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RESEARCH ARTICLE

Altered autonomic control in preterm newborns with impaired neurological outcomes

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Abstract
Purpose Very preterm newborns are at high risk of neurological injury. The objective of this work was to study the impact of neurological aggression on the autonomic nervous system.
Methods We studied polysomnography recordings, at term corrected gestational age, for 38 preterm infants born at less than 28 weeks or weighing less than 1 kg. These infants were seen by a neuropediatrician, average age at follow up was 54.4 months. We created two groups: one with children who did not have any neurological disorder, including cerebral palsy (CP), language or mental retardation, visual or hearing disability, and attention disorder; the second group contained children with at least one of these impairments. From the polysomnography recordings, using coarse-graining spectral analysis, we compared heart rate variability indices between preterm infants with normal and abnormal neurological outcomes.
Results Twenty infants had an impaired neurological outcome. Regarding the clinical characteristics, there were more babies born from smoking mothers ($p = 0.025$), with early-onset neonatal sepsis ($p = 0.04$), and abnormal results on cerebral magnetic resonance imaging ($p = 0.014$) in the group with impaired neurological outcomes. Spectral parameters were significantly different between active and quiet sleep. Total powers, harmonic and non-harmonic powers, high frequency and low frequency powers were higher in active sleep compared with those in quiet sleep. Preterm babies with impaired neurological development, in particular those with CP, had lower total power and non-harmonic power especially in active sleep than those with normal neurological outcome.

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Conclusion These findings suggest that, in very preterm infants, perinatal neurological injuries could be associated with abnormal maturation of the autonomic nervous system.

Keywords Autonomic nervous system · Heart rate variability · Developmental disabilities · Preterm infants · Sudden infant death syndrome

Abbreviations

GA	Gestational age at birth
PCA	Post-conceptional age
HRV	Heart rate variability
LF	Low frequency band of HRV (0–0.2 Hz)
HF	High frequency band of HRV (0.2–2 Hz)
TP	Total spectral power of HRV
HF/TP	HF power normalized to the total spectral power
LF/TP	LF power normalized to the total spectral power
SIDS	Sudden infant death syndrome

Introduction

More than one out of every ten infants born in the US is born prematurely. In European countries, the incidence of premature birth has increased in the last decades due to to an increase in birth mother's age and improvements in assisted reproductive technologies. Moreover, 1.1–1.6 % of live births are very preterm (i.e, born before 33 weeks' gestation). In France, almost 10 000 infants are born at less than 33 weeks gestation every year [19]. The number of neonates surviving very preterm birth has gradually increased due to improvements in therapy and quality of care, but this increasing survival has also raised issues regarding the increasing rate of adverse developmental outcomes [18]. Most studies have focused on severe sequelae, such as cerebral palsy (CP), with or without associated mental retardation. Deficits in cognitive performance, delayed language skills, visual-spatial or perceptual problems, behavioral difficulties, and learning difficulties at school have also been described [18]. In the French study, EPIPAGE 1 initiated in 1997, a total of 1817 very preterm infants were examined at 5 years of age: 49 % of those born at 24-28 weeks gestation and 36 % of those born at 29–32 weeks gestation had various neurological impairments [18]. In that population, the impaired neurological outcomes are mainly explained by abnormal cerebral development during the critical period of premature adaptation to extra-uterine life.

Heart rate and heart rate variability (HRV) calculated in the time or frequency domain are potent tools for studying not only the cardiovascular [24] but also the central nervous system [21]. Decreased HR variability and vagal activity, and increased sympathovagal balance have been reported in newborn infants with infection [2], and future victims of sudden infant death syndrome [12]. Among the various clinical applications, HRV measurements in newborns could have the potential to predict future outcome in several pathologic situations [16] and to predict future mental development [8]. New analysis methods of heart rate (HR) variability based on non-linear system theory may reveal features and abnormalities in R–R interval behavior, which are not detectable by traditional analysis methods [2].

The present study compares, in very preterm infants, the HRV (calculated in the time, linear and non-linear frequency domains) of infants who later had an impaired neurological outcome to the HRV of those who had a normal neurological outcome, in an attempt to establish a link between those injuries and the abnormal autonomic maturation. Although often associated, we distinguished the patients with CP, suffering from motor dysfunction, from those with other abnormal neurological outcomes (language or mental retardation, vision or hearing disability, and attention disorders). The hypothesis was that CP could be more related to brain parenchyma lesions, whereas other abnormalities could also involve environmental, genetic, or epigenetic mechanisms. As HRV and their spectral parameters are significantly different for active and quiet sleep [5], these HRV analyses were also evaluated according to the sleep stages.

