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Seizures in Pre-term Infants Less than 29 Weeks: Incidence, Etiology, and Response to Treatment

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ABSTRACT

Introduction. Seizures are neurological emergencies with short- and long-term adverse effects in pre-term infants. They may present with or without abnormal movements (clinical versus subclinical). Thus, the true incidence of seizures may be under-reported. Current research indicates that most seizures occur in the first few days of life, are associated with intraventricular hemorrhage (IVH), and show low response to anticonvulsant drugs. The purpose of this study was to evaluate incidence, etiology, clinical antecedents, mortality, and response to treatment of seizures in extremely pre-term infants.

Methods. This is a retrospective cohort study of pre-term infants < 29 weeks gestation from January 2011 to December 2013. Presence or absence of seizure was the outcome. Data extraction included demographics, medications, co-morbidities, mortality, and details of seizures. A multivariable prediction model was developed to evaluate risk for seizures.

Results. Analysis included 269 pre-term infants. Incidence of EEG-confirmed seizures was 40% (108/269); 49% were clinical and 51% were subclinical. Seizures occurred in 72% of infants ≤ 24 weeks, 57% of those 25-26 weeks, and 23% of those 27-28 weeks. Most seizures (85%) occurred after day eight of life. Mortality was 14% in those with seizures versus 5% in those without (p = 0.019). The model showed seizures were associated significantly with gestational age and medications, while controlling for sex, APGAR score, and co-morbidities, including IVH. At discharge, anticonvulsants were continued in 66% (72/108) of infants with seizures.

Conclusion. The incidence of seizures was highest in infants born most premature. Contrary to previous research, nearly two-thirds of pre-term infants with seizures did not have IVH or cystic periventricular leukomalacia; apnea of prematurity was a common presentation of subclinical seizures; and the majority of treated infants responded to Phenobarbital. These findings need be explored in future research.

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INTRODUCTION

Any seizure occurring in a term or pre-term infant may indicate a neurological emergency demanding urgent diagnosis and management.[1] However, the definition and identification of seizures, especially in pre-term infants, is an area of ongoing controversy.[2] Seizures are difficult to diagnose in pre-term infants because they may have uncoordinated movements unrelated to seizures, repetitive movements that may or may not indicate seizures, or subclinical seizures with no abnormal movements.

Volpe outlined five main types of neonatal seizures: subtle (subclinical), tonic, clonic, myoclonic, and non-paroxysmal repetitive behaviors.[3] Subclinical seizures may be the most difficult to detect because they imitate normal behaviors. Conversely, tonic, clonic, and myoclonic seizures are clinical seizures involving motor movements, presenting as rhythmic jerks or sustained contractions that are repetitive and may last one to two minutes or longer.[1]

Hypoxic-ischemic encephalopathy in full term infants is responsible for as much as 80% of all seizures in the first two days of life.[1] Davis et al.[4] found an increased risk of clinical seizures in extremely low birth weight infants (birth weight 401-1000 grams) who had severe intra-ventricular hemorrhage (IVH), sepsis, meningitis, and/or cystic periventricular leukomalacia (PVL). Research is ongoing to understand the various causes of seizures in pre-term infants.

Acutely, seizures (clinical/subclinical) in pre-term infants can result in significant decrease in oxygenation, bradycardia, apnea, and deterioration of cardiorespiratory status. Pre-term infants with seizures are at significantly higher risk for mortality, and in the long-term are at risk for neuro-developmental impairment (cognitive, motor, language skills), moderate to severe cerebral palsy, and epilepsy.[4,5]

The incidence of seizures in pre-term infants may vary widely and is dependent on the method used to diagnose. The incidence of clinical seizures in pre-term infants was 4 to 58% in studies without electroencephalogram (EEG) confirmation.[6-9] It is difficult to diagnose seizures in infants based solely on clinical features without the aid of EEG.[10] There is also a high frequency of electro-clinical dissociation in infants with seizures, meaning that infants may not show abnormal clinical movements in the presence of seizures.[11] Hence, there is a significant risk of under-diagnosis of seizures in pre-term infants without the use of EEG.

