Autonomic control in preterm infants

- What we can learn from mathematical descriptions of vital signs –

Inaugural dissertation

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Cover images:
Wunder Mensch by Alexander Tsiaras; 2005, Droemer Verlag
Top: Fractal structure of the cerebellum and of a saltwater coral (left)
and of blood vessels and leave veins (right)
ECG trace from webpage: http://www.swharden.com/
Lowest line: pictures from webpage
https://beyondbasicplay.wordpress.com/ ; http://www.texaschildrensblog.org

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2. Abstract

Background: Preterm birth is a major burden, affecting approximately 15 million infants each year. Recent advances in reproductive medicine increases that number even more. The population of preterm infants in particular suffers from autonomic dysregulation that manifests as temperature instability and poor control of heart rate and breathing. Thermal care, monitoring of vital signs in a neonatal intensive care unit, pharmacotherapy, and respiratory support over weeks to months is necessary. Improvements in neonatal care in the past years lead to a decrease in mortality, especially in very preterm infants. However, former preterm infants still are a high-risk population for acute and chronic sequelae as a result of the interruption of the physiological development.
A better understanding of the pathophysiology of the autonomic dysregulation in that population would be very useful. Unfortunately, accurate diagnostic tools that objectively assess and quantify the immature autonomic control in neonates are lacking.

Methods: In this PhD thesis we examined different effects of the immature autonomic control in very preterm infants under clinically relevant conditions. We conducted a prospective single center observational study, where we assessed fluctuations in body temperature, sleep behavior, and heart rate variability in very preterm infants. We described the different regulatory systems using distinct mathematical expressions, such as detrended fluctuation analysis and sample entropy. We assessed associations between these outcome parameters and relevant factors of the infant's history, such as demographic parameters and co-morbidities.
Besides that, we analyzed lung function measurements of preterm infants and healthy term controls at a comparable postconceptional age, to describe respiratory control.

Results: This study is systematically assessing different physiological signals of autonomic dysregulation in preterm infants during their first days of life. We found associations between parameters describing the complexity of time series analysis and maturity or relevant co-morbidities of the infants. In the analysis of heart rate variability we even found that parameters derived from time series analysis, assessed during the infants first days of life, improve our ability to predict future evolution of the infants' autonomic stability. Lastly, several weeks after the expected due date, tidal breathing pattern of preterm infants showed a different reaction in response to a sigh when compared to term born controls at equivalent postmenstrual age indicating that autonomic dysregulation in preterm infants is still present well beyond the expected due date.

Conclusion: A better understanding about the resolution of autonomic dysregulation in this population is not only important for the infant and its family but has the potential to support resource allocation and identification of patients with elevated risk for future deterioration. We thus think that the insights about the immature autonomic control in preterm infants, gained through this PhD work, are of substantial scientific and clinical relevance.

2.2. Zusammenfassung

Hintergrund: Jedes Jahr kommen ca. 15 Millionen Kinder zu früh auf die Welt. Die neuerlichen Fortschritte in der Reproduktionsmedizin lassen diese Zahlen noch mehr ansteigen. Frühgeborene Kinder leiden unter einem unreifen autonomen Nervensystem, welches sich als Temperaturinstabilität und verminderte Kontrolle der Herz- und Atmungsregulierung äussert. Diese Kinder brauchen über Wochen bis Monate eine Unterstützung dieser Funktionen mittels Pflege in einem Brutkasten, Überwachung auf einer Neugeborenen-Intensivstation, pharmakologische Therapie und Atemunterstützung. Fortschritte im Bereich der Neonatologie haben die Mortalität von frühgeborenen Kindern in den letzten Jahren zwar gesenkt, sie sind jedoch immer noch eine Hochrisiko-Population für akute und chronische Folgeerscheinungen der unterbrochenen, natürlichen Entwicklung. Ein besseres Verständnis der pathophysiologischen Mechanismen der unreifen autonomen Kontrolle in dieser Population wäre deshalb sehr wünschenswert. Leider mangelt es aktuell noch an objektiven Parametern, die diese unreifen Funktionen messen.