Patients and methods

Participant selection and data collection

The study was approved by the Ethics Committee of Erasmus Hospital (Brussels, Belgium) and carried out in infants born at that hospital between September 1996 and December 2003. Infants born at less than 28 weeks gestation or weighing less than 1 kg were eligible if they had no major congenital malformation, if they had had a PSG before discharge (common practice at Erasmus Hospital during the study period), and if data on follow-up were available. Among the 65 eligible infants, 45 were finally included: the others were either discharged before PSG or lost to follow-up. The characteristics of these patients and the method of this study have been already reported [29].

The clinical data were collected from the medical records by a neonatologist (DV).

Follow-up visits were planned at 1, 2, and 5 years old. The neurological outcome was determined by a neuropediatrician (VW) based on six criteria: CP, language or mental retardation, vision or hearing disability, and attention disorder [31]. Infants with at least one adverse

criterion at the last follow-up visit were considered as having an impaired neurological outcome and the others as having a normal neurological outcome.

Sleep recordings and parameters

Sleep data were collected on computerized polygraph recorders (Morpheus System, Medatec, Brussels, Belgium) at full-term corrected age, before discharge from the neonatal unit [29]. The following signals were simultaneously recorded: eight scalp electroencephalograms, two electrooculograms, a chin electromyogram, an electrocardiogram, thoracic and abdominal respiratory movements by inductance plethysmography, and airflow by thermistors taped under each nostril and facing the mouth. Oxygen saturation was continuously recorded using a transcutaneous sensor (Ohmeda Biox, Hayward, CA).

The 9-h recording sessions were made in supine position during the night or the day in an environment controlled for sound, light, humidity, and tactile stimulation. Infants were fed on demand and their behavior and any nursing intervention were recorded.

The recordings were randomly coded and analyzed by a scorer (GT) who was blind to the participants' identities and neurological outcomes. According to conventional criteria, [1, 6] each thirty-second recording epoch was labeled as quiet sleep (QS), active sleep (AS), indeterminate sleep, or wakefulness. A change in sleep state was any interruption of one state by another for 1 min or more.

The total sleep period was the delay from the time the infant fell asleep (or, if already asleep, from the beginning of the recording) to the end of the recording or to child's wake up. The total sleep time was the total sleep period minus the duration of wakefulness. Sleep efficiency was the ratio of the total sleep time to the total sleep period, expressed as a percentage.

The percentage of QS, AS, or indeterminate sleep was the duration of each stage divided by the total sleep time and multiplied by 100. The percentage of wakefulness was the duration of wakefulness divided by the total sleep time and multiplied by 100.

The sleep cycle length was the duration, in minutes, between two periods of at least three consecutive minutes of the same sleep state (QS or AS) interrupted by less than 15 min of continuous wakefulness [25]. The median duration of QS cycles was the median, in minutes, of all QS periods present between two periods of AS interrupted by less than 15 min of continuous wakefulness.

Sleep fragmentation was expressed by the number of awakenings and movements per hour of QS and per hour of AS. A thirty-second recording epoch was labeled "movement" when body movements were detected by movement sensors or seen as movement artifact in the somatic channels (electrocardiogram, electroencephalograms, and respiratory parameters) during more than 15 s. Two consecutive thirty-second epochs of movement were labeled "wakefulness." An awakening corresponded to a transition from a sleep stage to a wakefulness stage.

Cardiorespiratory parameters

Sleep apneas were scored only if they lasted 3 s or more, the duration usually used at this age [15]. A central apnea was scored when flat tracings were simultaneously obtained from thoracic or abdominal movements and from thermistors. An obstructive apnea was scored when continuous deflections were obtained from thoracic and abdominal movements, while a flat tracing was recorded from thermistors. To avoid artificial scoring due to thermistor displacement, obstructive apneas preceded by body movements, crying, or sighs were rejected. Mixed apneas were defined as central apnoeas directly followed by obstructive episodes; these were scored as "obstructive events." Hypopneas were not considered because we used thermistors, and because there is no consensus definition for hypopnoea in this age group. Awakenings that occurred 15 s or less after an apnea were taken into account as respiratory-related arousals.

