Brain State of the Newborn as a Biomarker for Brain Injury in Infants with Hypoxic-Ischemic Encephalopathy

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Objective To evaluate the use of a fully automated trend measure of cortical activity, Brain State of the Newborn (BSN), in early stratification of infants for add-on neuroprotective therapies during therapeutic hypothermia (TH).

Study design Our retrospective cohort study included 167 infants with moderate-to-severe hypoxic-ischemic encephalopathy who underwent TH and continuous electroencephalography monitoring. The BSN trends were computed using fully automated pipelines, and we used a priori–defined thresholds at 6, 12, 24, and 36 hours after birth to assess prediction of an adverse postrewarming magnetic resonance imaging finding, defined as moderate-to-severe cortical or deep gray matter injury and/or severe white matter injury. Adverse outcome at 2 years of age was defined as death, cerebral palsy, or cognitive/motor scores <85 on the Bayley Scales of Infant Development-III.

Results BSN-based prediction of an adverse outcome on MRI at 12-24 hours after birth showed high sensitivity (81%-87%) and specificity (73%-81%), and the corresponding area under the curve (AUC) ranged from 83% at 6 hours to 93% at 24 hours, stabilizing at 91% by 36 hours. In contrast, the prediction of adverse outcome at 2 years of age at 12-24 hours showed a moderate sensitivity (73%-77%) and specificity (70%-78%, AUC: 70%), whereas mortality prediction achieved high sensitivity (94%-99%) and specificity (69%-75%, AUC: 96%).

Conclusions BSN offers a fully automated and unbiased measure of recovery in spontaneous cortical activity, holding promise as a bedside biomarker in identifying infants who could benefit from early add-on neuroprotective therapies. *(J Pediatr 2025;285:114702)*.

Neonatal hypoxic-ischemic encephalopathy (HIE) is an important cause of perinatal mortality and morbidity.[1] Athough therapeutic hypothermia (TH) is the standard of care, this treatment often is less effective in patients with severe HIE.[2] Several promising experimental studies and preclinical human studies have explored the use of stem cell therapy (SCT), melatonin, erythropoietin, xenon, and cannabidiol as early add-on neuroprotective therapies for infants with HIE.[1,3] This approach could lead to decreased brain injury with improvement of cognitive and/or motor function.[1,3-6] Studies in animals demonstrate that early add-on SCT (24 hours vs 72 hours) after a hypoxic-ischemic insult results in better neuroprotection compared with delayed treatment, emphasizing the potential benefit of early add-on SCT.[1,7] However, the criteria and optimal timing for initiating neuroprotective therapies in infants are controversial and open for debate.

It is well acknowledged that future success of clinical trials on early add-on neuroprotective therapies requires that patients are early and accurately stratified for their likely outcome.[8,9] In practice, it is imperative to identify scalable bedside surrogate markers to predict infants' upcoming brain injury that can both ascertain eligibility for neuroprotective therapies and support statistically stronger study designs.[10] The use of magnetic resonance imaging (MRI) after rewarming is the gold standard to evaluate the severity of brain injury.[11] However, earlier identification of infants eligible for add-on neuroprotective therapies is essential to support their timely initiation, thereby improving the therapeutic efficacy. Scalp-recorded electroencephalography (EEG) is a widely used bedside tool for continuous monitoring of recovering cerebral functions. It typically is performed from a few hours of age through the days of TH, and bedside clinicians mostly review it visually using the time-compressed amplitude-integrated EEG trend (aEEG).[12] The most important prognostic characteristic in EEG or aEEG monitoring is the recovery of spontaneous cortical activity, known as background activity. A fully automated deep learning-based trend measure, Brain State of the Newborn

(a)EEG	Amplitude-integrated electroencephalography	DGM	Deep gray matter
AUC	Area under the curve	DNV	Discontinuous normal voltage
BS	Burst suppression	EEG	Electroencephalography
BSID-III	Bayley Scales of Infant and Toddler Development, third edition	HIE	Hypoxic-ischemic encephalopathy
BSN	Brain State of the Newborn	MRI	Magnetic resonance imaging
CGM	Cortical gray matter	ROC	Receiver operator curve
CP	Cerebral palsy	SCT	Stem cell therapy
		TH	Therapeutic hypothermia
		WM	White matter

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(BSN), recently was developed to provide an objective, quantitative, and continuous measure of the spontaneous cerebral activity in newborns.[13-16] Studies already have shown that early BSN recovery may be associated strongly with early and late outcome measures.[14-16] It was therefore feasible to hypothesize that BSN also could provide an effective early bedside biomarker for identifying infants who may benefit from early add-on neuroprotective therapies.[16]

In this study, we aimed to assess the predictive capacity of the BSN trend for a very early prediction of adverse outcomes on the postrewarming MRI scan, the current benchmark in selecting infants eligible for early add-on neuroprotective therapies. The secondary aims were to evaluate the predictive accuracy for any adversity in neurodevelopmental outcome at 2 years of age and to validate the relationships between experts' visual aEEG assessment and the BSN trends.

Methods

Study Overview Design and Setting

This retrospective, observational study recruited infants who were treated with TH as the result of HIE in the neonatal intensive care unit at Leiden University Medical Center, Leiden, the Netherlands. The study assessed infants' continuous brain monitoring with 2-channel aEEG with respect to their postwarming MRI scan and 2-year neurodevelopmental outcome. A priori thresholds for outcome prediction were adopted from previously published cohorts in another center. The institutional review board approved this study with respect to the Medical Research Involving Human Subjects Act (reference number 23-3117), and the use of retrospective clinical data was approved unless the parents had specifically objected to the use of data for research purposes. This study protocol was reviewed and approved by the non-WMO committee of the Leiden University Medical Center with approval number [22-3078].