Methodik: In dieser PhD Arbeit untersuchten wir unterschiedliche Auswirkungen der unreifen autonomen Regulation in Frühgeborenen. Wir führten eine prospektive Observationstudie auf der Neonatologie am Universitäts-Kinderspital beider Basel durch. In dieser Studie haben wir die Schwankungen der Körpertemperatur, das Schlafverhalten und die Variabilität der Herzfrequenz von Frühgeborenen Kindern gemessen. Wir haben diese Funktionen mit verschiedenen mathematischen Methoden, wie etwa der trendbereinigten Fluktuationsanalyse und der Entropie-Messung, beschrieben und Ihre Zusammenhänge mit Eigenschaften der Frühgeborenen analysiert. Wir schauten dabei auf die demographischen Faktoren der Studienteilnehmer, insbesondere den Grad der Unreife bei Geburt, sowie auch auf relevante Co-Morbiditäten. Ausserdem haben wir Lungenfunktionsanalysen von frühgeborenen und termingeborenen Kindern kurz nach dem errechneten Geburtstermin in Bezug auf die Atemmuster miteinander verglichen.

Resultate: Diese Studie misst systematisch verschiedene physiologische Signale von frühgeborenen Kindern während Ihren ersten Lebenstagen und beschreibt diese mit mathematischen Modellen. Wir konnten Zusammenhänge zwischen den Zeitreihen Analysen von verschiedenen, unreifen autonomen Systemen und dem Ausmass der Unreife und assoziierten, relevanten Co-Morbiditäten der Kinder aufzeigen. Die Beschreibung der Variabilität der Herzfrequenz während der ersten Lebenstage hat einen prädiktiven Wert für die Entwicklung des Kindes in Bezug auf die Ausreifung der autonomen Kontrolle. Auch nach dem errechneten Termin zeigen frühgeborene Kinder eine andere Reaktion im Atemmuster auf einen Seufzer als gesunde termingeborene Kontrollkinder.

Schlussfolgerung: Ein besseres Verständnis über die Ausreifung des autonomen Nervensystems bei Frühgeborenen ist nicht nur wichtig für das Kind und seine Familie. Es besteht durchaus Potential, daraus Erkenntnisse für eine Optimierung in der Ressourcen-Nutzung oder für individuelle Risikoerhebung zu gewinnen. Wir sind deshalb davon überzeugt, dass der Erkenntnisgewinn über unreife autonome Kontrolle bei Frühgeborenen einen hohen wissenschaftlichen und klinischen Stellenwert hat.

3. Introduction

3.1. Prematurity

Preterm birth, defined as prior to 37 weeks of gestation, accounts for more than 10% of births worldwide. This reflects approximately 15 million births every year[1].
The degree of prematurity is by definition divided in the following categories[2]:

1. Late preterm - those born between 32 and 37 weeks.
2. Moderately preterm- those born between 32 and 35 weeks.
3. Very preterm - those born between 28 and 32 weeks.
4. Extremely preterm - those born before 28 weeks.

In Switzerland, about 800 infants are born very preterm each year. Advances in reproductive medicine are leading to an increasing number of multiple pregnancies, a major risk factor for preterm birth, and to an increase in the number of medically indicated preterm births[3].
Survival rates of very preterm infants increased in the past years thanks to introduction of antenatal steroids, surfactant replacement therapy, and improvements in assisted ventilation [4]. Nevertheless, preterm birth remains a high-risk condition during the first days of life and also for later deterioration. Former preterm infants often suffer from bronchopulmonary dysplasia (BPD), they have an elevated risk for sudden infant death syndrome (SIDS), neurodevelopmental sequelae, and cardiorespiratory and metabolic morbidity like high blood pressure and insulin resistance in adulthood[4-6]. “Foetal origins of adult disease” has first been described 20 years ago by Baker et al, showing that being born with a low birth weight was associated with a substantially increased risk of death due to ischemic heart disease in adult men[7].