HRV spectral analysis

The analysis of the heart rate variability (HRV) was obtained as follows: digitized ECG signals were sampled at 300 Hz. The intervals between the successive R waves were measured with AcqKnowledge® software for signal analysis (Biopac Systems, Inc., Santa Barbara, CA, USA). For each subject, the analysis of the HRV spectrum was generated from artifact-free and stationary recording segments of 256 successive RR intervals (around 2 min), selected by visual inspection. This choice was blind of subject characteristics. Undesirable beats accounted for <1 % in each recording segment or subject. Using coarse-graining spectral analysis (CGSA) [32], the RR-interval series were analyzed to determine the power spectrum of HRV for each infant. CGSA has shown great sensitivity for the analysis of the HRV spectrum during exercise and is more consistent for short RR-interval series than conventional HRV analysis. In newborns and particularly preterm newborns, sympathetic influence on HRV modulation is predominant, leading to a HRV spectrum that is closer to that of an exercising adult than an adult at rest [28]. Moreover, in premature newborns, long-term stationary ECG recording is difficult. Even in quiet sleep, premature newborns show frequent muscular bursts with concomitant heart rate accelerations. CGSA therefore appeared to be a good technique for HRV spectral analysis in our

population. The total power of HRV (TP) contains the non-harmonic (or fractal) power of HRV and the harmonic (sinusoidal like) power of HRV. In the CGSA method, the non-harmonic variability of RR intervals series is subtracted before performing Fast Fourier transform (FFT) decomposition of signal. The power spectrum was then calculated and the analysis of results focused on the powers in the domains of low frequency (LF 0–0.2 Hz) and high frequency (HF 0.2–2 Hz) [28]. The limits of HF variability components were made higher than the usual upper limit to take into account the high respiratory rate of infants [28]. With Yamamoto and Hughson CGSA software, the upper limit is in fact half the lower heart rate of each series (for example 1.17 Hz for 140 bpm) [32]. The components of spectral power were considered separately and their normalized indicators were calculated. HF/TP was used as an indicator of vagal cardiac control. LF/TP reflects cardiac sympathetic modulation and also contains some parasympathetic modulation. LF/HF is usually considered as an indicator of the sympathetic modulation of heart rate and as a marker of sympathovagal balance [28, 32]. Recordings were divided into three successive periods of 3 h (i.e.: 9:00 p.m. to 12:00 p.m., 12:01 p.m. to 3:00 a.m., and 3:01 a.m. to 6:00 a.m.) in order to evaluate autonomic nervous system values throughout the night. Indeed, nycthemeral variations in the autonomic nervous system have been found in more adults than in infants [12, 30]. For each period, one series of 256 successive RR intervals was selected in AS and in QS. For each infant, the mean values of the three HRV analyses in QS and AS in the three parts of the recording was calculated. Among the 45 preterm babies included in this study, 38 infants had good signal ECG for spectral analyses.

Statistical analysis

Median values were calculated for demographic data. For each infant, a mean value of HRSA analyses including RR-interval duration, total power (TP), non-harmonic power, harmonic power (HF and LF powers), HF/TP, LF/TP, and LF/HF ratios during the recording was done in both AS and QS.

Maternal and infant characteristics, infant sleep characteristics, as well as the values of HRV measurements were compared between normal and impaired neurological outcome groups using Mann and Whitney tests for continuous variables and Chi-square tests for categorical variables. Wilcoxon paired test was used to compare values of HRV measurements between quiet and active sleep. The same statistical analyses were done to compare the preterm infants with normal outcome and those who will develop CP. All statistical analyses were conducted using SPSS 22.0 for Windows. Differences were considered to be statistically significant when the p value was lower than 0.05.

Results

Participants' characteristics

Table 1 presents the clinical and demographic data. The median (range) gestational age was 27 weeks [25–32], the median birth weight was 880 g (520–1180), and the median post-menstrual age at PSG was 39 weeks (36.3–44.4).

The neurological outcome was normal in 18 infants and impaired in the remaining 20 infants. The median age at last follow-up was 54.4 months (21.3–107.3) with no significant difference between the two groups. Among the preterm infants with abnormal outcome, 10 had CP, 4 had mental retardation (2 with CP), 11 had language retardation (5 with CP), 8 had attention disorders (4 with CP), and 10 had visual disabilities (4 with CP). Among the 10 preterm infants with CP, 4 had abnormal MRI (3 leukomalacia and 1 intra-ventricular ventricular hemorrhage stage 4).

There were no statistically different demographic or clinical characteristics between the two groups, except a higher rate of early-onset neonatal sepsis in the impaired outcome group ($p = 0.04$). All except one infant had a cerebral magnetic resonance imaging examination before discharge from the neonatal unit: there were more infants with abnormal results in the impaired outcome group than in the normal group (42.1 vs 11.1 %) ($p = 0.014$). Among the preterm newborns with abnormal development, 5 newborns had periventricular leukomalacia [parieto-occipital leukomalacia ($n = 3$), one patient with cystic lesions ($n = 1$), one patient had a cyst in the frontal lobe ($n = 1$)], and 3 patients had intra-ventricular hemorrhage [stage 3 ($n = 1$) or stage 4 ($n = 2$)]. Among the preterm newborns with normal development, two of them had periventricular leukomalacia (very discrete lesion, $n = 1$) and in the frontal lobes ($n = 1$). Smoking habits during pregnancy were reported for 32 mothers. All infants born to smoking mothers ($n = 5$) belonged to the impaired outcome group ($p = 0.025$). No differences were found for sleep and cardiorespiratory characteristics (Table 2).