Patients

The study reviewed all infants admitted to the neonatal intensive care unit between January 1, 2008, and January 1, 2024, and the infants were included if they underwent TH for HIE according to the national guidelines.[17,18] Eligibility required a gestational age ≥35 weeks and birth weight >1800 g. Perinatal asphyxia was defined as at least 1 of the following: a 5-minute Apgar score ≤5, prolonged resuscitation and ventilation for ≥10 minutes after birth, pH <7.0, base excess < −16 mmol/L, or lactate >10.0 mmol/L from either cord blood or within the first hour after birth. In addition, there had to be evidence of moderate-to-severe encephalopathy, determined by either a Thompson score ≥7 between 1 and 3 hours after birth or an abnormal aEEG background pattern within 6 hours after birth with either discontinuous normal voltage (DNV) or a more severe background pattern. Exclusion criteria included gestational age <35 weeks, congenital metabolic or genetic disorders, no neonatal MRI of the brain (only postmortem MRI), death within 84 hours after birth, and/or insufficient-quality aEEG recordings. TH was initiated within 6 hours (median 268 minutes, IQR 219-333 minutes) after birth and continued for 72 hours. The target rectal core temperature was set at 33.5°C using a whole-body cooling device (Criticool; Belmont Medical Technologies). After 72 hours, TH was followed by a gradual rewarming at 0.4°C/hour, and afterwards body temperature was stabilized at 36.5°C for 24 hours.[19,20] Demographic and clinical data were collected from electronic medical records. Data included gestational age; birth weight; sex; mode of delivery; Apgar scores at 1, 5, and 10 minutes; cord blood pH and base excess; first blood gas within 1 hour of birth; highest lactate level before the start of hypothermia; and highest Thompson score 1-3 hours after birth.[21]

During TH, all infants received analgesic or sedative medication with continuous morphine infusion as the first-line therapy and midazolam as a possible second-line therapy. In case of seizures, infants received antiseizure medication according to national guidelines.[22]

MRI Outcomes

MRI of the brain was performed in the postrewarming phase, with the aim of postnatal day 4-5. All MRI studies were acquired using a 3.0-Tesla MR system (Achieva; Philips Medical Systems), with a neonatal head coil. The scan protocol included a 3-dimensional T1-weighted sequence (slice thickness 1 mm) and T2-weighted sequence (axial plane, slice thickness 2 mm), diffusion-weighted imaging (axial plane, slice thickness 3-5 mm, and b values of 0 and 1000 s/mm^2), susceptibility-weighted imaging (axial plane, slice thickness 2-4 mm), and multivoxel magnetic resonance spectroscopy of the deep gray matter (DGM, long TE 288).[23,24] The severity of abnormalities on MRI was assessed on the basis of the Rutherford scoring system for DGM and the appearance of the posterior limb of the internal capsule, white matter (WM), and cortical gray matter (CGM).[23] In addition to this scoring system, diffusion-weighted imaging and spectroscopy findings were evaluated. The MRI findings in respectively DGM, CGM, and WM were categorized as normal (no abnormalities in DGM, CGM, WM), mild (focal signal intensity abnormalities with a maximum in 1 site in DGM, 1-2 sites in CGM, or exaggerated long T1/T2 signal in periventricular WM), moderate (multifocal DGM or CGM abnormalities; exaggerated long T1/T2 signal in subcortical WM or punctate WM lesions), or severe (widespread signal intensity abnormalities for DGM or CGM; widespread ischemic/hemorrhagic WM abnormalities). Infants with moderate-to-severe DGM, moderate-to-severe CGM, and/or severe WM MRI findings were categorized as having an adverse outcome on MRI.[23,24]

Assessment of Neurodevelopmental Outcome

For the prediction of 2-year neurodevelopmental outcome, we included all infants (n = 134) who were born before 2022. Neurodevelopmental outcome was routinely assessed at 2 years using the Dutch version of the Bayley Scales of

Infant and Toddler Development third edition (BSID-III).[25] Cerebral palsy (CP) was defined according to the General Motor Function Classification System.[26] Composite adverse neurodevelopmental outcome was defined as any of the following: motor or cognitive scores being <85 (corresponds to −1 SD), presence of CP, or neonatal death. This composite of a wide range of unfavorable clinical conditions jointly to represent an adverse outcomes was deliberately aimed to obtain maximally conservative estimates for the BSN-based prediction. In addition, we performed a subgroup analysis of infants who died.

Analysis of Brain Monitoring with aEEG

Infants were monitored using 2-channel aEEG (NicoletOne or Brainz, Natus Medical) at a sampling rate of 256 Hz from 4 needle electrodes (F3, F4, P3, and P4). Recording was initiated within 6 hours after birth (median 248 minutes, IQR 191-296 minutes) and continued throughout the 72-hour period of TH and approximately 12 hours of rewarming. The anonymized data were exported in the conventional bipolar montage (F3-P3, F4-P4, P3-P4) to a generic European Data Format for further processing.

Computation of BSN Trends

The signals in European Data Format were processed further using a fully automated pipeline available via a computational cloud service (https://babacloud.fi/) that delivers the BSN trends and their CIs for each minute of the recordings, and identifies seizure activity and a range of typical EEG artifacts for each second of the recording.[13] The BSN values range between 0 and 100: values near 0 correspond to an inactive EEG, whereas increasing BSN levels reflect improvement via burst suppression (BS) to increasing degrees of continuity until a fully continuous background activity (BSN near 100) that is typically observed in healthy infants during active sleep or wake states.[13,14] The automated Babacloud service requires no previous experience from the users, who simply upload the anonymized EEG file and download the analysis results afterward.[13,14,16] Notably, there is no user-dependent access to modify the analysis protocol, and both the raw data and analysis outputs are seen only by the given researchers to ensure full data security. The rationale, technical design, training, and external validations of BSN have been described previously.[13,14,16]

Postprocessing of BSN Outputs

An important part of the BSN-based analyses is postprocessing, in which 1-minute segments with more than 30-second artifacts or more than 6-second seizures are removed before averaging of the 1-minute BSN levels across all available channels. The hourly BSN values are derived as the median of the 1-minute BSN values within each hour, and the given 1-hour epochs are included only if >50% (or >30 minutes) of its 1-minute segments are retained after artifact and seizure detectors.

Assessing BSN-Based Outcome Predictions

The BSN-based predictions were assessed for adverse outcomes using 3 hierarchical approaches. For the first 2 approaches, we used the median BSN value from +/−1 hour around the 4 fixed time points (6, 12, 24, and 36 hours of postnatal age), whereas the third approach used the median BSN value over the given time windows.