Clinicians and researchers have used one of three types of EEG to monitor and diagnose seizures in pre-term infants. These three include: conventional 21 channel EEG (with or without video), continuous video EEG (cEEG), and amplitude-integrated EEG (aEEG). While cEEG may be the preferred method ("gold standard") for diagnosing neonatal seizures, there are significant practical barriers to implementation, such as availability of expensive equipment, technologists to do the test, and clinical neurophysiologists to interpret the EEG.[12,13]

Results from EEG monitoring show variability in the incidence of seizures. For example, the incidence in pre-term infants using cEEG in the first 72 hours of life was 5%,[14] while incidence with aEEG ranged from 20% to 48%.[15-17] A recent study compared simultaneous use of conventional EEG and aEEG monitoring and found that aEEG had a low sensitivity (50%) and low specificity (46%), and misclassified some events as seizures.[18] Moreover, aEEG might miss seizures that occur far from the electrodes or show false positive seizures due to artifacts.[19]

With the increased survival for extremely pre-term infants, there is an increased number at risk for seizures. Given the difficulty of

diagnosis of seizures in this population, the true incidence of seizures may be under-reported. Therefore, the purpose of this study was to evaluate extremely pre-term infants (< 29 weeks gestation) and measure the incidence and risk of EEG confirmed seizures, retrospectively. Etiology, clinical antecedents, mortality, and response to treatment of seizures also were explored.

METHODS

A retrospective cohort study was conducted with pre-term infants < 29 weeks gestation who were admitted to the neonatal intensive care unit (NICU) between January 1, 2011 and December 31, 2013. The institutional review board at the Wichita Medical Research and Education Foundation approved the study. Figure 1 shows the patient flowchart. First, all infants admitted to the NICU were assessed for gestational age and the presence or absence of clinical seizures. Second, pre-term infants < 29 weeks gestation were identified for inclusion into the analyses.

Suspected Seizures. Clinical seizure was suspected based on abnormal movements (tonic, clonic, myoclonic, or subtle tongue thrusting/cycling). In addition, seizures were suspected in infants with refractory apnea defined as apnea (without any abnormal movements) persisting despite treatment with Caffeine (20 mg/kg loading and 10 mg/kg maintenance), appropriate respiratory support, and when other causes of apnea, such as sepsis, were ruled out. The outcome used for the analysis was a dichotomous variable that represented presence or absence of seizures confirmed by conventional EEG.

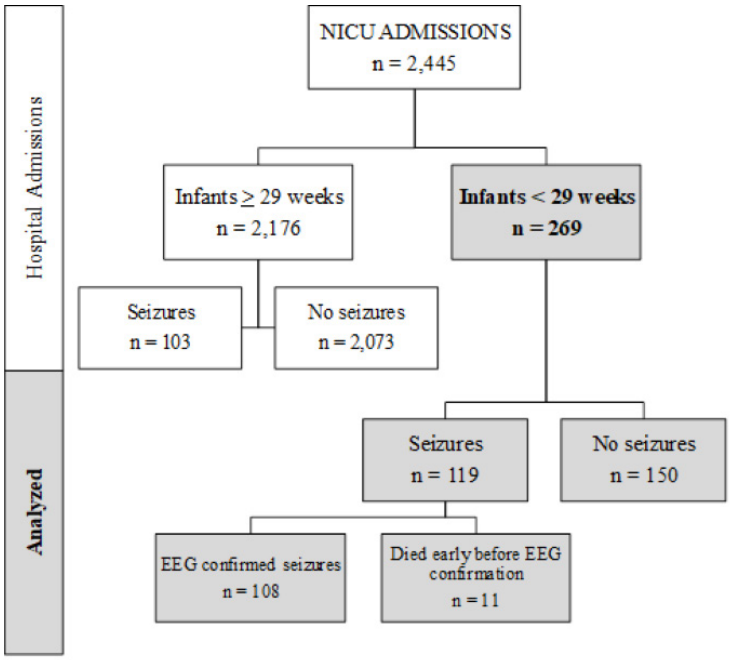

Figure 1. Participant flowchart.

Diagnostic Criteria for Seizure Using Conventional EEG. Conventional electroencephalography (Nihon-Kohden, Tokyo, Japan) was performed in pre-term infants suspected to have seizures, using the 10/20 international system of electrode placement and the neonatal electrode montage and recording through 17 to 21 channels for 30 to 60 minutes. The conventional EEG diagnostic criteria for electrical seizures were rhythmic, repetitive, stereotypic waveforms with an evolution of morphologic features, amplitude, or electric field, lasting greater than 10 seconds with minimum amplitude of 2 μV.[12] Seizures also were diagnosed when the EEG was positive for multiple electrical seizures and/or focal/multifocal sharp wave discharges. In select cases, continuous EEG (cEEG) was performed for 24 hours. Amplitude-integrated EEG (aEEG) was not used in this study.