3.2. Autonomic control

The autonomic nervous system

The autonomic nervous system (ANS) is, among other things, responsible for control of body temperature, cardiovascular function, and respiration. It is divided into two parts, the sympathetic branch and the parasympathetic branch. These two branches undergo different maturation during fetal life. The sympathetic branch, responsible for acceleration of heart rate and respiratory rate, is mostly developed during the first trimester and develops more slowly in later stages. The parasympathetic branch, also called the vagal branch, is mainly developed between 25 and 30 weeks of gestation[8].

Autonomic dysregulation in preterm infants

In preterm infants, the physiological process of intrauterine maturation of the ANS is interrupted due to delivery and transition from intra-uterine to extra-uterine life weeks to months prior to the expected due date. As a result, this population in particular suffers from temperature instability, and poor control of heart rate and breathing[9-11].

Hypothermia after birth is a main predictor of mortality in infants[12]. Therefore, maintaining thermal balance is a fundamental objective of neonatal care and small preterm infants are typically nursed in incubators. Moreover, stable body temperature has a positive influence on other regulatory systems, such as heart rate and breathing[13-15].
Apnea and bradycardia of prematurity (AOP) is defined as cessation of breathing lasting for at least 20 seconds or one lasting for less than 20 seconds that is associated with bradycardia and/or cyanosis[9]. AOP affects at least 80% of very preterm infants and can lead to life-threatening events[9, 10]. AOP represents immaturity of multiple interacting systems responsible for the control of rate and depth of breathing: involving the central brainstem generator[16], peripheral chemoreceptors[17], lungs and upper airways mechanics[18, 19] and respiratory muscle activity[20]. Current treatment strategies of AOP include the use of methylxanthines- typically caffeine, and positive pressure respiratory support[21, 22].
Consequently, temperature instability and AOP of very preterm infants require thermal care and necessitate close monitoring of vital signs, pharmacotherapy, and respiratory support over weeks to months[9, 10]. This results in a significant impact not only for the individual family but also on resource allocation in neonatal intensive care units (NICUs)[23]. In a recent US study in infants born before 26 weeks gestational age (GA), each additional day of monitoring cost approximately US$ 40,000 to 130,000 per quality-adjusted life year saved[23]. Clinical decisions related to very preterm infants are largely focused on the level of maturation an individual patient has achieved (e.g., choice of level of intensive care, choice of thermal environment, duration of monitoring, necessity and type of respiratory support, requirement of pharmacological support). Finally, complete resolution of autonomic dysregulation is a crucial prerequisite for discharge of preterm infants from the hospital[24].

3.2.1. Temperature

Optimized temperature control leads to a decrease in both morbidity and mortality in preterm infants[12, 25]. Consequently, there is a high interest in temperature control in preterm infants but also in infants at risk for sudden infant death syndrome (SIDS)[26-29]. A better understanding of thermoregulation is particularly important in the first months of life, where the autonomic control system of infants undergoes a critical maturational transition[30]. Stern et al showed that healthy term infants develop a less variable, smoother temperature regulation with increasing age[30]. They found fractality in nighttime rectal temperature time series of term born infants between 4 - 20 weeks of age and used detrended fluctuation analysis (DFA) to show increasing determinism[30]. Preterm infants are known to be a high-risk population for both poor temperature control during transition from intra-uterine to extra-uterine life and for SIDS beyond the due date but data assessing long-range correlations in time series of temperature measurements in this population are lacking. Studying the dynamics and complexity of time series of body temperature in preterm infants is a first step to better understand the mechanisms and interactions in the autonomic system. This could help to develop objective strategies for individualized thermal care, such as to identify patients who are ready for transfer from an incubator to an open cot based on the complexity of their body temperature fluctuations. Such an approach could be beneficial in terms of noise exposure[31]

and language development[32], given the current uncertainties regarding readiness to leave the incubator and potentially helps to prevent hypothermia in infants who are transferred into an open cot before being ready for it.