The 3 approaches were as follows. First, we used strictly a priori–defined thresholds (THR 1 and THR 2) that were obtained from a reanalysis of a previously published and fully independent cohort in Finland.[14] These BSN thresholds were computed so that they could maximally distinguish the adverse MRI outcome at different time points, and thus the threshold varied with postnatal age, respectively. Infants with a BSN value below the threshold at each timepoint were predicted to have an adverse outcome. THR 1 was defined as BSN <50 at 6 hours, <60 at 12 hours, <75 at 24 hours, and <80 at 36 hours. THR 2 was defined as BSN <30 at 6 hours, <40 at 12 hours, <40 at 24 hours, and <50 at 36 hours. The use of an independent international cohort provided us with the possibility to establish strong, evidence-based BSN thresholds for future therapeutic trials. The estimated prediction metrics included sensitivity, specificity, positive predictive value, and negative predictive value, as well as ORs with 95% CIs in logistic regression models. Because the MRI scoring system in the previous work was not fully equivalent with the MRI scoring of our present cohort, we chose to study 2 alternatives: Our primary candidate was THR 1 (moderate/severe = scores 2A, 2B, and 3), and the secondary candidate was THR 2 (moderate/severe = scores 2B and 3).[14] THR 1 reflected a broader definition of moderate-to-severe brain injury, including score 2A (any involvement of the basal ganglia, thalamus, anterior or posterior limb of the internal capsule, or watershed infarction without other cerebral lesions), score 2B (2A plus additional cerebral lesions), and score 3 (cerebral hemispheric devastation), and was associated with greater BSN thresholds. THR 2 was added to have criteria without MRI score 2A ("any involvement of the basal ganglia, thalamus, anterior or posterior limb of the internal capsule or watershed infarction; no other cerebral lesions"), which was found partly ambiguous because that approach may mix white and gray matter injuries, the core of our presently used MRI scoring.[23,27] Second, the overall potential of BSN trends to distinguish the adverse outcomes was assessed using receiver operator curves (ROC) and their associated area under the curve (AUC) values. This approach complements the a priori–defined thresholds, aiming to uncover predictive potential that was possibly neglected with fixed thresholds. Third, as a post hoc analysis, we also computed the median BSN levels over longer time windows (6-12 hours, 12-24 hours, 6-36 hours, and 12-36 hours postnatal age) to assess how well the overall BSN trajectory could distinguish between outcome categories. This approach aimed to complement the previous assessments in case the long-term BSN level was better than a specific time window. On the basis of previous literature, we selected

BSN <40 as a conveniently practical, round threshold for adverse MRI outcome.[14-16]

Visual Assessment of aEEG Background Patterns

aEEG recordings were extracted from an electronic aEEG database and reviewed offline by consensus between 2 neonatologists with more than 15 years of experience. They assessed the aEEG background patterns and sleep-wake cycling using the established aEEG scores: continuous normal voltage, DNV, BS, continuous low voltage, and flat trace.[21]

Statistical Analysis

Descriptive statistics are presented as means with 95% CIs or medians with IQR for continuous variables and as percentages for categorical variables. Student *t* test or Mann–Whitney *U* test was used to compare continuous variables, as appropriate, and the Fisher exact test was used for categorical variables. Statistical significance was set at P value < .05. MATLAB (Version 2022b, the MathWorks Inc), Python (version 3.12.2; Python Software Foundation), and SPSS (version 28.0.1.0; IBM Corp) were used for data analysis and plotting.

Results

Clinical Characteristics

A total of 167 infants with moderate-to-severe HIE were included in this study, with 53 of 167 (32%) classified as having an adverse outcome on MRI (Table I, Figure 1). The median Thompson score was 11 (IQR 9-15) for the group with adverse outcome on MRI and 9 (IQR 7-11) for favorable outcome on MRI group (P < .001). Blood gas values (pH, base deficit, lactate) were significantly different between the outcome groups (P < .01). For the 149 infants born before 2022, 118 infants were eligible for 2-year outcome evaluation; 31 infants died. Of the 118 infants eligible for follow-up data regarding 2-year outcome, 15 (13%) were lost to follow-up. A total of 20 infants had a composite cognitive or motor score <85 for the BSID-III at 2 years of age. Among the surviving infants, 4 (3%) were diagnosed with CP (2 General Motor Function Classification System grade II, 2 grade V). The overall rate of adverse outcomes at 2 years of age was 55 of 134 (41%). The median composite cognitive score was 75 (IQR 72-82) for the adverse outcome group and 101 (IQR 96-110) for the favorable outcome group. The composite motor score was 83 (IQR 72-89) for the adverse outcome group (without infants with CP) and 107 (IQR 98-112) for the favorable outcome group.

Overview of Outcome-Related BSN Trends

Altogether, of the expected 12 661 hours of aEEG recordings from 167 included infants, 11 142 hours of BSN recordings were available for analysis after exclusion of artifacts and periods of insufficient data quality, representing 88% of the total expected recording time across the cohort. Both outcome groups had a similar proportion of missing data. Comparison of infant-wide BSN trends showed substantial

Table I. Clinical characteristics and MRI findings for the total sample and infants with favorable and adverse MRI outcomes

Characteristics	Total population (n = 167)	Favorable MRI outcome (n = 114)	Adverse MRI outcome (n = 53)	*P* value
Gestational age, wk	39.3 (39.0-39.6)	39.7 (38.1-41.0)	39.6 (38.1-40.4)	.28
Birth weight, g	3430 (2900-3810)	3450 (2870-3900)	3380 (3050-3600)	.44
Female, No. (%)	73 (44%)	49 (43%)	24 (45%)	.78
Apgar at 5 min	3 (2-4)	3 (2-4)	3 (1-4)	.17
Cooling start, min	268 (219-333)	270 (219-339)	261 (224-326)	.43
Thompson score	10 (8-12)	9 (7-11)	11 (9-15)	<.001
pH	6.89 (6.80-7.00)	6.90 (6.82-7.02)	6.80 (6.71-6.96)	<.001
Base deficit, mmol/L	18 (14-22)	17 (14-21)	20 (15-24)	.009
Lactate, mmol/L	13 (10-16)	12 (9-15)	15 (12-18)	<.001
Neonatal death, No. (%)	35 (21%)	0 (0%)	35 (66%)	<.001
Age at MRI, d	6 (5-6)	6 (6-6)	5 (5-6)	.036
MRI findings, No. (%)				
DGM score, No. (%)				
Normal	98	95	3	<.001
Mild	27	19	8	
Moderate	16	0	16	
Severe	26	0	26	
CGM score, No. (%)				
Normal	76	72	4	<.001
Mild	54	42	12	
Moderate	16	0	16	
Severe	21	0	21	
White matter score, No. (%)				
Normal	8	8	0	<.001
Mild	75	68	7	
Moderate	61	38	23	
Severe	23	0	23	

Descriptive statistics are presented as medians with IQRs for continuous variables and as percentages for categorical variables.

