

Autonomic Nervous System: A Biomarker of Neurodevelopmental Comportment- the AuBE Study

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Abstract

Background: While a dysfunction of the autonomic nervous system (ANS) determined from the cardio-respiratory rhythms in children may represent a key criteria in the physiopathology of possible neurodevelopmental disorders, the normal thresholds and profile of the ANS maturation during the two first years of life have not been established.

Method : The Autonomic Baby Evaluation (AuBE) study is a prospective observational prospective single-center cohort following a population of 302 consecutive term and preterm newborns.

The primary objective of the "AuBE" prospective cohort is to define the autonomic nervous system maturity profile obtained, during the two first years of life, by repeated polysomnography and 24-hour ECG recordings, with a time scale values for each autonomic indices of interest and the secondary objective is to determine, the potential influence of this autonomic profile, on sleep disorders and on cognitive development at the age of 3. Parental questionnaires on sleep quality of children and mood status of the mother are collected at birth and at 6, 12, 18 and 24 months (i.e. M0, M6, M12, M18 and M24). A psychometric status is measured at 3 years for all children.

Results: Study population was included between September 2009 and September 2011. During these two years, a cohort of 302 children was recruited, including 271 (89.7%) term, and 31 (10.3%) preterm newborns. After an initial polysomnography at birth (M0), Holter ECG recordings ECG Holter recordings were performed at M6, M12 M18 and M24. A temporal and frequency domain analysis of the heart rate variability is being performed on each recording.

Conclusion: The strength of this study, is based on the longitudinal organization of a large cohort of newborns (n=302) including physiological maturation of cardiorespiratory systems drive, together with the quantitative and qualitative analysis of sleep and neurological and psychomotor outcome.

The demonstration of such a link between autonomic disorders in the neonatal period and the subsequent onset of sleep and/or psychomotor disorders 3 years later may improve the monitoring of neonates and help schedule early and adapted therapeutic interventions.

Keywords: Neonate; infant; autonomic nervous system; heart rate variability; sleep disorders; neuropsychological assessment; biomarker.

Introduction

Heart rate variability is a robust tool to evaluate the autonomic nervous system (ANS) function with recognized clinical applications at any age [1]. The Autonomic Nervous System (ANS) provides a permanent control over the internal environment to ensure homeostasis and plays a key role in regulating many critical physiological functions, and particularly at the cardiovascular system level to adjust quickly and finely heart rate and blood pressure levels as needed by the body. Conversely, heart rate variability (HRV) is a validated tool to assess the level of overall ANS activity and of the sympathetic-parasympathetic balance.

For neonates, this tool allows us to understand the central mechanisms of cardiorespiratory control implicated in the pathophysiology of severe heart attacks and unexpected infant death syndrome [2-6] A lack of autonomic function may also reflect a lack of central neuronal function, and basic fundamental premise of subsequent neurocognitive

However, while knowledge continues to grow in this area, the

physiological maturational profile of the sympathovagal control is not yet precisely established in the first months of life.

It is assumed that 37 weeks of fetal development are necessary for optimal autonomic maturation at birth [7-9]. Compared to children born at term the cardiac parasympathetic and sympathetic baroreflex responsiveness of premature babies are still altered with postnatal age, with a strengthening of the cholinergic and a reduced adrenergic activity. This pronounced autonomic imbalance may facilitate the occurrence of rhythm disorders and inadequate cardiorespiratory responses in case of internal or environmental stress and may explain

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the particular window of vulnerability in the first year of life [3-6]. If cardiorespiratory adjustment may initially altered, a dysautonomia could also be the precursor of poor future neural control development.

However, up to now, the longitudinal evolution of autonomic activity level with maturation, have not been established [10]. Considering the clinical implications, it seems important to better define the physiological standards of autonomic nervous system activity, which are of importance for cardiorespiratory control. Such analysis of physiological norms requires to take into account adjustment variables inherent to the newborn, such as birth weight, blood pressure level, stages of consciousness, [11-17] and environmental variables such as the position during assessment [18], nicotinic fetal exposition [19-24], feeding choice, psychological state of the mother, temperature, and stress situations that may be potential factors for autonomic modulation [3].