A pediatric neurologist (SS), with 25 years' experience, interpreted the EEG studies (conventional and continuous). Repeat EEG was done at periodic intervals to diagnose resolution of seizures.

Identifying Etiology of Seizures. To identify etiology of seizures, the patient history, examination, laboratory tests, and imaging studies were reviewed. Laboratory studies included serum electrolytes, sepsis workup, cerebrospinal fluid studies (bacterial and viral), acid-base analysis, and state newborn screen. Workup for metabolic disorder (serum ammonia, urine and serum organic acids, serum amino-acid analysis, lactate, and pyruvate levels) was done when indicated. To diagnose IVH, a cranial ultrasound study (Logic-S8, GE Ultrasound Korea, Ltd, Gyeonggi-do, Korea with a 10c probe) was done on day seven of life. Infants with IVH were monitored with repeat cranial ultrasound at periodic intervals to assess resolution or worsening, development of post-hemorrhagic hydrocephalus, and PVL. When close to discharge, infants underwent brain magnetic resonance imaging (GE 1.5-Tesla 5X or LX, Milwaukee, WI) to confirm presence or absence of PVL.

Treatment of Seizures. Phenobarbital was used as the first line drug to treat seizures (20 mg/kg loading dose and 5 mg/kg maintenance dose). If clinical seizures persisted, the dose of Phenobarbital was increased to attain serum level of ≥ 30. The following drugs were added to Phenobarbital, in sequence, if clinical seizures persisted: 1) Fosphenytoin (20 mg/kg loading dose and 5 mg/kg maintenance dose); 2) Levetiracetam (20 mg/kg loading dose and 10 to 20 mg/kg/dose every 12 hours, maintenance dose); and 3) Lorazepam was used to control breakthrough seizures. Anticonvulsants were reduced gradually and discontinued when a follow-up EEG confirmed resolution of seizures.

Statistical Analysis. The primary outcome was presence or absence of seizure. Gestation, birth weight, and age at seizure diagnosis were evaluated for normality with the Kolmogorov-Smirnov test and summarized with medians, means, and standard deviations. Categorical data (sex, cesarean birth, medications, co-morbidities, procedures, and death) were summarized with frequencies and percentages. Where data were sparse, categories were collapsed. For example, gestation was grouped by < 24 weeks, 25-26 weeks, and 27-28 weeks; birth weight was categorized as < 749 g, 750-999 g, and 1000 g or more; Apgar scores were categorized as 0-3, 4-6, and 7+; age of seizure diagnosis was categorized as 0-7 days, 8-14 days, 15-21 days, and 22+ days. Bivariable analyses by seizure outcome were conducted: continuous data were evaluated with t-tests or with Mann Whitney U tests; categorical data were evaluated with Chi-square tests. Exact tests were used when data were sparse. Unadjusted prevalence ratios were calculated by seizure outcome for each variable.

Model Building Strategy to Assess Risk for Seizures. To understand risk for EEG confirmed seizures, a diagnostic, multivariable binomial regression model was developed using criteria from TRIPOD.[20] Selection of independent variables for model inclusion were based on bivariable analysis results and factors that occurred in the early part of clinical course (prior to the diagnosis of seizure). To avoid the potential for model over- or under-fitting, the number of predictors was kept to a minimum; thus, those variables with fewer than 10 outcome events were not included in the model. Generalized linear models utilizing the binomial distribution were conducted using a variety of linking functions. Collinearity was evaluated with correlation coefficients and Variance Inflation Factor (VIF). Criterion for multicollinearity was mean VIF > 1. Model inclusion among the correlated predictor variables was based on clinical importance, strongest correlation to seizure, and VIF. Goodness of fit measures, such as Deviance and Akaike's Information Criterion, were used to describe how well the model fit the observed values. Model calibration was evaluated graphically using observed versus predicted values. Discrimination was determined by the concordance index (c-index). Internal validation of the final statistical model involved a bias correcting bootstrap technique with 5,000 samples. All statistical analyses were conducted in IBM SPSS Statistics for Windows, Version 23.0, Armonk, NY: IBM Corp.