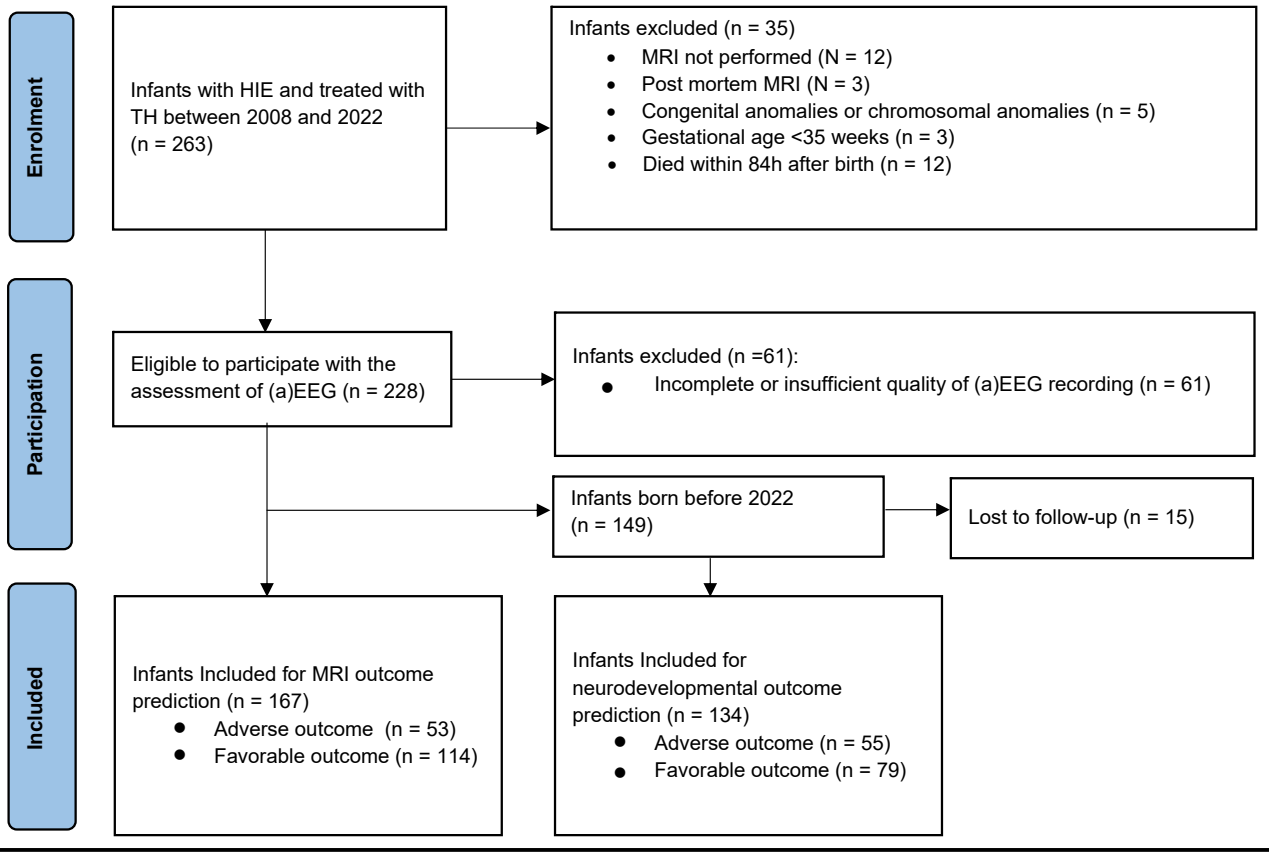

Figure 1. Flow diagram of study population.

interindividual variability across the whole cohort; however, there was also a salient group-level difference, with infants in the adverse outcome on MRI group showing generally lower BSN levels (**Figure 2**, A and B). The median BSN values within each 1-hour epoch over the first 36 hours after birth were significantly lower in the adverse outcome on MRI group (median BSN = 23: IQR 11–36, $P < .001$, **Figure 2**, C) compared with the infants with a favorable outcome on MRI (median BSN = 69.2; IQR 45–86). The group difference was also significant for the specific timepoints at 6, 12, 24 and 36 hours after birth (**Figure 2**, D). The BSN levels over longer time windows or at specific time points were less discriminating between adverse and favorable outcomes at 2 years of age when using the broad category of all adverse outcomes together (**Supplementary Figure 1**; available at www.jpeds.com).

BSN-Based Prediction of Adverse MRI Outcome

When using the a priori–defined BSN thresholds, the prediction performance varied substantially with time and the choice of threshold (**Figure 3**, A and B). The generally best sensitivity was observed at 24 hours (THR 1: 98%; THR 2: 86%), with corresponding specificity of 41% (THR 1) and 81% (THR 2), respectively (**Table II**). However, threshold THR 1 showed even greater sensitivity (100%) at 18 hours at the cost of lower (30%) specificity, whereas the threshold THR 2 showed the highest sensitivity (94%) and specificity (73%) several hours later, at 27 hours after birth. Lower BSN values were consistently associated with a greater likelihood of adverse MRI outcome (**Figure 3**, C). The OR for a low BSN to predict adverse MRI outcome peaked at 19 hours after birth (mean OR 0.89; CI 95% 0.86–0.93, $P < .001$).

These time- and threshold-dependent dynamics also were reflected in the ROCs (**Figure 3**, D), which showed generally very high AUC values using the THR 2, increasing from 83% (95% CI 75%-90%) at 6 hours to 93% (95% CI 88%-97%) at 24 hours, and stabilizing at 91% (95% CI 86%-95%) by 36 hours. The greatest AUC result was observed at 27 hours with an AUC of 93% (95% CI 89%-96%, **Figure 3**, E).