If anatomically, macroscopic encephalopathy lesions including periventricular leukomalacia, cortical or subcortical lesions may explain the occurrence of neurodevelopmental disorders, [25-32] symptomatic moderate lesions are not always detectable through conventional brain imaging performed routinely in neonatology units [30] (transfontanelar ultrasound, CT scan, magnetic resonance imaging). In the French EPIPAGE 1 study, launched in 1997, whose aim was to monitor long-term (two to five years) evolution of a cohort of 2,000 children born before 33 weeks, 4.4% of children with a totally normal brain CT-scan had also cerebral palsy, [33-37] showing that conventional imaging is not sufficient in the neonatal period to fully assess the neurological risk.

Taking into account the subcortical anatomical structures as brainstem nuclei, cortical areas and hypothalamic projections constituting the autonomic nervous system, we hypothesize a possible correlation between a neonatal dysautonomia and a later occurrence of subsequent neurodevelopmental alteration with neuromotor, cognitive or attentional problems and possibly sleep disorders [38]. These assumptions are supported by a positive correlation already shown between dysautonomia and deficit/hyperactivity attention disorder (DHAD) or oppositional defiant disorder shown between 3 and 6 years, in several transversal studies [39-43].

Thus, sympathovagal control dysfunction in the first months of life may be a biomarker of a possible brain injury with later consequences in childhood, although unrecognizable with imaging. However, such a prospective study of this neuro-biomarker and of the potentially dependent clinical features has never been conducted. The primary objective of the "AuBE" prospective cohort is to define the autonomic nervous system maturity profile from birth to the age of 2 years, with a time scale values for each autonomic indices of interest and secondary objectives are to determine, the potential influence of this autonomic profile, and psychological status of mothers on infant sleep disorders and on cognitive development at the age of 3.

Polysomnographic recordings and 24-hour ECG recordings are the actual best validated methods to detect a cardiorespiratory dysautonomia during the quiet sleep of newborns and infants, and the more adapted non-invasive tools to answer precisely those fundamental questions.

The demonstration of such a link between autonomic balance in the neonatal period and the subsequent onset of sleep and/or psychomotor disorders 3 years later may improve the monitoring of neonates and help schedule early and adapted therapeutic interventions [38,39].

Materials and Methods

Study design

Autonomic Baby Evaluation (AuBE) study is a prospective observational follow-up of a single-center cohort to assess in a population of consecutive term and preterm newborns the main effect of the autonomic maturation profile during the first two years of life on psychometric development assessed at 3 years.

For the Heart rate variability analysis during quiet sleep, we realized polysomnographic recordings at 0 and 6 months and because of the increased motor ability of children, we relayed the analysis by an ambulatory complete 24 hours-Electrocardiogram (ECG) at 12,18, and 24 months. Parental questionnaires on sleep quality of children and on psychological and mood status of the mother are collected at birth and at 6, 12, 18 and 24 months. A psychometric assessment is carried out at 3 years for all children.

Population

The AuBE study is conducted at the University Hospital of Saint-Etienne (France), a level III university maternity managing 3,500 births annually and with a Neonatal Intensive Care Unit. The inclusions were performed over a 24 months period, from September 2009 to September 2011. Monitoring follow-up of the cohort will be completed in September 2014.

The inclusion criteria were:

- Every inborn child at term consecutively included from birth date.
- Any inborn premature child, down to a 37 weeks corrected postnatal age, consecutively included from birth date.
- Signature of informed consent and affiliation to a health system required from the parents.

The exclusion criteria were as follows:

- Less than 37 weeks corrected postnatal age,
- History of familial dysautonomia, documented by parental questioning and genetically confirmed [Riley-Day syndrome, CIPA (congenital insensitivity to pain and anhidrosis), Allgrove (achalasia, alacrima, adrenergic deficiency), and congenital central hypoventilation syndrome].
- Congenital Heart disease malformation, congenital abnormality of the brainstem, Pierre Robin sequence (glossoptosis, micrognathia, cleft palate and dysautonomia).
- Permanent Cardiac arrhythmias, of ventricular and supraventricular origin.
- Any treatment at the time of the study or taken in the week before the study, known to alter cardiac or respiratory activity. The following non-exhaustive treatments were considered: caffeine citrate, antiarrhythmics, sympatholytic or parasympathomimetics such as pilocarpine nitrate, cisapride, α or β blockers, adrenergic sympathomimetic (epinephrine, dobutamine, dopamine, norepinephrine, isoproterenol, and salbutamol), parasympatholytics (atropine), antihistaminics, anticholinergics (ipratropium bromide, hydroxyzine, and dexchlorpheniramine), opioid analgesics...
- General anesthesia in the 2 weeks before recording.