RESULTS

Figure 1 shows all infants admitted to the NICU during the study period. The overall incidence of seizures was 9% (222 of 2,445). There were 269 infants born < 29 weeks gestation, and seizures in these infants accounted for more than 53% (119/222) of all seizures observed in the NICU. The proportion of seizures in pre-term infants was 44% (119/269) and 40% (108/269) with EEG confirmation. A downward, linear trend was observed between seizure and gestational age: for infants born at ≤ 24 weeks the incidence was 72% (39/54), for 25-26 weeks it was 57% (49/85), and for those born at 27-28 weeks the incidence was 23% (31/130).

Table 1 shows the bivariable results for characteristics of pre-term infants < 29 weeks gestation by outcome. Median gestation in weeks was significantly lower for those with seizures 25.0 versus 27.0 (p < 001). The prevalence ratio (PR) of seizure (PR = 3.03) was significantly higher for those born most premature at < 24 weeks gestation compared to those born at 27 to 28 weeks; p < 0.001. Likewise, prevalence ratio was greatest when birth weight was lowest: PR = 2.55 for those < 749 g compared with 1000+ g (p < 0.001). Prevalence ratios were greatest for lower APGAR scores, 0-3, at one minute (PR = 1.89) compared with higher scores of 7+ (p = 0.015). Conversely, infants born via C-section were less likely to experience seizures (PR = 0.64; p = 0.002). In addition, infants with seizures received dopamine more often (33% vs. 6%; p < 0.001). Fewer infants with seizures received Furosemide (10% vs. 29%); however, this drug was given after red cell transfusions and later in the course of hospital stay. There was no difference in exposure to Caffeine between the groups, 99% vs. 96%.

Infants with IVH were 1.5 times more likely to have seizures compared to those without (p = 0.003), with the greatest difference of proportion occurring when IVH was severe (i.e., Grade 3 to 4; 46% versus 7%). PVL occurred more often in the seizure group compared to the non-seizure group (13% vs. 0.7%), though data were sparse (PVL was classified as cystic in 50% of cases; data not shown). Not shown in Table 1 are 46 infants with seizures and IVH, PVL, or both: IVH only = 30 infants; PVL only = 3 infants; IVH plus PVL = 13 infants. Thus, 38.7% of all seizures were associated with IVH and/or PVL. The overall mortality for the cohort was 9% (25/269). Mortality was significantly higher in infants with seizures compared to those without seizures (14% vs. 5%, p = 0.019).

The median age when seizures were diagnosed was day 16 (0, 47; Table 2). All 119 infants with seizures received Phenobarbital. When clinical seizures persisted despite attainment of therapeutic levels of Phenobarbital, additional drugs were used. In all, 10 (8%) received Fosphenytoin, 14 (12%) Lorazepam, and 3 (2%) Levetiracetam. Phenobarbital, as monotherapy, controlled seizures in 85% of infants (data not shown). At discharge, over 66% (72/108) of surviving infants with seizures continued to receive one or more anticonvulsant. Anticonvulsants were discontinued within two months after discharge in most of these infants (data not shown).

Table 3 compares clinical versus subclinical seizures. Clinical seizures occurred in 49% of infants (58/119) compared to 51% (61/119) with isolated subclinical seizures. Seizure type differed significantly by gestation and birth weight. Pre-term infants ≤ 24 weeks presented more often with clinical seizures than with subclinical seizures, 50% vs. 16%, respectively; while those born at 27 to 28 weeks presented more often with subclinical seizures, 38% vs. 14% (p < 0.001). Infants with birth weight < 749 grams were diagnosed more often with clinical seizures (65.5%), while those 1,000 grams or more were diagnosed with subclinical seizures (28% vs. 9%; p = 0.002). More infants with clinical seizures died compared to those with subclinical seizures (12 vs. 5; p = 0.067). Infants with subclinical seizures were significantly more likely to continue anticonvulsants at discharge (72% vs. 48%; p = 0.009).

Two infants diagnosed with seizures and no evidence of meningitis at the time of diagnosis of seizures developed meningitis later in their hospital course (one culture negative and one Escherichia Coli). Three infants in the non-seizure group were diagnosed with meningitis (late onset Group B Streptococcus; data not shown). Neither group had any infant with viral encephalitis. There were no metabolic causes identified for seizures in this cohort (hypoglycemia, hypocalcemia, hypo- or hypernatremia, and inborn errors of metabolism).