BSN-Based Prediction of Adverse Outcome at 2 Years of Age and Mortality

When using the a priori–defined BSN thresholds, the predictive performance of BSN for adverse outcome at 2 years of age varied considerably with time and the choice of threshold. The best sensitivity was observed at 24 hours after birth for both THR 1 (90%) and THR 2 (77%), whereas these thresholds showed substantially different specificity (THR 1: 37%; THR 2: 77%, **Supplementary Figure 2**; available at www.jpeds.com). The greatest sensitivity (94%) for THR 1 was observed at 18 hours at a cost of lower specificity

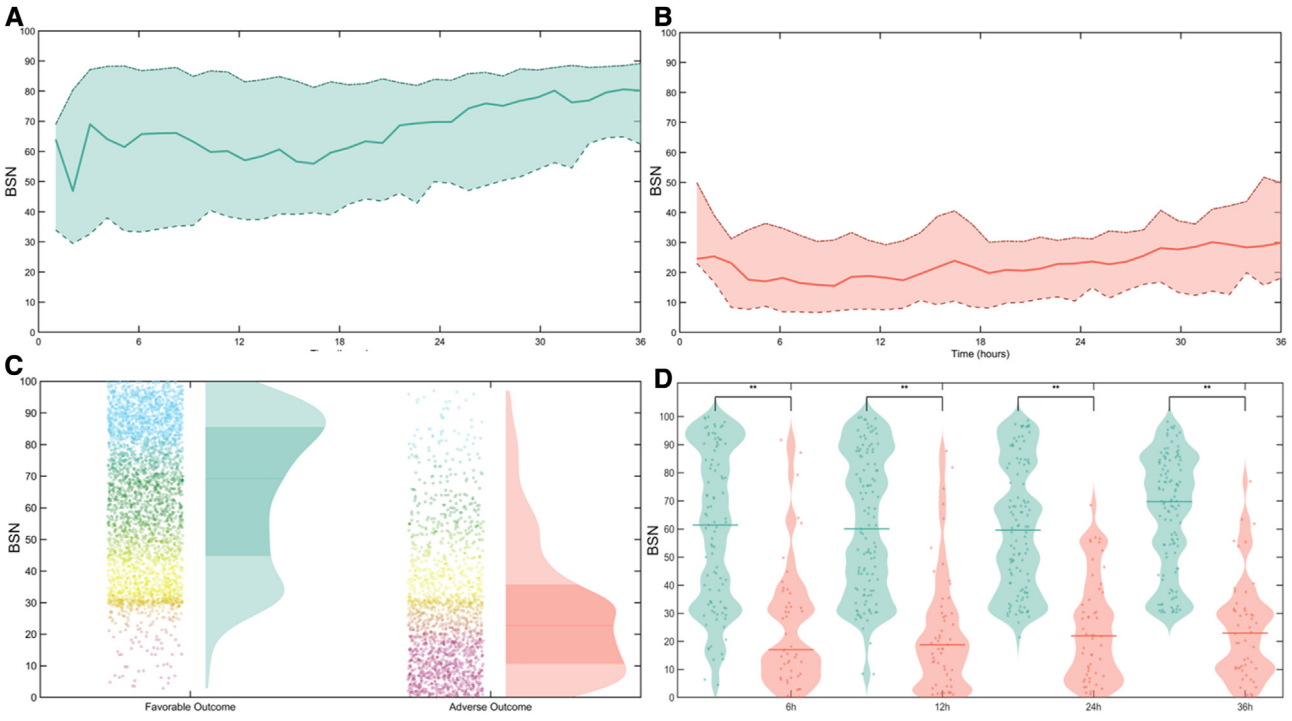

Figure 2. BSN trend distributions over time in the MRI outcome groups. Group-level summary of BSN trends over the first 36 hours after birth for infants with **A,** favorable (*green*) and **B,** adverse (*red*) MRI outcomes. The *solid lines* represent the median BSN values, whereas the *shaded regions* indicate the 25th to 75th percentiles. **C,** Distributions of all BSN values for the first 36 hours after birth in infants with favorable and adverse MRI outcomes. Individual BSN values (epochs of 1 hour in first 36 hours after birth) are color-coded from *blue* (low severity) to *red* (high severity), with density distributions shown for each group to highlight the concentration and spread of BSN values. **D,** The distributions of BSN values from the fixed time points at 6, 12, 24, and 36 hours after birth (each *dot* represents 1-hour median BSN) in each outcome group. Each violin plot shows group-wise median BSN levels and interquartile ranges. Significant differences ($P < .001$) are marked by asterisks (**).

(25%). Meanwhile, THR 2 performed best later, at 26 hours, showing the greatest sensitivity of 83% with a relatively high concomitant specificity of 70%. The prediction of mortality showed the greatest sensitivity at 24 hours, achieving 100% (THR 1) and 97% (THR 2), with corresponding specificity of 37% and 68%, respectively. The optimal time point for maximizing the combined sensitivity and specificity was identified at 6 hours after birth for THR 1 (98% sensitivity and 56% specificity) and 36 hours after birth for THR 2 (85% sensitivity and 84% specificity). Lower BSN values were consistently associated with a greater likelihood of adverse outcome at 2 years of age or mortality, peaking at 21 hours after birth (mean OR 0.94; 95% CI 95% 0.92–0.96, $P < .001$) and 25 hours after birth (mean OR 0.85; 95% CI 0.80- 0.91), respectively.

These time- and threshold-dependent dynamics also were reflected in the ROCs, which showed relatively stable AUC values using THR 2 ranging from 67% (95% CI 58%-75%) at 6 hours, to 70% (95% CI 63-79%) at 24 hours, and 69% (95% CI 61%-77%) at 36 hours. In contrast, the prediction of mortality showed very high AUCs using THR 2, ranging from 86% (95% CI 78-93%) at 6 hours, to 96% (95% CI 92-98%) at 24 hours, and 93% (95% CI 87-97%).

