpretation of aEEG using various methods. One method, the al Naqeeb classification system,[19] is based on the numeric value of the upper and lower voltage margins of the aEEG trace. This simple but accurate method is currently used in the assessment of infants with encephalopathy. However, it has limitations in the evaluation of development of preterm infant aEEG tracings. Most previous studies of aEEG in preterm infants have adopted pre-established criteria by Burdjalov et al.[10], Olischar et al.[16], and Hellström-Westas et al.[17]. These may have been applied independently [5,20], in a modified form[21], or combined with other methods [7,11,18,22]. However, many studies have focused on preterm infants with abnormalities that represent a deviation from normal rather than baseline value. In this study, we aimed to

identify the baseline aEEG activity of preterm infants. As such, we chose the previously described criteria by Burdjalov et al. as we considered them objective and reproducible in repeated assessments of aEEG tracings. Moreover, these criteria have been used widely in developmental evaluations of aEEG because they evaluate overall brain function[10]. Using these criteria, we found that inter-rater agreement was high for most components of the tracing, and the results corresponded with previously reported subjective visual observations of aEEG patterns [9,11,18]. Therefore, we believe that aEEG can be used to compare different tracings in a variety of ways when evaluating preterm infants using this scoring system. However, the proposed scoring system has some inherent subjectivity, especially when assessing cycling, which has the largest impact on differences between raters. We expect that with technological advances, such as proposed methods for automatic computation of various measurements, the problem of subjectivity can likely be overcome.

All preterm and term neonates physiologically show ontogenic maturation in the background activity of EEG. Three major determinants of ontogenic maturation are continuity, symmetry, and synchronicity. Therefore, the most important factor to consider when interpreting neonatal EEG and aEEG is the GA of the infant. In this study, we found that continuity increased progressively, reaching its maximum by 30 to 32 weeks' postmenstrual age, and this continuous background pattern was established in the earlier development stages. Furthermore, with advancing GA, cyclical periods reached a more mature pattern by 36 weeks' postmenstrual age (score of 4), but we did not observe a completely mature pattern until 36 weeks' postmenstrual age (score of 5). After 27 weeks' postmenstrual age, the lower border amplitude of the aEEG band increased and the bandwidth gradually became narrower, between postmenstrual ages of 30 to 34 weeks, with scores of 3.

One major feature of EEG in extremely preterm infants is discontinuity. Several previous aEEG studies evaluating preterm infant brain maturation have focused on continuity. Olischar et al.[16] published reference aEEG values for continuity in preterm infants within the first 2 weeks of life. Andre et al.[23] showed that in preterm infants with increasing GA, the duration of EEG bursts increases and the inter-burst interval decreases. Connell[24] reported that the proportion of EEG tracings with continuous activity increases from GA between 26 and 37 weeks, whereas, the rate of discontinuous activity decreases with increasing GA. Additionally, it was proposed that the continuity of aEEG may depend more on the general status of CNS electrical activity than on maturity[10]. In agreement with their conclusions, we found that with increasing GA, continuous background activity increased, but discontinuous low-voltage activity decreased.

Burdjalov et al.[10] reported that the emergence of a cycling pattern seemed to have the highest correlation with postconceptional age and could be considered the single best determinant of cerebral maturity. This finding might be explained by the emergence and establishment of sleep-wake cycles determined by integration of higher CNS functions. In our study, we observed cyclical changes in aEEG background activity with advancing GA and PNA in all the infants. We were able to observe sleep-state differentiation at 26 weeks' postmenstrual age. These findings are in accordance with previous study by Selton D et al. [25]. In a study by Kuhle

Table III. Reference Values of aEEG According to Gestational Age in the First Week of Life.

	Group 1 24–26 weeks	Group 2 27–29 weeks	Group 3 30–32 weeks	Group 4 33–35 weeks
Continuity (0–2)	0	1	2	2
Cycling (0–5)	0–1	1–2	2–3	3–4
ALB (0–2)	1	2	2	2
BS and ALB (0–4)	1	2	2–3	3–4
Total Score (0–13)	2–3	6–7	8–10	10–12

Figures in parentheses are the range of possible scores. ALB: amplitude of lower border; BS and ALB: bandwidth span and amplitude of lower border.

S et al. [26] aEEG recordings have shown cyclic organization of cortical activity in premature newborns of less than 30 weeks' GA and even as early as 24 weeks. In their aEEG study, Sisman et al.[8] found that continuity and cycling are accelerated in extremely preterm infants. In a conventional EEG study with preterm infants between 24 and 27 weeks' GA by Scher MS et al.[27], the alternation between periods of wakefulness/active sleep and quiet sleep was observed. Similarly, Hellström-Westas et al.[17] showed that intact sleep organization and an increase in the minimum amplitude during quiet sleep are good prognostic factors in preterm infants. In our study, we found that the sleep-wake cycle improved at a gradual constant rate even if other components had already reached a mature pattern earlier. Even though we did not observe a fully developed cycling pattern (due to the length of the monitoring period), we believe the sleep-wake cycle may be a good indicator of brain maturation.

Amplitude is another important component of aEEG evaluation. In a conventional EEG study by Vecchierini MF et al.[28], the amplitude of electrical activity decreases with CNS maturation. Hellström-Westas et al.[17] have reported minimum and maximum amplitude values for aEEG tracings at different GA and PNA, showing a clear gradual increase in the former and a decrease in the latter. In our study, we assessed both amplitude of the lower border and bandwidth, and our findings are in accordance with the above-mentioned studies.

In our study, there was a high proportion of SGA in Group 4. According to our NICU admission policy, healthy preterm infants with a GA ≥35 weeks and a weight ≥2 kg at birth are not hospitalized. Thus, there was a bias, with Group 4 comprising a SGA preterm population. Previous studies by Ozdemir OM et al.[29] and Yerushalmy-Feler A et al. [20] have reported the effects of intrauterine growth restriction (IUGR) on EEG findings in term infants and the effects of IUGR on neonatal aEEG data in preterm infants. Both of the studies report altered EEG activity and delayed maturity in infants with IUGR. Conversely, one case report by Kazanci E et al. [30], which studied functional brain maturity in discordant twins, reported that the IUGR brain may preserve itself from the deleterious effects of nutrient deprivation. In this study, we did not consider SGA in our aEEG scoring. However, Group 4, which included a high percentage of SGA, showed high scores of both individual Burdjalov scores and total scores. This result might support those of the case report cited previously, suggesting that further studies on the effects of IUGR are needed.

The present study has several limitations. First, the sample size was small. This was due to our exclusion criteria, particularly because many unstable infants were transferred after birth from other hospitals to our hospital. Further studies with larger numbers are needed. A second potential limitation of our study is that the duration of the aEEG recording was rather short to observe full maturation. Finally, there was a selection bias with regard to SGA in the population of late preterm infants as discussed above.

Our results confirm that the maturation of the aEEG tracing in stable premature infants depends on both GA and PNA. The greater the GA, the more mature the aEEG tracing is at birth. In addition, with increasing PNA, a gradual maturation of the aEEG pattern becomes apparent. These preliminary results enable us to ascertain some approximate reference values for the development of aEEG tracings in preterm infants with respect to varying GA and PNA. However, to establish standardized aEEG reference criteria for preterm infants, further studies in larger cohorts of premature infants should be performed over longer periods. In particular, the aEEG recording should be performed at least once after 36 weeks' postmenstrual age to cover the complete maturational process.

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