Results from the final multivariable, diagnostic model for predicting risk for seizure are shown in Table 4. Infants have less risk of seizures if born more mature (PRadj = 0.66, p < 0.001) and with exposure to morphine (PRadj = 0.55, p = 0.019). In other words, the earlier an infant was born, the more likely they were to have seizures; although, Morphine administered early may reduce the risk. Conversely, those who received Dopamine had a two-fold greater risk for seizure. Cesarean birth was associated significantly with less seizure

in the bivariable analysis (p = 0.002; see Table 1). However, when taking all other factors into consideration, the multivariable results showed that cesarean birth was not associated significantly with seizures (p = 0.071).

Performance measures of the final model were not optimal; AIC = 245.25, c-index = 0.789, correct classification = 73.6%, with sensitivity = 61% and specificity < 16%. These results may be due to the overall sample size being small. However, to account for potential overfitting or uncertainty brought about by the sample size, a bias correcting bootstrap technique was conducted to quantify optimism of the model. For example, with regards to weeks gestation, the bias corrected 95% confidence interval for the adjusted prevalence of seizures indicated that as gestation increased, the prevalence ratio for seizures decreased to between 0.60 and 0.70. The line for predicting seizures formed a downward trend such that for every one unit increase in gestational age, the line dropped between 0.33 to 0.57 units. Thus, gestation was associated inversely with the incidence of seizures. A similar interpretation can be made for morphine.

DISCUSSION

In this study, the incidence of conventional EEG-confirmed seizures in extremely pre-term infants was 40%. Compared to the results from our study, Pisani et al.[21], using conventional EEG but with video, reported that the incidence of seizures was 5.49% for pre-term infants 28 to 30 weeks and 8.56% for those less than 28 weeks gestation. The results from our study were similar to the 48% incidence shown by Vesoulis et al.[2] who used a two-channel amplitude-integrated EEG (aEEG) to monitor for seizures. As shown by previous research, incidence of seizures in pre-term infants is dependent on method of diagnosis. In addition, the incidence of seizures was high as we evaluated infants with refractory apnea extensively, which may not have been done in other studies.

With regards to timing of seizures, the mean age of diagnosis was 17.9 days (range: 0-47 days, median was day 16). This is similar to the study by Pisani et al.[22] who found a mean seizure onset of 14.6 days (range: 1-120 days) in pre-term infants < 29 weeks and 5.2 days (range: 1-37 days) in those > 29 weeks gestation. Other studies have evaluated for seizures in the first 72 hours of life,[14] whereas we evaluated infants throughout the hospital stay, much longer evaluation period compared to other studies.

Table 1. Characteristics of pre-term infants < 29 weeks gestation by presence or absence of seizure.

	Seizure		No seizure			
Description, f %	**n = 119**	**44.2%**	**n = 150**	**55.8%**	**PR_{unadj} ***	**P**
Demographics at birth						
Male	54	45.4	75	50.0	0.9	0.451
Median gestation, weeks; mean (sd)	25.0; 25.4 (1.5)		27.0; 26.6 (1.4)		--	< 0.001
Gestation (weeks)						
≤ 24	39	32.8	15	10.0	3.03	< 0.001
25 to 26	49	41.2	36	24.0	2.42	
27 to 28	31	26.1	99	66.0	Ref	
Median birth weight (g); mean (sd)	740.0; 793.8 (228.2)		959.5; 957.3 (242.6)			< 0.001
Birth weight (g)						< 0.001
≤ 749	60	50.4	32	21.3	2.55	
750 to 999	37	31.1	54	36.0	1.59	
1000+	22	18.5	64	42.7	Ref	
Cesarean birth	78	65.5	123	82.0	0.64	0.002
APGAR score at 1 minute						0.015
A1: 0-3	55	46.2	51	34.0	1.89	
A1: 4-6	50	42.0	62	41.3	1.63	
A1: 7+	14	11.8	37	24.7	Ref	
APGAR at 5 minutes						0.063
A5: 0-3	18	15.1	12	8.0	1.53	
A5: 4-6	36	30.3	37	24.7	1.26	
A5: 7+	65	54.6	101	67.3	Ref	
Positive pressure ventilation	119	100.0	149	99.3	--	0.999
Chest compressions	14	11.8	7	4.7	1.58	0.031

Table 1. Characteristics of pre-term infants < 29 weeks gestation by presence or absence of seizure. *cont.*