3.2.2. Sleep

The sleep architecture of newborns is different when compared to adults. Healthy newborn infants sleep about 70% of the time, this amount is even higher in preterm infants[33]. The origin of circadian rhythms can be found at fetal age: the biological clock has been shown to be responsive to maternal entraining signals in the last trimester of pregnancy. Fetal heart rate is at that point synchronized with maternal activity, hormone levels, and body temperature[34]. At birth, this interaction fails and a free-run, ultradian rhythm, dependent only on the internal clock of the newborn, appears[35]. Preterm neonates in particular undergo this dramatic change with early loss of maternal fetal interactions. Ultradian sleep cycles were found in premature infants as young as 25 weeks postconceptional age (PCA)[36], although clear discrimination of sleep stages is only visible via electroencephalogram (EEG) after 30 weeks of gestation[33]. In infants, behavioral states are divided into awake, active sleep and quiet sleep using the adapted criteria from Anders, Emde and Parmelee[37], validated by Mirmiran et al[33].
Sleep, and especially the cycling of sleep states, is vital for neurosensory motor development, as several studies have shown[38-42]. To what extend the internal ultradian rhythm and the consecutive acquirement of a circadian rhythm is influenced by external factors is not fully understood. Hence, to allow sleep regulation to be as natural as possible, minimal handling of NICU patients is recommended and routinely performed at the University of Basel Children's Hospital[43]. A recent study showed that entrainment of the circadian sleep wake rhythm of preterm infants appeared earlier than in term born infants[44]. Acquired knowledge about sleep promotion could possibly lead to better neonatal care. Additionally, we believe it is crucial to consider sleep behavior when analyzing physiological regulatory systems in human beings because such systems are highly sensitive to sleep stage[45-47].

3.2.3. Heart rate

Heart rate variability (HRV), a quantitative marker of autonomic activity, is known to be associated with cardiovascular mortality, including sudden cardiac death[48-52]. Time series of electrocardiographic (ECG) records are analyzed to identify heartbeats (QRS-complexes) and to derive the distance between two consecutive beats, the interbeat interval (IBI), also called normal-to-normal (NN) interval when resulting from sinus node depolarization (see Figure 1).

Some of the most popular, descriptive time domain variables that can be calculated from NN (IBI) are the mean, standard deviation (SDNN), and coefficient of variation (CV). In 1965 Hon and Lee[53, 54] showed that fetal distress was preceded by changes in IBI before any noticeable change in the heart rate itself. This was the beginning of the appraisal of HRV as clinically useful marker. Since then, characterization of dynamics and variability in physiological systems, such as HRV, is getting more attention in clinical studies in children and was recently shown to be a useful diagnostic tool for prediction of neonatal sepsis [55-59].