Post Hoc Analyses and False Predictions

Assessment of mean BSN levels over longer time intervals confirmed that the aforementioned prediction measures at discrete time points do indeed represent a stable pattern rather than an occult chance prediction. The median BSN level during the latter half of the first postnatal day (hours 12-24) showed the greatest sensitivity (87%) and specificity (81%) for adverse MRI outcomes. Alternative time windows 6-36 hours and 12-36 hours after birth showed the greatest specificity (both 89%) and sensitivity (83% and 79%, respectively). In 20 of 22 (91%) of the false positives, a clear decrease in the aEEG background score and BSN level was observed after the administration of antiseizure medication or sedation within 36 hours after birth. Phenobarbital induced an abrupt change in 13 of 20 (65%) infants, of whom 10 developed a BS pattern and 3 a DNV pattern after administration. The administration of midazolam induced a change in 7 of 20 (35%) infants, with 5 showing a BS and 2 a

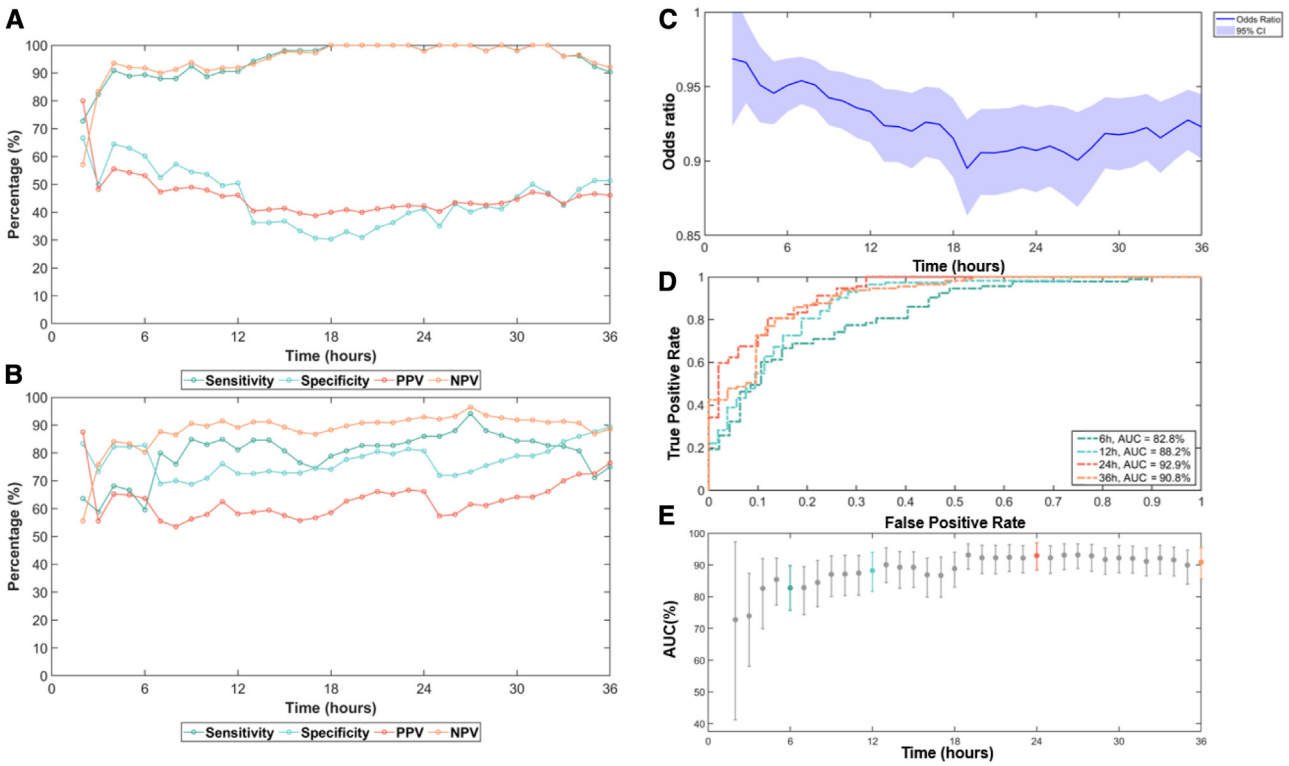

Figure 3. Predictive metrics over time for MRI outcome prediction **A,** Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for predicting adverse MRI outcome over time using THR. **B,** Sensitivity, specificity, PPV, and NPV for predicting adverse MRI outcome over time using THR 2 at fixed time points. For the remaining time points, the following thresholds were applied: THR 1: 1-6 hours (BSN <50), 7-12 hours (BSN <60), 13-24 hours (BSN <75), and 25-36 hours (BSN <80); or THR 2: 1-6 hours (BSN <30), 7-24 hours (BSN <40), and 25-36 hours (BSN <50). **C,** Lower BSN values were associated with greater odds of adverse MRI outcome over time (0–36 hours), with a 95% CI represented by the *shaded region*. **D,** An OR and 95% CI below 1 indicates an inverse relation that lower BSN values are associated with a greater likelihood of adverse MRI outcome. ROC curves for predicting adverse MRI outcome using BSN values at fixed time points (6, 12, 24, and 36 hours postbirth. Hourly AUC values with 95% confidence intervals from 0 to 36 hours after birth. The colors correspond to the ROC curves in **D,** linking them to their respective AUC values (**E**).

DNV pattern after administration. Notably, of these false-positive results after medication, 11 of 20 (55%) infants showed a recovery to the continuous normal voltage pattern within 48 hours. This suggests that the observed "false positives" (ie, lower BSN levels) were likely the result of a medication effect rather than a genuine deterioration of the cerebral state. We therefore assessed the effect of these retrospectively identified infants with a medication-related background change (aEEG/BSN) on our results. Removing these infants from the cohort resulted in a greater sensitivity of 86% and a specificity of 94% for predicting adverse MRI outcome at 24 hours after birth using THR 2 (AUC 96%). For predicting adverse outcomes at 2 years of age using THR 2, the sensitivity at 24 hours after birth was 76%, with a specificity of 85% (AUC 75%).

Visual Assessment of aEEG Background Patterns

After visual assessment of aEEG background patterns and sleep-wake cycling, a relationship was found between BSN values and the classification aEEG background scores. A greater aEEG score, indicative of a worse background, was strongly correlated with lower BSN levels (Spearman rho = 0.82, $P < .001$; **Figure 4**).