Ethical consideration

In accordance with the requirements of the ethics committee, a protocol information leaflet was given to the parents within 24 hours after birth. If they agreed, the parents were given a personal interview with the investigating doctor to detail the study aim and procedures. Then the doctor gave them a written consent form. A 24 hour time to think was given before collecting acceptance signatures. The study was registered in the International Clinical Trials Registry under the label ClinicalTrials.gov ID NCT01583335.

Assessments proposed during the first 2 years of life

Recordings: The first recording was a full polysomnographic recordings performed in the maternity (i.e. month "0"=M0), then the following 24-hour recordings were performed at M6 at home using a full polysomnographic recordings or, when difficulty arised for that PSG, a simple 24-hour ECG recording. These 24-hour ECG were repeated 12, 18 or 24 months later (i.e. M12, M18 and M24). The recordings at home allowed benefiting from the usual environmental conditions. The polysomnograph (*Dream Medatec, Belgium*) recorded simultaneously frontal, central and occipital leads (FP2, C4, O2, A1), two electrooculogram, one chin electromyogram, one electrocardiogram lead (two electrodes), chest and abdominal respiratory movements by inductance plethysmography, as well as the noninvasive arterial oxygen saturation using an oximetry probe placed on the foot. Sleep position was noted. Alternatively when the polysomnographic monitoring was impossible, it was proposed a three leads Holter electrocardiogram (*Vista, Novacor, France*) connected through five thoracic electrodes.

Autonomic assessment and spectral analysis: For this study, we chose a power spectral density analysis method of HRV considered as a reliable technique to evaluate ANS functions in published studies concerning child and newborn, with absolute values of the results expressed in ms^2/Hz . For assessing autonomic activity this method requires a short but stationary signal (5-15 minutes) from the nocturnal polysomnographic recordings or a longer signal (up to 24 hours) to measure the autonomic activity at different stages of awakesness or sleep. The RR signal is sampled at 1000 Hz. Each RR interval is extracted with a precision of 1 ms and manually validated. An interpolation process is first applied to correct for missing intervals or for ectopic beats on each windowed stationary period. Respiratory rate is precisely known and is considered stable during quiet sleep recording period. The obtained RR lists are analyzed using a spectral-domain analysis using Fourier Transform determining a spectrum with several components: 1) high frequency (HF, 0.15-1.40 Hz), known as Traube-Hering wave, reflecting parasympathetic activity, mainly modulated by ventilatory cycles (respiratory sinus arrhythmia), 2) low frequency (LF, 0.04-0.15 Hz), mainly representing sympathetic activity (but also some parasympathetic activity) related to the baroreflex system), 3) very low frequency (VLF, lower than 0.03 Hz), representing mechanisms of long-term regulation (thermoregulation, peripheral vasomotor tonicity, renin angiotensin system), 4) total spectral power (Ptot, 0.0-1.4 Hz), which represents the sum of VLF, LF and HF values, and 5) the ratio LF/HF reflecting the state of the autonomic balance. These indices are frequently used in contemporary research studies to assess the modulation and symptho-vagal balance of the autonomic nervous system. The thresholds between components are adapted to the young age of the population [44].

Recent publications have highlighted the predictive interest of a real time or delayed HRV analysis in neonatal clinical situations as various

as growth restriction [45], Kangaroo care [46], pain assessment [47], and sepsis prediction [48-50]. If age-dependent thresholds values have not been determined to date for child of any age, they have however been specified for the newborn in time and frequency domain [51] and may work well when the analyzed signal is stationary. In case of unstable signal other real-time analysis, including the complexity and responsiveness of the cardiac signal (wavelets, simple or approximate entropy) will be used by ours signal processing laboratories of Rennes and Saint-Etienne [52,53].

Clinical assessment: The systematic clinical exam, including somatic and neurological assessment, was initially made for all newborn term or preterm, during the "discharge visit" in the maternity. At 6, 12, 18 and 24 months of life, a consultation related to the research program establishes a new clinical evaluation including questioning about the child's sleep and psychological status of the mother.

More specifically, maternal clinical data gathered were: date of birth, weight before and after pregnancy, height, number of pregnancy, single or twin, term and mode of delivery, premature rupture of membranes, the presence of a cardiovascular, lung, nephrologic, neurological, endocrine, or oncologic chronic disease, the use of tobacco or nicotine substitutes, alcohol, drugs, beverages containing caffeine, and the assessment of a depression trend by the Hospital Anxiety and Depression scale (HAD) self-administered questionnaire (see questionnaires).