Spectral parameters are significantly different for active and quiet sleep. Total power, non-harmonic parameters (power and %), harmonic Power including HF and LF powers were larger in active sleep compared with those of quiet sleep (Table 3).

Preterm babies with impaired neurological development had lower total power and non-harmonic (fractal) power especially in active sleep than those with normal neurological outcome (Table 3; Fig. 1). The harmonic power, the LF power, and the HF components (power and HF/TP)

Table 1 Demographic and clinical characteristics of the participant very preterm infants according to their neurological development status

Variables	All infants	Normal neurological outcome	Impaired neurological outcome	*p* value
Number of infants	38	18	20	
Maternal age, year	30 [20–40]	29 [23–38]	30 [20–40]	0.63
Maternal smoking during pregnancy	5/32 (15.6)	0/16 (0)	5/16 (31.2)	0.025
Use of antenatal steroids	31 (81.6)	16 (88.9)	15 (75)	1
Inborn	35 (92.1)	18 (100)	17 (85)	0.16
Cesarean section	26 (68.4)	12 (66.7)	14 (70)	1
Gestational age at birth, week	27 [25–32]	27 [25–32]	27 [25–30]	0.37
Female	18 (47.4)	9 (50)	9 (45)	1
Birth weight, g	880 [520–1080]	865 [605–1030]	890 [520–1080]	0.44
Small for gestational age	9 (23.7)	6 (33.3)	3 (15)	0.16
Five-min APGAR score	8 [5–10]	8 [6–10]	8 [5–10]	0.22
Use of surfactant	24 (63.2)	11 (61.1)	13 (65)	1
Mechanical ventilation, days	10.5 [0–54]	10 [0–40]	11 [0–54]	0.69
Bronchopulmonary dysplasia	21 (55.3)	9 (50)	12 (60)	1
Postnatal steroids for BPD	21 (55.3)	9 (50)	12 (60)	1
Persistent ductus arteriosus	15 (39.5)	9 (50)	6 (30)	0.16
Early-onset sepsis	6 (15.8)	0 (0)	6 (30)	<0.01
Nosocomial infection	20 (52.6)	9 (50)	11 (55)	1
Hemodynamic shock	22 (57.9)	11 (61.1)	11 (55)	1
Necrotizing enterocolitis	19 (50)	9 (50)	10 (50)	1
Blood transfusion	31 (81.6)	15 (83.3)	16 (80)	1
Doxapram for apnea syndrome	18 (47.4)	8 (44.4)	10 (50)	1
Gastroesophageal reflux	32 (84.2)	14 (77.8)	18 (90)	1
MRI abnormalities*	10/37 (27)	2/18 (11.1)	8/19 (42.1)	0.014
Age at follow-up, month	54.4 [21.3–107.3]	49 [21.3–101.7]	59.2 [26–107.3]	0.14
Post-menstrual age at PSG, week	39 [36.3–44.4]	38.6 [36.3–42]	39.3 [37–44.4]	0.35
Daytime PSG	14 (36.8)	9 (50)	5 (25)	0.16

Data are expressed as median [range] or n (%). "*p* value" for comparisons using the Mann & Whitney or Chi-Square test

NS non-significant, *BPD* bronchopulmonary dysplasia, *PSG* polysomnography, *MRI* magnetic resonance imaging

*An abnormal MRI was defined as the presence of any white matter disorder or intraventricular hemorrhage, except germinal matrix hemorrhage

were decreased, and the LF/TP and LF/HF ratios increased but the differences were not statistically significant. When the newborns from smoking mothers were excluded, we found a tendency for the same results, respectively, for total power ($p = 0.11$) and for non-harmonic power ($p = 0.099$) in active sleep.

Compared to preterm infants with normal neurological outcome, preterm infants with CP more frequently had smoking mothers during pregnancy (30 vs 0 %, $p = 0.014$), more early-onset sepsis during neonatal period (30 vs 0 %, $p = 0.014$), and more MRI abnormalities (40 vs 11.1 %, $p = 0.04$) than the preterm infants with normal outcome. Three of the preterm infants with CP had periventricular leukomalacia and one intra-ventricular hemorrhage stage 4). These preterm infants with CP tended also to have better sleep efficiency during PSG recording 81 % (range 48–87 %) vs 70 % (range 41–81 %) ($p = 0.07$). Concerning spectral HR parameters, compared to the group control, the preterm infants with CP had the same results than the entire group of preterm infants with impaired neurological outcome (Table 4). They had also an increase of LF/HF ratio in total sleep time. When we compared the preterm infants with the other causes of neurological impairment and the group of normal neurological outcome, we did not find any differences for clinical, polysomnographic, and spectral analyses.