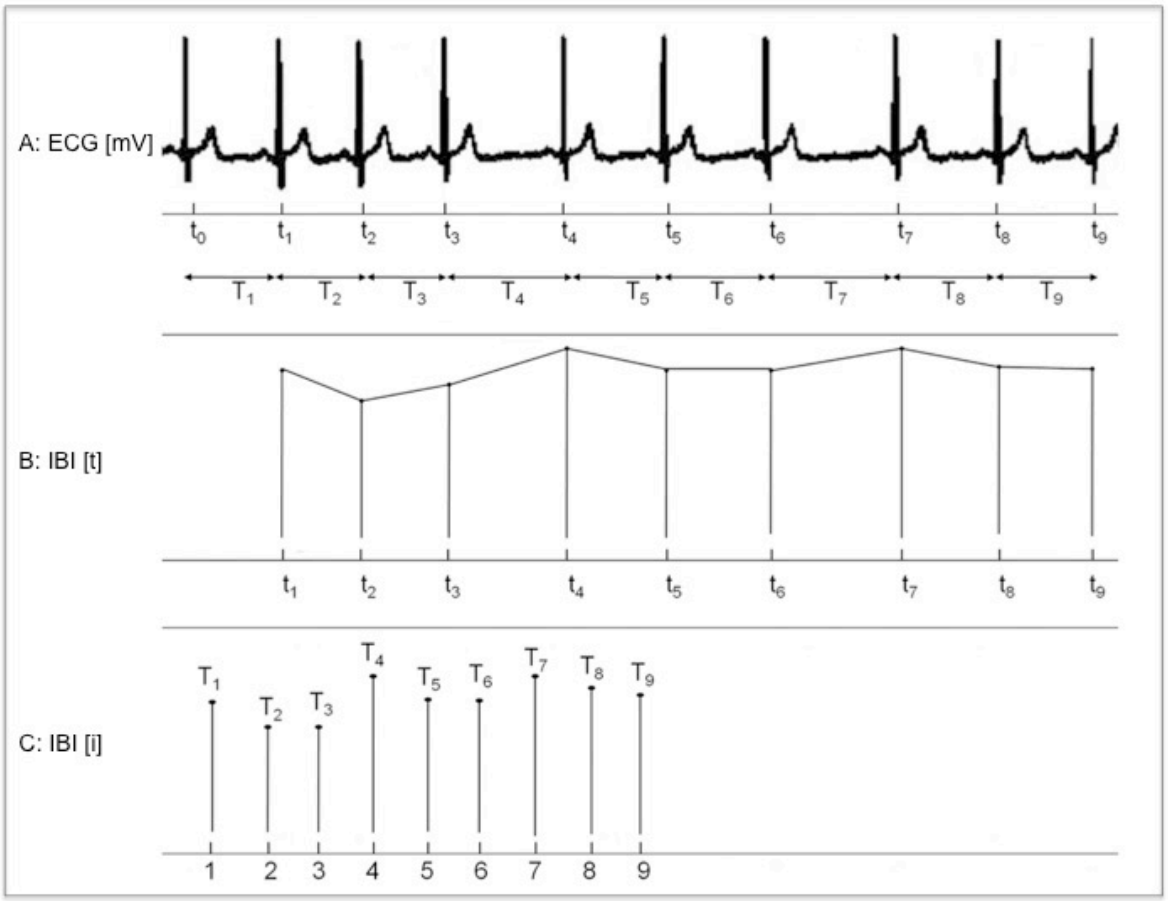

Figure 1. Interbeat interval (IBI); adapted from Peltola [60]:
A: ECG trace with marked QRS complexes
B: Interpolated time-series of distances between QRS complexes (IBI)
C: QRS interval (IBI) time-series

HRV analysis in infants has been pointed out to be important to gain additional knowledge about the physiological background and identify infants at risk[48]. Fluctuations in IBI of infants born preterm or with low birth weight have been described to possess different mathematical properties when compared with healthy term infants[55, 61]. Even within the group of preterm infants, several diseases such as sepsis and sepsis-like illnesses have been shown to result in altered behavior of HRV time series[56, 57, 59]. Whether HRV could be used as a predictive marker of an infant's autonomic maturation during its stay on the NICU has not yet been assessed.

3.2.4. Breathing

Lung development of a very preterm infant is between end of the canalicular and beginning of the saccular stage at the time-point when physiological, intrauterine maturation is disrupted (see Figure 2).