The collected data about the child concerned: the birth date, sex, weight, height, head circumference, Apgar score (1.5 and 10 minutes), and the gestational age at discharge. Diseases and therapeutics were recorded as well: immediate cardiac and pulmonary failure needing resuscitation in the delivery room (epinephrine, intubation); lung disease as hyaline membrane disease, bronchopulmonary dysplasia, apneic syndrome, needing ventilatory support (conventional ventilation, surfactant administration, Continue positive airway pressure, High frequency oscillation nitric oxide administration, later hemodynamic failure needing hemodynamic support (cardiac inotropic non-steroidal anti-inflammatory drugs, fluid restriction, patent ductus arteriosus surgery, transfusion); enterocolitis (parenteral nutrition); perinatal or nosocomial infection (antibiotics treatment); metabolic disequilibrium as hypoglycemia, glucose intolerance, hypocalcemia; severe neurological pathology as intraventricular hemorrhage, periventricular leukomalacia; dysmorphic syndrome. A CRIB score (Clinical Risk Index for Babies), a tool for assessing initial neonatal risk was performed.

Then, an in-hospital clinical consultation was proposed when babies reach their 36th month of life for a neuromotor and neuropsychological complete assessment, using *Wechsler Preschool and Primary Scale of Intelligence -III test* (WPPSI-III) performed by a trained neuropsychologist.

Anthropometric measurements (weight, height, head circumference) and general clinical health problems were noted at each step at M0, M6, M12, M18, M24, and M36, including the concept of hospital stay, chronic or acute pathology, medication., food type, family events, current disease, neurological or sensory syndrome and the type of institutional care that they could possibly have required (physiotherapist, psychologist, and dedicated centers).

Questionnaires: Four questionnaires concerning the mother's health and the infant were proposed at each visit, M0, M6, M12 and M24. A later complementary clinical questionnaire was performed at M24.

1. Firstly a “maternal sleep” Questionnaire including the following items: insomnia, difficulty falling asleep, nocturnal awakening, early awakening, bad dreams, pain, snoring, sleep apnea, tiredness or drowsiness, estimation of hours of sleep, restless legs syndrome, and the need for specific treatment. Pregnant women are at particular risk for sleep deprivation. We studied sleep duration patterns and sleep trajectories during pregnancy, their associated risk factors and risks for birth outcome.

2. Secondly, the French version of the Hospital Anxiety and Depression scale (HAD), [42] a structured self-administered questionnaire of 14 items, 7 for anxiety and 7 for depression, proposed by Zigmond and Snaith to detect the most common neurotic manifestations of anxiety and depression according to DSM-IV criteria. The aim of this approach is to screen depressive symptoms after delivery and to compare sleep disturbances during pregnancy in women with a lifetime diagnosis of Major Depressive Disorder (MDD) among those with postpartum major depression (PPMD) and controls. Each item is scored on a semi-quantitative scale going from 0 to 3 to qualify the intensity of symptoms during the past week. The range of global scores thus extends for each scale from 0 to 21, the highest score corresponding to the presence of more severe symptoms. Thresholds subscales to retain a diagnosis of depression or anxiety are usually 8, as chosen here, or 10 depending on the study. Mothers positive for the HAD depression scale are afterwards called by telephone to confirm or invalidate the diagnosis of depression by a MINI semi-structured interview.

3. Thirdly a Brief Infant Sleep Questionnaire (BISQ) [43] was also proposed up to define the infants’ sleep characteristics and assess potential links with their autonomic control and their mother sleep disturbances. It includes 1) nocturnal sleep duration (between 7 pm and 7 am), 2) daytime sleep duration (between 7 am and 7 pm), 3) the number of night waking, 4) the duration of wakefulness during night hours (from 10 pm to 6 am), 5) the nocturnal sleep-onset time (the clock time at which the child falls asleep for the night), 6) the settling time (latency to falling asleep for the night), and 7) the method of falling asleep.

4. Finally, at the age of 2 years, a complementary clinical questionnaire about the child health is proposed to assess potential cardiopulmonary, digestive, and neurological chronic diseases as well as data on the duration of sleep, the presence of sleep disorders, difficult breathing during sleep, the presence of nocturnal awakenings, behavior during the day, the type of feeding, the number of TV hours per day.