Discussion

Preterm newborns with abnormal neurological development, especially with CP, had lower total power with lower non-harmonic power than those with normal neurological outcome in AS.

Non-invasive measurement of heart rate variability (HRV) has been successfully used to estimate cardiac

Table 2 Sleep measures and cardiorespiratory indices in the participant very preterm infants according to their neurological development status

Variables	All preterm infants	Normal neurological outcome	Impaired neurological outcome	*p* value
Number of infants	38	18	20	
Total sleep period, min	469 [159–599]	407.5 [304–599]	503.5 [159–599]	0.15
Total sleep time, min	366 [128–487]	309 [133–459]	377.5 [128–487]	0.12
Sleep efficiency, %	73.6 [41.1–91.3]	69.7 [41.1–91.3]	77.9 [47.8–86.8]	0.19
Quiet sleep, %	28.1 [16.8–43.2]	27 [19.1–43.2]	29.6 [16.8–43.1]	0.33
Active sleep, %	61.2 [45.9–73.7]	62.5 [45.9–73.7]	60.2 [47.5–72.2]	0.22
Active sleep/quiet sleep	2.2 [1.1–4.3]	2.3 [1.1–3.7]	2.0 [1.1–4.3]	0.26
Indeterminate sleep, %	5.1 [1.8–9.2]	5.4 [1.8–9.2]	4.9 [2.7–8.8]	0.48
Wakefulness, %	26.4 [8.7–58.9]	30.3 [8.7–58.9]	22.1 [13.2–52.2]	0.19
Median duration of QS sleep, min	18.5 [10–29.5]	17.7 [10–28]	19.0 [10.7–29.5]	0.65
Movements and awakenings in QS (/h)	3.2 [0.5–16.1]	5 [0.5–16.1]	2.2 [0.7–9]	0.22
Movements and awakenings in AS (/h)	19.4 [7.9–35.1]	19.3 [7.9–35.1]	19.5 [11.6–27.6]	0.69
Ratio of movements and awakenings during AS/QS	5.7 [1.5–39]	4.4 [1.6–39]	6.4 [2.2–23.5]	0.23
Central apnea frequency (/h)	14.9 [1.3–42.3]	14.9 [1.4–33.3]	14.1 [1.3–42.3]	0.96
Central apnea duration (s)	4.3 [3.7–5.5]	4.3 [3.7–5.5]	4.3 [3.8–5.2]	0.59
Obstructive events (/h)	6.4 [0.4–44.3]	5.6 [0.4–44.3]	8.4 [0.5–32.8]	0.13
Obstructive events duration (s)	5.3 [3.5–8.3]	5.2 [4.0–7.0]	5.5 [3.5–8.3]	0.46
Awakenings $\leq$15 s after apnea (/h)	1.1 [0–4.8]	1.2 [0.6–4.8]	0.95 [0–4.7]	0.13
QS breathing rate (/min)	55 [44.1–71.1]	57.9 [39.1–71.7]	55.6 [38.1–70.7]	0.57
AS breathing rate (/min)	56 [38.1–71.7]	58.6 [44.1–70.7]	53.5 [44.3–71.1]	0.57
Oxygen saturation values, %	95.5 [91.1–98.5]	96.7 [92.7–98.6]	95.5 [91.1–98.5]	0.98
QS basal heart rate (bpm)	139 [118–162]	137 [118–162]	141 [121–158]	0.42
AS basal heart rate (bpm)	140 [108–158]	139.5 [108–158]	142 [120–158]	0.27

Data are expressed as median [range]. "*p* value" for between groups comparisons using the Mann–Whitney or the Chi-Square test