	Seizure		No seizure			
Description, f %	**n = 119**	**44.2%**	**n = 150**	**55.8%**	**PR_{unadj} ***	**P**
Demographics at birth						
Medications						
Caffeine[a]	118	99.2	144	96.0	3.15	0.106
Dopamine[a]	39	32.8	9	6.0	2.25	< 0.001
Furosemide	12	10.1	44	29.3	0.43	< 0.001
Indomethacin[a]	97	81.5	111	74.0	1.29	0.144
Gentamicin	64	53.8	84	56.0	0.95	0.196
Morphine[a]	33	27.7	38	25.3	1.07	0.658
Piperacillin-Tazobactam[a]	107	89.9	142	94.7	0.72	0.140
Co-morbidities						
Bronchopulmonary dysplasia[b]	104	87.4	84	56.0	2.99	< 0.001
Hydrocephalus (post-hemorrhagic)	10	8.4	0	0.0	--	< 0.001
Necrotizing enterocolitis	29	24.4	11	7.3	1.85	< 0.001
Patent ductus arteriosus	69	58.5	69	48.3	1.26	< 0.001
Retinopathy of prematurity[c]	62	52.1	35	25.2	1.81	0.008
Intraventricular hemorrhage (IVH)	43**	36.1	30	20.0	1.52	0.003
Grade 1 to 2	23	53.5	28	93.3	--	< 0.001
Grade 3 to 4	20	46.5	2	6.7	--	
Periventricular leukomalacia (PVL)	16**	13.4	1	0.7	--	< 0.001
Surgery[d]	45	37.8	16	10.7	2.07	< 0.001
Ventriculoperitoneal shunting	6	5.0	0	0.0	--	0.005
Death[e]	17	14.3	8	5.3	1.82	0.019

Frequencies and percentages are reported unless otherwise stated.
*PRunadj: Unadjusted prevalence ratio.
**Infants with IVH only = 30; PVL only = 3; IVH plus PVL = 13; thus, 38.7% of seizures were associated with IVH and/or PVL.
[a]Medications before the onset of seizures in seizures group.
[b]Bronchopulmonary dysplasia: defined as need for oxygen and/or mechanical ventilation at day of life 28.
[c]Severe ROP treated with Avastin: 17 infants in the seizures group versus 3 infants in the no seizure group.
[d]Surgery for necrotizing enterocolitis, inguinal hernia repair, patent ductus arteriosus ligation.
[e]Of the 17 infants with seizures who died, 11 died before EEG confirmation.

Table 2. Seizure characteristics of pre-term infants < 29 weeks gestation.

	EEG confirmed		No EEG*	
Description	**N = 108**	**90.8%**	**N = 11**	**9.2%**
Median age in days when seizure was diagnosed (min, max)	16.0 (0, 47)		7.0 (0, 18)	
Age in days when seizure was diagnosed				
Day 0 to 7	16	14.8	6	54.5
Day 8 to 14	28	25.9	2	18.2
Day 15 to 21	27	25.0	3	27.3
Day 22+	37	34.3	0	0.0
Seizure type				
Subclinical	61	56.5	0	0.0
Clinical (tonic/clonic)	47	43.5	11	100.0
Medications administered during hospital stay				
Phenobarbital	108	100.0	11	100.0
Fosphenytoin	7	6.5	3	27.3

Table 2. Seizure characteristics of pre-term infants < 29 weeks gestation. *cont.*

	EEG confirmed		No EEG*	
Description	**N = 108**	**90.8%**	**N = 11**	**9.2%**
Lorazepam (Ativan)	10	9.3	4	36.4
Levetiracetam (Keppra)	1	0.9	2	18.2
Anticonvulsant at discharge	72	66.7	N/A	--

*Infants who died before EEG confirmation of seizure.

Table 3. Comparing clinical versus subclinical seizures in pre-term infants < 29 weeks gestation.