Neuropsychological test at the age of 3 years (primary endpoint of the study).

The *Wechsler Preschool and Primary Scale of Intelligence* (WPPSI-III), approved for children aged from 2 years 6 months to 7 years 3 months, includes 14 subtests. For 3 years old children, 5 of these subtests are usable: 1) Understanding of words (COM), where the child looks at a group of 4 images and shows the one that the psychologist calls, 2) Informations (INF) where the child answers to a question by choosing from 4 images (items in picture), or the child clearly answers to a question relating to general knowledge (verbal items), 3) Name of images (DIM) where the child names images that are presented, 4) Cubes (CUB), where the child uses one color or two colors cubes to reproduce a construction determined from a model built before him or presented in the book, and 5) assembly of objects (AOB), where the child must bring up in 90 seconds, the pieces of a puzzle placed before him in a standardized manner.

The subtests are divided into three general categories, main, additional and optional. 1) The main subtests are COM, INF, CUB, and AOB. Standard scores are obtained from a conversion of raw score for each main subtest, adapted to the age of the child. The sum of the scores of the main subtests provides a value of verbal IQ (VIQ), performance IQ (PIQ) and total IQ (TIQ) this last being calculated as the average of VIQ and PIQ, with a 95% confidence interval. 2) Additional subtests may provide a broader view of intellectual and cognitive functioning and may allow the practitioner to determine a composite score of language (CSL). 3) The optional subtests in children older than 4 years will in fact be routinely used in our 3-year old cohort and considered as an additional testing.

Sleep characteristics analysis

Polysomnographic recordings from newborn children were analyzed on 30-second intervals and classified as Non-Rapid-Eye-Movement (NREM), Rapid-Eye-Movement (REM), or indeterminate sleep, according to Guilleminault and Souquet’s criteria [54]. As we want to evaluate sleep maturation from birth to 6 months [14], we did not modify our scoring of sleep stages at 6 months according to the new rules published in 2007 [55]. Sleep stages were expressed as a percentage of total sleep time. Times awake were calculated as a percentage of total recording time. Sleep efficiency was defined by dividing total sleep time by total recording time, multiplied by 100. Only central apneas were scored [56]. It states both, but lists 3 things: the simultaneous recording of flat tracings by both thoracic and abdominal movements and the thermistors. An apneic event is counted if a pause in breathing of 3 s or more occurred. The frequencies of apneas is measured by dividing the total number of episodes by the total sleep time in minutes and multiplied by 60. In this study, we did not have the recordings of nasal cannulas or thermistors. So obstructive, mixed apneas, partial upper airway obstructions such as obstructive hypopnea or upper resistance syndrome were not taken into account [55]. A cortical arousal was scored if there is an occurrence of an abrupt change in EEG background frequency of at least 1 Hz for a minimum of 3 seconds, while at least two of the following changes occurred at the same time: 1) a large body movement detected by movement sensors or seen as an artifact movement in the somatic channels (ECG, EEG, respiratory parameters) or by direct observation, 2) changes in heart rate, at least 10% of baseline values; changes in breathing pattern, frequency and/or amplitude, in NREM sleep, or increase in chin EMG tonus in REM sleep [15]. The frequencies of arousal events were measured by dividing the total number of episodes by the total sleep time in minutes and multiplied by 60. Baseline sleep states that preceded arousals were established during 30-sec time periods. At least 10 seconds of uninterrupted state was required between arousals. Awakening was defined as a cortical arousal, as defined above, lasting 1 minute or more and meeting the Anders, Emde, and Parmelee criteria for wakefulness [57].

Statistical method

Data quality has been checked as missing data, and improbabilities values for each variable. We have made cross-checks between variables. A description of the included population was performed using the following parameters: 1) for quantitative variables: number of observations, mean, standard deviation or median, interquartile range, minimum and maximum, depending on the shape of the distribution, 2) for qualitative variables: frequency (expressed in%).

To meet the primary objectives, we define a time scale values for each autonomic linear and nonlinear indices of interest in the main

domains of short term (rMSSD, HF) and long term (SD, LF, VLF) variability or to define the autonomic balance (total spectral power, ratio LF/HF). For this, each criterion is summarized each time by its logarithmic variable to correct the asymmetry of distributions. Furthermore, in order to observe the evolution of these criteria on a precise time scale, graphical representations such clouds are made to measure indices of slope and identify a type of distribution. To test a significant linear trend in the indices, we use the analysis of variance (ANOVA) for repeated measures and a generalized linear model (GLM) or a mixed model. Analyzes will be adjusted (multivariate) on the characteristics of the study population (gestational age at birth, nicotinic fetal exposure, etc...). The interactions between the characteristics will be studied to determine if the indices are similar or not according to the characteristics considered.