NS non-significant, *AS* active sleep, *QS* quiet sleep

autonomic modulation in neonates [20]. However, caution must be taken to adapt this tool for this specific population [24]. Our ECG recordings and processing complied with the recommendations of the Task Force with appropriate frequency bands for HRV spectral analysis of newborn infants [28]. The use of CGSA technique gave us a more consistent tool for short RR-interval series than conventional HRV analysis [32]. Even more, CGSA discriminated fractal (non-linear) random walks from simple harmonic motion [23]. Compared to more mature infants ($\geq$35 weeks GA), non-linear heart rhythms are lacking in preterm infants (gestation $\leq$27 weeks) where parasympathetic-sympathetic interaction and function are presumed to be less well developed [3]. Other studies comparing preterm to full-term infants have found a delay in autonomic maturation in preterm infants [22]. It is not known, if it is the interruption of relations with the mother or the more external stimulations of the ex-utero life, which predominantly contributes to the observed changes in the maturation of cardiac autonomic control for this population. In these studies, the neurological outcome of preterm infants was not specified; it was then difficult to determine whether the differences were mainly influenced by immaturity or by neurological injuries. The present work raises the hypothesis that a part of the alteration of autonomic maturation in preterm infants could be explained by neurological injuries. The fact that HRV alterations were specifically found in the preterm infants with CP was also in favor of this hypothesis. Due to prenatal insults, lesions of the periventricular white matter affecting the motor corticospinal tracts were often found in preterm infants with CP (periventricular leukomalacia, intra-ventricular hemorrhage, history of perinatal sepsis, etc.). However, the relations between HRV alterations and periventricular lesions have to be explored more.

One limitation of this study is the small sample size, which did not allow us to examine the effects of different environmental conditions and treatment including mode of ventilation, electrolyte changes, and physical interventions. However, our data were homogenous regarding gestational age at birth and PSG recordings. The selection of very preterm infants allowed us to consider two groups with

Table 3 Heart rate spectral parameters according sleep stages and neurological outcome

Variables	Normal neurological outcome ($n = 18$)	Impaired neurological outcome ($n = 20$)	p value
RR (ms) total sleep	425.8 [365.2–495.4]	421.0 [368.3–466.8]	0.534
Quiet sleep	429.1 [358.4–512.5]	423.7 [369.9–481.2]	0.851
Active sleep	422.0 [371.9–478.4]	413.5 [366.7–469.9]	0.290
p QS vs AS	0.078	0.002	
TP (ms^2) total sleep	71.2 [12.8–168.8]	46.5 [11.8–137.7]	0.048
Quiet sleep	28.2 [3.1–115.1]	16.2 [4.0–99.6]	0.290
Active sleep	117.6 [22.6–280.6]	69.7 [13.2–175.8]	0.019
p QS vs AS	<0.001	<0.001	
Fract (ms^2) total sleep	57.5 [10.0–143.1]	36.4 [9.5–113.9]	0.038
Quiet sleep	23.8 [2.4–93.8]	12.6 [3.3–72.8]	0.236
Active sleep	94.8 [17.6–252.3]	56.3 [10.9–154.9]	0.015
p QS vs AS	<0.001	<0.001	
Fract (%) total sleep	82.8 [70.7–87.3]	81.9 [77.9–88.8]	0.718
Quiet sleep	79.9 [70.7–87.3]	82.7 [74.0–93.7]	0.158
Active sleep	85.7 [71.3–93.1]	83.3 [76.7–92.7]	0.290
p QS vs AS	0.003	0.204	
Har (m^2) total sleep	9.1 [2.3–36.6]	7.8 [10.9–154.9]	0.141
Quiet sleep	5.5 [0.7–21.2]	2.9 [0.5–26.8]	0.196
Active sleep	12.5 [3.4–58.1]	11.3 [2.0–25.9]	0.141
p QS vs AS	<0.001	0.005	
HF (ms^2) total sleep	0.45 [0–2.8]	0.13 [0–3.2]	0.361
Quiet sleep	0.07 [0–1.7]	0 [0–3.2]	0.251
Active sleep	0.37 [0–5.5]	0.2 [0–4.6]	0.426
p QS vs AS	0.039	0.041	
LF (ms^2) total sleep	7.5 [1.5–32.8]	5.1 [0.9–21.7]	0.167
Quiet sleep	4.7 [0.3–21.6]	1.1 [0.1–23.1]	0.176
Active sleep	10.2 [2.2–52.1]	7.5 [0.9–23.1]	0.176
p QS vs AS	<0.001	0.004	
LF/HF total sleep	11.6 [2.6–104.7]	40.7 [2.4–1670]	0.075
Quiet sleep	12.0 [1.8–76.3]	15.3 [2.4–253.9]	0.656
Active sleep	7.8 [1.2–142.2]	26.3 [2.9–3332.3]	0.156
p QS vs AS	0.53	0.39	

The mean values of the HRV analyses selected in the three parts of the recording were calculated for QS and AS. Data are expressed as median [range]. "p value" between groups comparisons using the non-matched Mann–Whitney for normal and impaired outcome groups and the matched Wilcoxon tests for comparison between quiet sleep (QS) and active sleep (AS)