	Clinical*		Subclinical		
Description	**n=58**	**48.7%**	**n=61**	**51.3%**	**P**
Baby's sex					
Female	32	55.2	33	54.1	0.999
Male	26	44.8	28	45.9	
Gestation (weeks)					< 0.001
<= 24	29	50.0	10	16.4	
25 to 26	21	36.2	28	45.9	
27 to 28	8	13.8	23	37.7	
Birth weight (g)					0.002
<= 749	38	65.5	22	36.1	
750 to 999	15	25.9	22	36.1	
1000+	5	8.6	17	27.9	
Intraventricular hemorrhage	23	39.7	20	32.8	0.851
Grade 1 to 2	12	52.2	11	55.0	0.999
Grade 3 to 4	11	47.8	9	45.0	
Periventricular leukomalacia	9	15.5	7	11.5	0.596
Death	12	20.7	5	8.2	0.067
Age in days when seizure was diagnosed					0.002
day 0 to 7	18	31.0	4	6.6	
day 8 to 14	16	27.6	14	23.0	
day 15 to 21	11	19.0	19	31.1	
day 22+	13	22.4	24	39.3	
Anticonvulsants administered during hospital stay					
Phenobarbital	58	100.0	61	100.0	--
Fosphenytoin	7	12.1	3	4.9	0.197
Ativan	9	15.5	5	8.2	0.262
Keppra	3	5.2	0	0.0	0.113
Anticonvulsant at discharge	28	48.3	44	72.1	0.009

*Clinical seizures included tonic, clonic, myoclonic seizures.

Table 4. Multivariable diagnostic model for predicting EEG confirmed seizures from clinical antecedents.

			Bca* 95% CI of B					Bca 95% CI of PR_{adj}	
Clinical antecedent	**n = 261***	**B**	**Lower**	**Upper**	**VIF**	**P**	**PR_{adj}**	**Lower**	**Upper**
Male	125	-0.13	-0.60	0.26	1.02	0.519	0.88	0.50	1.30
Gestation of baby (weeks)	261	-0.41	-0.57	-0.33	1.53	< 0.001	0.66	0.60	0.70
Cesarean birth	196	-0.42	-0.94	0.11	1.13	0.071	0.65	0.40	1.10
APGAR score at 1 minute					1.23				

Table 4. Multivariable diagnostic model for predicting EEG confirmed seizures from clinical antecedents. *cont.*

			Bca* 95% CI of B					Bca 95% CI of PR_{adj}	
Clinical antecedent	**n = 261***	**B**	**Lower**	**Upper**	**VIF**	**P**	**PR_{adj}**	**Lower**	**Upper**
A1: 0-3	99	0.15	-0.61	0.97	--	0.659	1.16	0.50	2.60
A1: 4-6	111	0.47	-0.28	1.40	--	0.139	1.60	0.80	4.10
A1: 7+	51	ref	--	--	--	--	--	--	--
Medications									
Dopamine	48	0.86	0.23	1.85	1.28	0.001	2.37	1.30	6.30
Morphine	69	-0.60	-1.21	-0.10	1.11	0.019	0.55	0.30	0.90
Piperacillin-Tazobactam	241	-0.49	-1.48	0.20	1.05	0.175	0.61	0.20	1.20
Co-morbidities									
Patent ductus arteriosus	138	0.30	-0.20	0.90	1.08	0.178	1.35	0.80	2.40
Intraventricular hemorrhage	72	0.18	-0.40	0.85	1.17	0.440	1.20	0.70	2.30

*BCa: Bias-corrected and accelerated with sample size of 5,000
Generalized Linear Model: Binomial probability distribution, Complementary log-log link function
**Eight infants were not included in the model due to missing data: 7 with no seizures who died within 2 days and 1 who had an EEG confirmed seizure.
Model assessment: Overall percentage correctly classified: 73.6%; Concordance Index C: 0.789
Omnibus test: Likelihood ratio: 82.87, df=10, p<0.001
Goodness of Fit: Deviance: 1.127; df=177; Pearson Chi-Square: 1.046; df-177; AIC: 254.25

Our results showed that the incidence of subclinical seizures was higher than reported in other studies (51% vs. 5%-23%).[14,23] This difference was not surprising when you consider that these infants were more mature, had higher birth weight, and were diagnosed significantly later than those with clinical seizures. Therefore, these infants might not have been suspected for seizures in other studies. This higher incidence of subclinical seizures in our study may be due to extensive use of EEG as part of the evaluation for infants with refractory apnea.