Discussion

The present study showed that the automated BSN trend of the aEEG monitoring data can be used to identify infants with the greatest risk of adverse outcomes who may benefit from add-on therapies, even as early as during the first 24 hours after birth. Our work was based on a large patient cohort, and we used strictly a priori–defined BSN criteria for outcome prediction, which allowed a genuinely prospective validation of the previous exploratory findings. The BSN trend within the first 36 hours after birth, during TH, is strongly associated with greater odds of moderate-to-severe brain injury on postrewarming MRI in infants with moderate-to-severe HIE. In our cohort of 167 infants, THR

Table II. BSN-based prediction of adverse MRI outcome

THR 1	6 h (BSN <50)	12 h (BSN <60)	24 h (BSN <75)	36 h (BSN <80)
Sensitivity, %	89.4	90.6	98.0	90.4
Specificity, %	60.2	50.4	41.2	51.3
PPV, %	53.1	46.2	42.2	46.1
NPV, %	91.8	91.9	97.9	92.1
THR 2	**6 h (BSN <30)**	**12 h (BSN <40)**	**24 h (BSN <40)**	**36 h (BSN <50)**
Sensitivity, %	59.6	81.1	86.0	75.0
Specificity, %	82.8	72.6	80.7	89.4
PPV, %	63.6	58.1	66.1	76.5
NPV, %	80.2	89.1	92.9	88.6
Mean interval	**6-12 h (BSN <40)**	**12-24 h (BSN <40)**	**6-36 h (BSN <40)**	**12-36 h (BSN <40)**
Sensitivity, %	81.1	86.8	83.1	79.3
Specificity, %	71.7	80.7	88.6	88.6
PPV, %	57.33	67.6	77.2	76.4
NPV, %	71.7	80.7	88.6	88.6

BGP, background pattern; *NPV*, negative predictive value; *PPV*, positive predictive value. Prediction performance values at different time points and with the 2 a priori–defined BSN thresholds (THR 1 and THR 2), as well as for the mean BSN levels using BSN <40 threshold. Mean interval is defined as the average BSN value calculated across all available data points within each specified time window (6-12 hours, 12-24 hours, or 6-36 hours postnatal age) for each infant.

2 provided the most balanced sensitivity and specificity and clinically applicable thresholds for predicting moderate-to-severe brain injury on MRI. The 24-hour fixed time point, using an easily applicable threshold in clinical practice, was identified as the optimal time point at an early stage for the prediction of moderate-to-severe brain injury, and therefore we suggest that this threshold can be used for future clinical studies. ROC analysis further supports the use of BSN values to effectively identify infants at risk of moderate-to-severe brain injury on MRI. The findings of this study suggest that BSN could serve as a reliable biomarker in future clinical trials to identify infants at risk of brain injury and adverse subsequent outcomes who may benefit from early add-on neuroprotective therapies.

Our present findings and previous studies have shown a close association between the levels of BSN trends and the visually interpreted aEEG scores. However, the fully automated and objective computation of the BSN trends offers several advantages compared with the visually assessed aEEG patterns.[14-16] Most importantly, BSN offers an alternative (or complementary) brain monitoring method that can be available at the bedside in a uniform manner around the world, and around the clock, potentially waiving the need for a continuous access to trained professionals.[28,29] Another advantage of BSN is the ability to eliminate the ambiguity of discrete categorization, reducing the interrater variability common in aEEG background assessments.[30-32] Furthermore, recent international collaborations showed that Babacloud is an easily accessible and effective platform for international collaborations.[14-16] Its potential to facilitate standardized large multicenter studies could lead to improved outcome prediction, seizure analysis, and treatment strategies, ultimately enhancing predictive models and clinical decision-making in HIE.

A sufficiently reliable and early patient selection before the postrewarming MRI is crucial to achieve the greatest efficacy in current and future clinical trials for the administration of

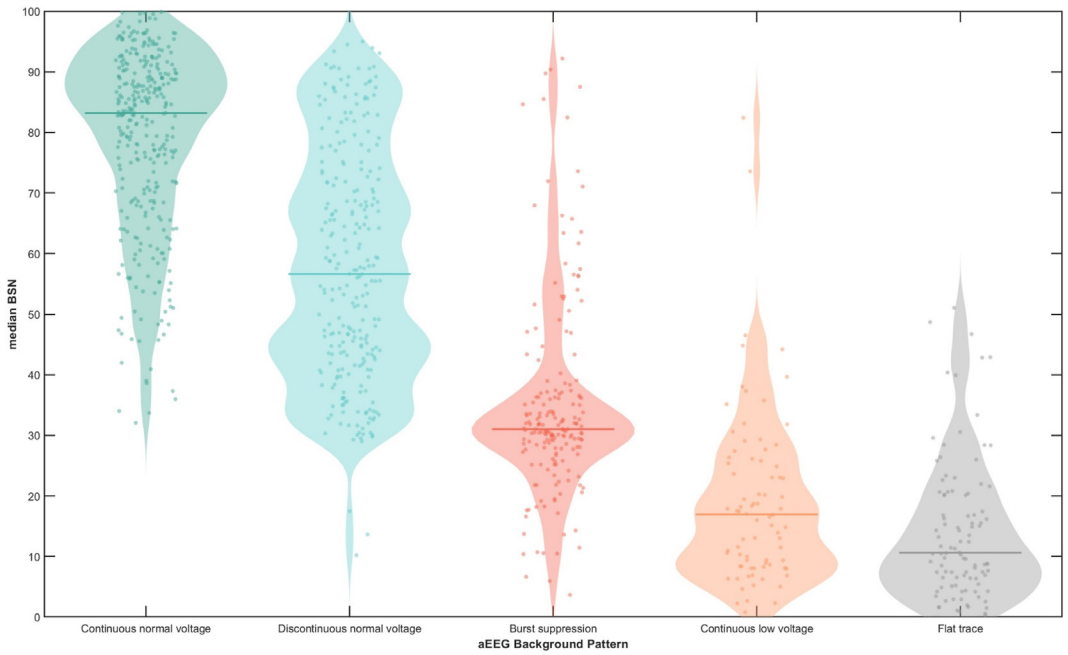

Figure 4. Comparison of BSN levels and the visual (a)EEG scores The box plots summarize the median and IQR of BSN values (median in each 6-hour epoch), grouped according to the 5 visually assessed (a)EEG background categories scored from the first 36 hours after birth: continuous normal voltage, DNV, BS, continuous low voltage, and flat trace.