Heart rate variability (HRV) spectral analysis—RR: RR intervals in ECG, TP: Total power, Fract (fractal or non-harmonic power), Fract (%): ratio of fractal power on total power, Har: harmonic power, LF: low frequency power (0–0.02 Hz), HF: high frequency power (0.2–2.2 Hz), LF/HF: ratio LF on HF powers reflecting sympathovagal balance

comparable demographic and clinical characteristics but different neurological outcomes. The two groups of preterm babies could not be differentiated by prenatal and postnatal conditions such as gestational or postnatal age [22], antenatal steroid or betamethasone treatment [26] or birth weight [5]. However, preterm babies with abnormal neurological outcome had more frequently early-onset infection than those with normal outcome. We know that reduced heart rate variability measurements have been significantly associated with sepsis or sepsis-like illness in premature infants [2]. Indeed, during a sepsis, a cascade of events due to the associated inflammatory process may participate in the brainstem deactivation. The earlier host immune response results in production of proinflammatory cytokines [e.g., tumor necrosis factor α (TNF-α), interferon γ, and IL6] especially during severe infections. These interleukins interact at different levels of autonomic neurotransmission. TNF-α may interfere in the vagal reflex through vagal fibers and catecholaminergic neurons of the nucleus tractus solitarius (nTS) after unlocking the blood–brain barrier and

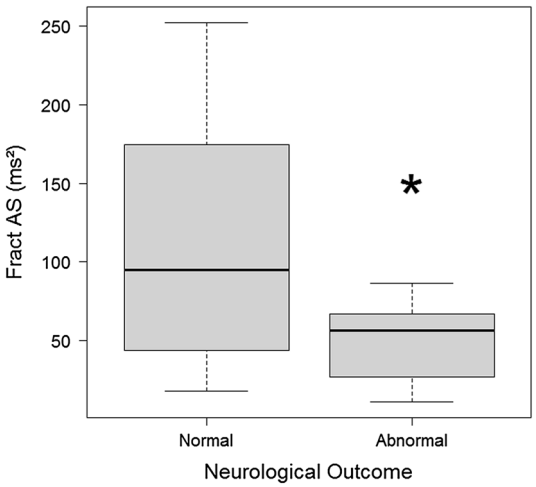

Fig. 1 Non-harmonic (fractal) power in active sleep in preterm infants with normal and abnormal neurological outcome (statistical significance *asterisk*)

determining a decreased neuronal activity through a provoked immediate early gene expression in astrocytes of the nTS. Other early inflammatory cytokines such as IL-1β facilitate also the synthesis and fixation of prostaglandin E2 in the solitary tract and rostroventromedulla brainstem nuclei, cardiac, and regulatory centers [2].

There were only smokers among the mothers of infants with impaired outcomes, and none of the mothers of infants with normal outcomes were smokers. Tobacco exposure during pregnancy could favor autonomic imbalance, with an alteration in the maturation of the two components of the autonomic nervous system [11]. The findings could result from various mechanisms such as alterations in the autonomic central pathways [17], a decrease in baroreflex [13], or nicotinic acetylcholine receptors sensitivity [4]. These findings suggest that prematurity especially with tobacco exposure might represent a risk factor for impaired maturation of autonomic cardiac control with potential long-term effects.

Table 4 Heart rate spectral parameters according sleep stages and cerebral palsy

Variables	Normal neurological outcome ($n = 18$)	Children with cerebral palsy ($n = 10$)	p value
RR (ms) total sleep	425.8 [365.2–495.4]	415.4 [380.9–459.1]	0.52
Quiet sleep	429.1 [358.4–512.5]	423.4 [369.3–461.3]	0.46
Active sleep	422.1 [371.9–478.4]	407.5 [380.9–456.9]	0.41
TP (ms^2) total sleep	71.2 [12.8–168.8]	36.2 [11.8–137.7]	0.06
Quiet sleep	28.2 [3.1–115.1]	18.3 [4.0–99.6]	0.24
Active sleep	117.6 [22.6–280.6]	52.1 [13.2–175.8]	0.02
Fract (ms^2) total sleep	57.5 [10.0–143.1]	27.1 [9.5–113.9]	0.04
Quiet sleep	23.8 [2.4–93.8]	13.7 [3.3–72.8]	0.19
Active sleep	94.8 [17.6–252.3]	41.7 [10.9–154.9]	0.02
Fract (%) total sleep	82.8 [70.7–87.3]	81.5 [77.9–88.8]	0.62
Quiet sleep	79.9 [70.7–87.3]	79.9 [74.0–93.7]	0.62
Active sleep	85.7 [71.3–93.1]	82.7 [76.7–88.6]	0.16
Har (m^2) total sleep	9.1 [2.3–36.6]	6.1 [2.2–23.8]	0.24
Quiet sleep	5.5 [0.7–21.2]	2.9 [0.5–26.8]	0.24
Active sleep	12.5 [3.4–58.1]	9.6 [2.3–25.9]	0.24
HF (ms^2) total sleep	0.45 [0–2.8]	0.13 [0–1.2]	0.31
Quiet sleep	0.07 [0–1.7]	0.01 [0–0.15]	0.19
Active sleep	0.37 [0–5.5]	0.24 [0–2.3]	0.44
LF (ms^2) total sleep	7.5 [1.5–32.8]	5.5 [0.9–21.7]	0.26
Quiet sleep	4.7 [0.3–21.6]	1.14 [0.1–23.1]	0.17
Active sleep	10.2 [2.2–52.1]	8.6 [0.9–23.4]	0.24
LF/HF total sleep	11.6 [2.6–104.7]	57.4 [9.7–1670]	0.04
Quiet sleep	12.0 [1.8–76.3]	21.1 [9.6–253.9]	0.26
Active sleep	7.8 [1.2–142.2]	36.1 [4.1–3332.3]	0.35