Findings from animal models might explain the association of subclinical seizures with refractory apnea. The neurons in the respiratory rhythmogenesis center (pre-Botzinger nucleus) show high expression of GABA receptor α-3 (depolarizing, excitatory) and low expression of α-1 (hyper-polarizing, inhibitory) subunits with high neuronal chloride.[24] In addition, there is expression of Adenosine receptors (A2A) in the neurons that express GABA in the respiratory centers in brain stem. Activation of GABA receptor α-3 stimulates Adenosine receptor A2A to inhibit breathing.[25] From these animal models, we can infer that initiation of seizures leads to apnea in pre-mature infants and these were the infants diagnosed with subclinical seizures in our study. Regardless, infants with subclinical seizures were more likely to continue with an anticonvulsant at discharge. As electrographic (subclinical) seizures may be associated with disturbed cerebral metabolism, prompt detection and treatment of subclinical seizures may decrease the risk of later epilepsy.[26]

The results of our study showed that when infants are more pre-mature (gestational age < 24 weeks), the incidence of seizure increases. This higher incidence of seizures in extremely pre-mature infants is physiologically possible due to Gamma Amino Butyric Acid (GABA) exerting a paradoxical depolarization of immature cortical neurons. The depolarizing action of GABA in the immature brain is due to a high intracellular chloride concentration, mediated by chloride co-transporters NKCC1 (importer) and KCC2 (exporter). However, these developmental changes only have been shown in animal models.[27]

With regards to etiology of seizures, our results showed a significantly lower incidence of IVH and/or PVL compared Scher et al.[28] (39% vs. 90%). In addition, there were no other identifiable causes (metabolic/infection) in 61% of infants with seizures. In contrast, other studies have found that in addition to brain injury there were transient metabolic disorders (9.2%) and infection (3.9%) causing seizures in pre-term infants.[22] However, seizures of genetic origin,[29] such as variants in ion channels (SCN1A, SCN2A, KCNQ2), were not investigated in this study.

While bivariable results showed a significant association between cesarean birth and decreased risk of seizures, the multivariable analysis conducted did not demonstrate this (probably due to the small sample size). There is, however, evidence from previous research showing that cesarean birth is associated with decreased risk of IVH.[30,31] It may be that cesarean birth is associated with decreased risk of seizure due to less risk of IVH, but this needs to be evaluated in future studies.

We found that early use of morphine decreased risk of seizures in pre-mature infants. In some animal models, morphine has been shown to have anti-convulsant effects via the nitric acid pathway.[32] Whether this mechanism is important in pre-term infants is not clear.

Conversely, dopamine administered to pre-mature infants was associated with an increased risk of seizures. Dopamine may be a surrogate marker for severity of illness. Indeed, close inspection of the

data revealed that infants who had seizures and received dopamine had: 1) lower APGAR scores at one minute, 2) were born at earlier gestation, 3) weighed less, and 4) had higher incidence of hydrocephalus or PVL. All infants with seizures, both clinical and subclinical, were treated with anticonvulsants in our study. Others, however, have used phenobarbital selectively for subclinical seizures.[17] In our cohort, Phenobarbital monotherapy controlled seizures in most (85%) infants. In other studies, complete response to Phenobarbital was observed in 62%, partial response in 16.5%, and no response in 20.9%.[33] There is concern that Phenobarbital can cause apoptotic neuro-degeneration in the developing brain, as shown in animal models.[34] However, Shankaran et al.[35] showed that antenatal exposure to Phenobarbital did not adversely affect the neuro-developmental outcome of pre-mature infants at 18 to 22 months of age. More recent data, from animal studies, have shown that Caffeine administered with Phenobarbital decreased the negative effects of Phenobarbital on neural cells.[36] In our study, nearly all infants with seizures treated with Phenobarbital were receiving Caffeine for apnea of prematurity.

The limitations of this study were the small sample size that originated from a single center, which may reduce generalizability, along with the retrospective design with little data on long-term neuro-developmental outcome. The strengths of this study were that all suspected seizures were confirmed with conventional EEG, the detailed workup to detect underlying etiology, seizure treatment per protocol, and adherence to TRIPOD for the model building process.

CONCLUSIONS

The incidence of neonatal seizures was high in pre-term infants < 29 weeks, most likely due to conventional EEG confirmation and inclusion of those with subclinical seizures. Significant number of infants with refractory apnea had subclinical seizures, which has not been highlighted before. The multivariable predictive model showed that extreme prematurity and dopamine increased the risk of seizures, while early exposure to morphine decreased the risk. There were no detectable brain lesions (IVH/PVL) in 61% of infants with seizures; this finding challenges the dogma that most seizures in pre-term infants are due to IVH or PVL or central nervous system infection. In addition, the response to Phenobarbital was higher than previously reported. Whether prompt detection and treatment of seizures (both clinical and subclinical) in extremely pre-term infants would improve long-term neuro-developmental outcome needs urgent study. A study of this nature could benefit from close co-operation between neonatologists and neurologists.

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