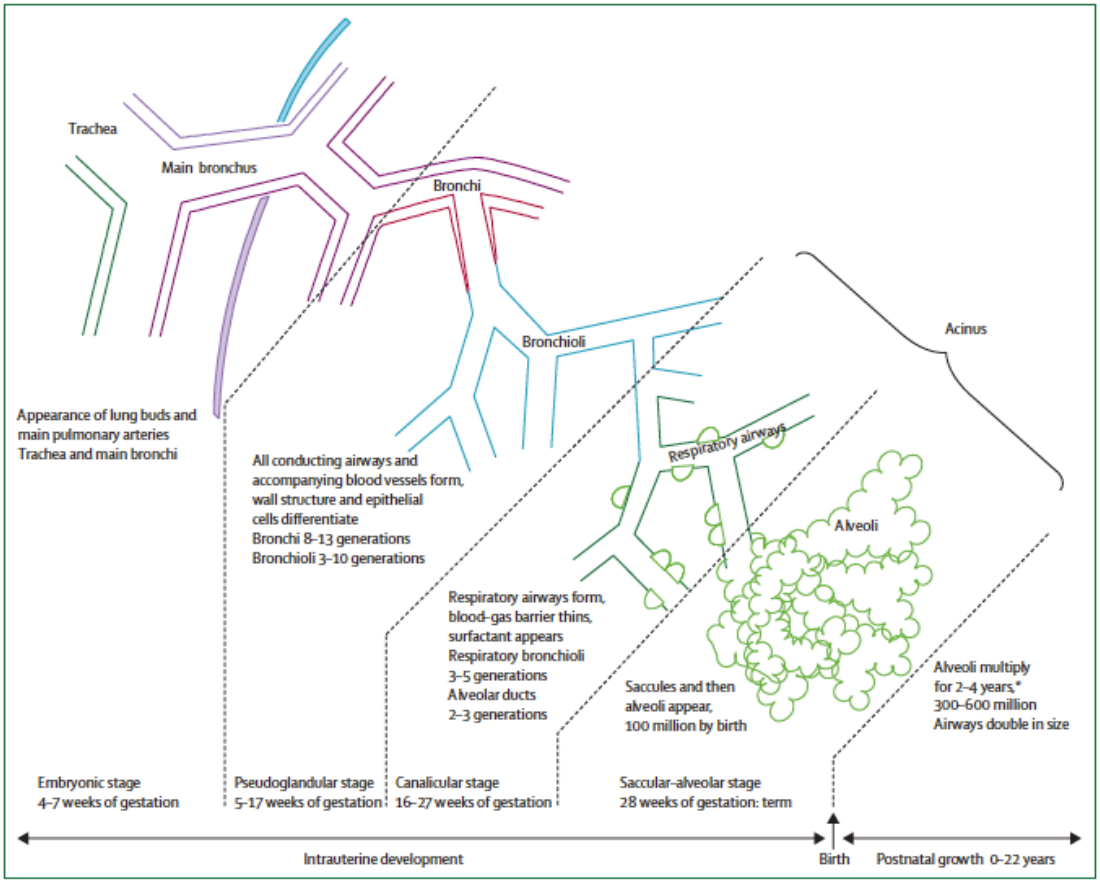

Figure 2: Lung development intra- and extrauterine, by Stocks et al[62]

Besides the difficulties, conditioned by the anatomical immaturity, preterm infants suffer from poor respiratory control[16, 63]. Apnea and consecutive decrease in oxygen levels and/ or bradycardia are one of the main problems preterm infants are facing within their first weeks of life[9, 10]. Improved neonatal intensive care, including prenatal glucocorticosteroids, use of surfactant, and gentle ventilation strategies, have led to improvements in respiratory outcome of former preterm infants[64]. Nevertheless, bronchopulmonary dysplasia (BPD) is still the most common morbidity among survivors of preterm birth[65]. The National Institutes of Child Health Consensus defines the categories none, mild, moderate, and severe BPD based on need of respiratory support and/ or oxygen at 28 days of life and 36 weeks PCA[66]. There are several other classifications for BPD, all having their own strengths and limitations[65]. The disruption of intra-uterine physiological development and the consecutive need for respiratory support during preterm extra-uterine life leads to measurable changes in breathing pattern in former preterm infants[67-69]. A more profound understanding of the pathophysiology of breathing regulation in preterm infants would be of great interest and could help to identify individual infants at risk for future deterioration.