To meet the secondary objectives we will perform Cox models with time-dependent variables and which variable of interest is represented by the occurrence of a sleep disorder or neurodevelopmental disorders to 3 years. The models will be adjusted on potential confounders.

In case of significant interactions between groups, analyzes is stratified.

For the longitudinal sleep study among pregnant women, we used a group-based trajectory modelling to 1) identify sleep duration patterns during pregnancy 2) identify fixed and time-dependent associated risk factors and 3) study relationship between sleep patterns and both term and birth weight.

To define the sleep pattern during pregnancy and the maternal depression we realized an analysis of correlation with Pearson's coefficient used to examine the links between depressive symptoms and sleep duration. A multivariate analysis determined for each sleep disturbance during pregnancy if it was linked to maternal postpartum depression or to MDD in another period or to both. Logistic regressions were conducted to calculate odds ratios (ORs) with 95% confidence intervals (CIs) to illustrate the strength and significance of associations.

Given that the autonomic indices are quantitative data, testing the ranks are recommended to study the distributions. All tests are two-sided. The results are considered significant for a p value less than 5%. The statistical software used will be Stata V11 (StattaCorp, Texas, USA).

First Results

Study population was included between September 2009 and September 2011. During these two years of inclusion 302 children were recruited, with 271 (89.7%) term newborns and 31 (10.3%) preterm newborns before 37 weeks of gestation. The size of this sample was considered realistic for the inclusion period and sufficient to provide an adequate description of the autonomic profile. The selection of subjects was consistent with consecutive inclusion of newborns according to their birthdate and the availability of recording devices regardless of choice based on race, term birth, sex or socio-cultural components.

Results for mothers

Given the twin pregnancies (n=5), 297 mothers are included in the study. The mean age at delivery was 30.3 years (18-43), the mean term was 39 wGA+1 d (24 wGA+5 d, 41 wGA+6 d). The mean weight before pregnancy was 62.4 kg (39-120) with 41 (14.7%) mothers overweighted and 25 (9%) obese. The non-instrumental delivery rate was 65.9% (196/297), instrumental 9.7% (29/297) and using cesarean section 24.6% (73/297). There were 83 (27.9%) premature rupture of membranes and 18 women have benefited from antenatal corticosteroids therapy

aimed at treating fetal lung maturation. In addition, 14 women (4.7%) were diabetic, 12 (4%) were treated for hypertension and 4 (1.3%) for epilepsy. No alcohol consumption during pregnancy was found but 86 mothers (28.9%) smoked including 14 of them more than 10 cigarettes per day. According the results of HAD questionnaires, 20 mothers were considered depressed during pregnancy and the postpartum period but only 2 of them were treated with inhibitors of serotonin reuptake (ISR). A major depressive episode (MDE) during pregnancy or in the postpartum period was observed in 28 mothers, and 36 presented a lifetime MDE. Regular consumption of caffeine involved 154 mothers (55.2%).

Then, a secondary analysis included 191 women with usable data on sleep duration and various sleep troubles before and in 1st, 2nd and 3rd trimester of pregnancy. We identified 3 distinct sleep trajectories during pregnancy: short and decreasing (SD, <6 h30/night, 10.8%), medium and decreasing (MD, 6 h30-8 h00/night, 57.6%) and long and increasing (LI, >8 h00/night, 31.6%) trajectories. Multivariate analyses showed that compared to MD, fixed risk factors associated with SD trajectory were older age (p=0.004), smoking (p=0.05) and working during pregnancy (p=0.03) while longer work duration in weeks tended to be protective (p=0.06). The only fixed factor associated with LI trajectory was depression (p=0.05). Decrease in sleep duration was associated with increased 1) nocturnal waking and/or early waking and/or and difficulties to fall asleep (=IWD score) (p<10⁻⁴) in the SD trajectory 2) restless legs syndrome (p=0.006) and nightmare (p=0.03) in the LI trajectory 3) IWD score (p<10⁻⁴) and pain (p=0.008) in the MD trajectory. Increase in sleep duration was only associated with nap in SD trajectory (p=0.04). Moreover, mean term was significantly lower in SD (34.8 WA, p<10⁻⁴) than in other trajectories; mean birth weight for gestational age was also lower in SD (2.2 kg, p<10⁻⁴) but also in LI (3.0 kg, p<10⁻⁴) compared to MD trajectory (3.3 kg).