The mean values of the HRV analyses selected in the three parts of the recording were calculated for QS and AS. Data are expressed as median [range]. "p value" between groups comparisons using the non-matched Mann–Whitney for normal neurological outcome group and preterm infants with cerebral palsy (CP), and the matched Wilcoxon tests for comparison between quiet sleep (QS) and active sleep (AS)

Heart rate variability (HRV) spectral analysis—RR: RR intervals in ECG, TP: total power, Fract (fractal or non-harmonic power), Fract (%): ratio of fractal power on total power, Har: harmonic power, LF: low frequency power (0–0.02 Hz), HF: high frequency power (0.2–2.2 Hz), LF/HF: ratio LF on HF powers reflecting sympathovagal balance

The infants in the two groups were treated by the same medical and nursing staff with the same medical protocols and were recorded in the similar ambient temperature [7] and physical environment [14]. We also considered the HRV variability according to the sleep stages. As others [5], we found that TP as a measure of heart rate variability with its harmonic and non-harmonic component was significantly larger during active sleep compared with quiet sleep. The significantly higher values for LF/HF, LF, and TP during active sleep compared with quiet sleep reflect the different autonomic sleep organization. In preterm babies with abnormal neurological outcome, lower total and non-harmonic power was only found during AS. The consequence of these changes is emphasized by the fact that REM sleep is the major sleep stage of preterm and newborn infants accounting for more than 50 % of total sleep [5]. These results also confirmed the importance to evaluate the HR variability according to the sleep stages.

For almost 25 years, research has consistently documented the positive relationship between heart rate variability (HRV) and clinical outcome [16]. Neonates who consistently displayed decreasing total spectral energies had a worse outcome than those whose spectral energies were high [16]. Sugihara et al. have shown that infants with clinical brain death and those treated with atropine exhibit a lack of non-linear feedback control, suggesting that central nervous input reflected by a well-balanced autonomic functioning is required for chaotic-like dynamics [27]. CNS injury could expose these preterm infants to more fatal events during their postnatal life. Altered HRV with reduced heart rate variation and lower high frequency spectral power of HRV (HF) have been reported in future SIDS victims [12]. The decreased HRV in preterm infants could reflect the relative risk of SIDS incidence 4–6 times higher in infants born prematurely.

If the evaluation of neural regulation of heart rate via a measure of HRV variability reflects CNS integrity, this measure can serve not only as an indicator of clinical status in the newborn but also as a predictor of development outcome [8, 10]. Follow-up data on 30 preterm infants indicated that heart rate variability and cardiac vagal tone was related to better outcomes in mental processing, social and motor skills, and to fewer behavior problems at 3 years [8, 16]. The use of HRV measures to assess the function of the nervous system, which may or may not be exhibiting physical signs of damage, can guide appropriate interventions for at-risk infants. HRV could be enhanced by environmental factors such as caregiving and nursery conditions [9] suggesting that these neurofunctions might be sensitive to intervention. The hypothesis was that these interventions operate on currently developing systems by altering their growth trajectory to more optimal levels.

In conclusion, most preterm infants escape major sensory, motor, or multiple other impairments, but are at high risk of long-term cognitive, academic, and behavioral problems, especially the smallest and less mature survivors when compared to term-born socially comparable peers. Earliest possible identification of those infants at greater risk would provide the opportunity of early intervention and effective use of available resources. The results of this study indicate a new direction in early identification of risk, namely, that early autonomic dysfunction may reflect neuromotor outcome in childhood. However, altered HRV is not yet able to identify preterm infants at higher risks of abnormal neurological outcomes.

Conflict of interest Patricia Franco had no conflict of interest for this work; she received payment for lectures by UCB (Xyrem, treatment for narcolepsy). The other authors declare no conflict of interest.

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