early add-on protective therapies.[9] Postnatal cranial ultrasound at 24 hours provides limited information, and MRI at 24 hours does not yet reveal the full extent of brain injury.[33-35] Preclinical studies suggest that early postinjury SCT enhances efficacy through anti-inflammatory and proangiogenic mechanisms; however, the efficacy of SCT during TH is still open for debate.[36-39] Animal models of stroke, intraventricular hemorrhage, and HIE suggested a narrow therapeutic window and thus administration closer to the HIE event might provide better therapeutic outcome.[40-42] Melatonin is another promising neuroprotective agent with antioxidant and anti-inflammatory properties and showed promising results in combination with TH, reducing seizures and white matter abnormalities on MRI as well as improved survival and cognitive scores in clinical trials.[5,43-45] Findings from our study indicate that these neuroprotective therapies could be administered as early as 12 hours postinjury, accurately selecting those infants who will have a high risk of moderate-to-severe brain injury on postrewarming MRI.[36,37] By 24 hours, this predictive accuracy increases to 87%, with only 13% who would have missed treatment and 19% of the infants being overtreated at this early stage. Despite the high predictive accuracies, our data also show that prediction of adverse MRI outcome is not absolute with BSN. Infants with false-negative prediction (ie, BSN indicates falsely good outcome) may still receive add-on therapies, if indicated by the postrewarming MRI at day 4-6.

It is encouraging to find that a priori–defined BSN thresholds can be transferred across datasets from different countries and EEG devices, even with some differences in the MRI scoring systems between the previous work and our present study.[27] Taken together, there is now evidence to suggest that lower BSN levels in the early days after birth are associated with a significantly greater likelihood of adverse MRI outcome, as defined by the modified version of the Rutherford scoring system.[23,24] Our choice of the Rutherford scoring system was due to its strong correlation with the composite cognitive score of BSID-III in infants, although some other MRI score alternatives have been reported to have even greater correlation with neurodevelopmental outcomes.[24,27] We used a modified version of the Rutherford scoring system, incorporating diffusion-weighted imaging and spectroscopy, to increase prognostic accuracy, and in order to establish categorical groups of brain injury rather than numerical classifications, as seen in the Weeke score.[24,27] Furthermore, Shankaran et al scored basal ganglia/thalamic injury and white matter injury in the same severity grade, although they do not have the same implications for outcome.[14,27,46,47]

Three recent studies are largely comparable with our present findings, showing the prognostic value of the BSN for the assessment of neurodevelopmental outcome or mortality in the first days after birth in infants with HIE receiving TH and/or healthy controls.[14-16] Montazeri et al showed high accuracy (AUC 96%-99%) for predicting mortality and CP with epilepsy but moderate accuracy (AUC 78%) for lower grades of CP or any impairment.[14] This aligns with our finding that severe impairment is generally easier to predict than milder impairment. Kota et al observed high sensitivity (77%) and specificity (97%) for distinguishing infants with HIE from healthy infants within 6-9 hours after birth; however, Kota et al's HIE sample predominantly showed adverse outcome at 2 years of age (n = 33) compared with a small group of favorable outcome (n = 5), making their findings less generalizable to our sample, which consisted predominantly of favorable neurodevelopmental outcome.[16] Lagacé et al reported excellent prediction of severe impairment or death (AUC 97%) at 24 hours and slightly greater accuracy (AUC 75%) for any impairment than our study (AUC 70%), probably because of the use of different thresholds (optimal thresholds vs a priori–defined thresholds), and differences in the study sample.[15]

Our study has some limitations. First, our retrospective case-by-case analysis indicated that one-half of the false-positive results (ie, falsely adverse outcome prediction) were associated with an acute use of antiseizure medications, which is well known to affect the background activity in both aEEG and EEG.[48,49] This obvious caveat was realized only in hindsight in our study, and therefore, future BSN-based predictions should be careful when such medications are administered. Moreover, the problem with medications may be less pressing at the bedside, where clinicians can always wait more hours to observe a rapid recovery of cerebral activity in infants in whom poor background was caused by medication. Furthermore, our cohort spanned over 15 years, during which clinical practice changed, including increased morphine use, reduced midazolam administration, and milder cases of neonatal encephalopathy, all of which may have impacted the predictions.[17] Second, our study was limited to short-term neurodevelopmental outcome and relied on composite adverse outcome scores, which may not fully capture the complexity of infant outcomes. To achieve a more comprehensive and precise outcome prediction, longer follow-up periods and domain-specific assessments are necessary. Third, this study did not perform a previous sample size calculation, because of its retrospective design, and all available data were used. Finally, knowledge regarding the efficacy and ideal timing of SCT or other neuroprotective therapies in infants with HIE remains limited, underscoring the need for further research to better define the eligibility and therapeutic windows, in order to maximize neuroprotective outcome. Future studies should investigate the added predictive value of combining BSN-derived features with established early clinical markers such as biochemical markers (eg, lactate, base excess, pH), seizure burden, Thompson scores, aEEG background patterns, and the presence of multiorgan dysfunction to further refine prognostic tools in neonatal encephalopathy, which could facilitate timely initiation of add-on neuroprotective therapies.[50]

To conclude, this study showed that the automated BSN trend of aEEG monitoring data can effectively identify infants eligible for add-on therapies as early as the first day of life. We showed that BSN values within the first 24 hours after birth in infants with HIE receiving TH have an important predictive value for moderate-to-severe brain injury on postrewarming outcomes on MRI. Our findings indicate that the selection of infants eligible for add-on neuroprotective intervention based on BSN is feasible as early as 24 hours postinjury, allowing timely intervention for infants at risk. Therefore, BSN may play an important role in guiding neuroprotective interventions and improving outcome in future clinical trials. ■

CRediT authorship contribution statement

Mathies Rondagh: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Willem J.J. Schrama:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Linda S. de Vries:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Andrea van Steenis:** Writing – review & editing, Resources, Methodology, Investigation, Data curation. **Saeed Montazeri:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sampsa Vanhatalo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sylke J. Steggerda:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

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Data Statement

Data sharing statement available at www.jpeds.com.

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