Finally, women in the "depressed" group presented more difficulties in falling asleep (p=0.030), more nocturnal awakening (p=0.021) and sleep deprivation (p=0.048) in the first trimester, more pain during sleep (p=0.025) and shorter sleep duration (p=0.012) in the second trimester and more early awakening in the second (p=0.037) and third (p=0.013) trimesters with more morning sleepiness in the first (p= 0.044) and second (p=0.034) trimesters of gestation. Among these sleep alterations several seemed to be associated with PPMD and not with MDD outside postpartum.

Child outcomes

In our population the M / F ratio was 1.25 (168 boys/134 girls).

Clinical data among preterm infants (n=31), the median age was 33 Wga+6 days (24 wGA+5 days; 36 wGA+6 d). The ratio M / F was 1.58 (19/12). No child had a dysmorphic syndrome. Anthropometric characteristics at birth were: mean birth weight=2007 g (755-3000), mean height=43.2 cm (31-50), mean head circumference=30.3 (22.6-35). The mean APGAR score at 1, 5 and 10', were respectively, 8.9 ± 0.2 and 10 ± 0.1 and the mean CRIB score was 0.4 ± 0.1. Of these, five were affected by resuscitation in the delivery room as 4 (12.9%) needing cardiac massage and 5 (16.1%) tubes.

The mean duration of mechanical ventilation of these five was 14.4 days (1-45). One child received nitric oxide and 8 children non-invasive ventilation on a CPAP mode. Finally, two children developed a chronic lung disease at 36 corrected weeks'GA. No child presented collapse, two had a patent ductus arteriosus, one received an anti-inflammatory treatment (ibuprofen) and 5 were transfused at least once. None of the children had presented a maternal-fetal infection and one child had a

probable microcatheter nosocomial infection. No case of necrotizing enterocolitis was compiled. Neurologically, only one child presented periventricular leukomalacia lesions. No high-grade intraventricular hemorrhage (3 or 4) was noted. On a metabolic level, two premature infants presented transient carbohydrate intolerance.

The average age at discharge was 38 corrected weeks'GA (37-43) and 29/31 children were considered as normal when the two others were hypotonic. No home monitoring was recommended at discharge.

For the longitudinal follow-up, usable recordings were distributed as follows: M0 (29 polysomnographic recordings + 1 Holter/24 h), M6 (22 polysomnographic recordings+1 Holter/24 h), M12 (20 Holter/24 h), M18 (17 Holter/24 h), M24 (18 Holter/24 h).

Clinical data among term newborns (n=271), the median age was 39 wGA+5 days (37 GA, 41 wGA+6 d). The ratio M/F was 1.22 (149/122). No child had dysmorphic syndrome. Anthropometric characteristics at birth were: mean birth weight = 3256 g (1640, 4410), mean height=49.6 cm (44,55), mean cephalic perimeter=34.2 (30, 38.5). The Apgar score was 9 ± 0.2, 10 and 10 at 1, 5 and 10', and the mean CRIB score was 0. A child received a brief resuscitation in the delivery room requiring a short cardiac massage with immediate hemodynamic recovery. No child had presented neonatal lung disease, or hemodynamics, gastrointestinal or neurological troubles. One child (0.2%) had been treated for a suspected neonatal infection.

The mean age at discharge was 40 wGA+2 days (37 wGA+4 days, 42 wGA+4 days) and 100% of the children were considered clinically normal. No home monitoring was recommended at discharge.

For the longitudinal follow-up, usable recordings were distributed as follows: M0 (270 polysomnographic recordings), M6 (210 polysomnographic recordings+11 Holter/24 h), M12 (210 Holter/24 h), M18 (197 Holter/24 h), M24 (190 Holter/24 h).

Heart rate variability analysis

Analysis of heart rate variability indices in children are long and time consuming. Normative data to be defined through this longitudinal study require multiple checks signal quality. The analysis also depends on the perfect selection of sleep stages (quiet sleep, rapid eye-movement sleep, and wakefulness). This requires a complex analysis. These results will be the subject of a future more detailed article.

Early exit from the study

In our cohort of 302 infants, one child (0.3%) died by sudden death in the third month of life, without explanation despite an extensive research of etiologic factors and an autopsic review. We note an early disengage from the study before the age 2 for 68/271 (25%) of term newborns and 13/31 (41.9%) preterm.

Discussion

The main objective of the AuBE cohort is to determine in a healthy term and preterm newborns population the profile of physiological autonomic maturation from 0 to 2 years of age and sleep characteristics with subjective and objective tools and to correlate the ANS indices to the occurrence of neurodevelopmental disorders at 3 years (on a psychomotor development scale basis).

Normative data at each stage of sleep should be well defined with this longitudinal study that requires a complex and careful analysis of cardiac and electroencephalographic physiological signals.

The strength of this original study, unique to our knowledge, is based on the longitudinal organization of a large cohort of newborns (n=302) including physiological maturation of cardiorespiratory systems drive, together with the quantitative and qualitative analysis of sleep and neurological and psychomotor outcome.

The pathophysiological basis that led to the establishment of such a cohort is also based on evidence that the activity of the autonomic nervous system (ANS), an essential regulator of homeostasis and cardiorespiratory adaptation correlates closely to the states of vigilance [11-18] (wakefulness, nREM and REM sleep) and vice versa. It is admitted that the evaluation of the ANS functioning is a solid tool to evaluate health state at each end of life, but no study to date has focused on physiological autonomic maturation profile and its correlation with sleep characteristics and later neuropsychological outcome in the first years of age. The measure of wakefulness and sleep states is easily accessible from the neonatal period with the EEG analysis. The same is true for the assessment of autonomic control that is quantifiable from time and frequency domain analysis of heart rate variability. Using these complementary tools will provide complete neurophysiological approach to cardiorespiratory adaptation and cortical and subcortical neural activity during the different states of vigilance.

Finally studying the evolutionary profile of these components during the early years of child development, allows exploring the possibility that these assessment tools could be used as markers of neurological dysfunction undetectable by conventional imaging.

Confounders

Of course results should be interpreted with caution and we should take into account the possibility of intra and inter-individual confounding factors which could modulate the autonomic balance of infants. For example, differences in findings could be due to availability of food and resources. Impact of nicotinic fetal exposure during pregnancy should be also evaluated. Nicotine is the principal agent of cigarette smoke responsible for the harmful effects of smoking [19-24]. Soluble nicotine acts directly on the placenta [23] which it will disrupt the composition and function. A phenomenon of placental adaptation to chronic hypoxia is then set up with ultra-structural changes, alteration of the cytotrophoblast proliferation, and abnormal angiogenesis. Nicotine also crosses the placenta and reaches the main target of the fetal organs, especially lung and central nervous system²⁴ by binding to nicotinic cholinergic neuronal receptors but also to non-neuronal cholinergic receptor present on very many cells (such as endothelial cells, bronchial epithelial cells ...) then modulating cell proliferation, differentiation and motility. The major metabolite of nicotine is cotinine which also has a vasoconstrictor action causing a decreased uterine and umbilical artery flow, hypoxemia, an increased fetal blood pressure and fetal heart rate and changes in acid-basic balance [19,20].

Microcephaly at birth is a possible early effect of nicotinic exposure during pregnancy in relation to secondary hypoxia and direct biochemical toxicity of nicotine on fetal brain development, but its effects are also delayed, particularly by increasing later in children the learning disabilities, or inducing an attention deficit and hyperactivity disorder (ADHD), or moderate disabilities [34,38].

In the same way, the mood status of the mother could affect the sleep characteristics of the child and its potentially autonomic physiological maturation.

The AuBE cohort analysis will take into account these confounding factors that could theoretically modulate standard autonomic indices.

Finally, if the results at 2 and 3 years confirm our hypothesis, further follow-up extended to adulthood could be justified.

Conclusion

In summary, this study by its design could have two major effects, allowing firstly for the first time establishing normative data of autonomic physiological indices in time and frequency domains from 0 to 2 years i.e. a key age for the child development, and allowing secondly assessing the potential link between dysautonomia in the neonatal period and later neurological disabilities.

However these results of HRV indices require a complex analysis of physiological signals and so a further detailed article. These results will be needed to analyze the different clinical situations of dysautonomia and indicate the risk-areas for child below whom a clinical follow-up and monitoring may be required.

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