Interaction between different regulatory systems

Instability of different autonomic control systems is interdependent. Some of these interdependencies are well described, as for example the drop in temperature during sleep or the increase of heart rate during inspiration. Breathing pattern of adults is more random during sleep than during wakefulness when applying nonlinear time series analysis[46]. However, assessment of complexity of breathing pattern in infants did not show any significant changes between sleep stages[47]. Generally, little is known about the pathophysiological mechanisms of the interactions in the ANS of preterm infants, however, it is consensus that excessive imbalances should be avoided[13, 63].

3.3. Mathematical description of biological systems

3.3.1. Current knowledge about complexity of vital signs

Time series of physiological parameters might look random, however, there are weak internal correlation properties, as an expression of order and a result of the internal regulation of the autonomic system. Recent work on complex biological regulatory systems using system control theory has highlighted the nonlinear nature of feedback controls[70, 71]. The degree of how much a value is predictable can be described by assessing the complexity of a signal. In a totally random signal (also called "white noise"), the complexity is very low. This means that single values are not depending on another and to describe the whole signal we need to know every single value. In a system with a higher complexity, values are interdependent, and an underlying pattern or 'memory' is detectable. In many natural network systems, these variability and correlation properties can be characterized by distinct mathematical expressions. In the following paragraph, two commonly used methods to describe complexity of a physiological signal, detrended fluctuation analysis (DFA) and sample entropy (SampEn), are explained in more detail.

Nonlinear systems

Physiological signals behave rarely purely periodic but rather fluctuate irregularly over time. Therefore, nonlinear equations are needed to describe the behavior of physiological systems. Glass described nonlinear systems, referencing the following book chapters[72-75], with three central concepts[76]:

- Bifurcations (changes in qualitative properties of dynamics);
- Stable limit-cycle oscillations (following a perturbation, a stable limit-cycle oscillation re-establish itself within the same amplitude and frequency as before the perturbation);
- Chaos (aperiodic dynamics in deterministic equations with a high sensitivity to initial conditions, the so called butterfly effect)

Fluctuations are seen as a combination of the fluctuating environment, the 'noise' that is inherent in biological systems, and deterministic, possibly chaotic, mechanisms[76]. The

normal heart rate for example displays complex fluctuations in time in response to various environmental factors (respiration, food intake, exercise, emotions). It is difficult to discriminate, whether the fluctuations are essential to the physiological function, or whether the function is carried out despite the fluctuation. Several diseases lead to extremely regular dynamics of physiological signals, including periodic (Cheyne-Stokes) breathing, certain abnormally rapid heart rhythms (atrial flutter), and epilepsy. However, regular periodicity can also be an expression for healthy dynamics system, as for example sleep-wake cycles, or the menstrual rhythm. Finally, pronounced irregularity can also be an expression of disease, as for example in atrial fibrillation. Summarized, we can say that abnormal rhythms, that can either be more regular or more irregular than what is considered specific optimum rhythmicity, are an expression of modification in physiological control systems that lead to bifurcations in the dynamics[77].

Detrended fluctuation analysis (DFA)

Temporal dynamics in biological systems with a hierarchy of feedback loops often reveal fractal-type statistical properties[45, 78, 79]. Fractality in the human body was first described by the French-American mathematician Benoît Mandelbrot[80]. He defined fractals as objects with *self-similar* organization, meaning that details of the structure are repeating itself in smaller scales. The fractal-like long-range correlation properties in the fluctuation of time series of physiological signals can be quantitatively described with a single scaling factor alpha, derived from detrended fluctuation analysis (DFA)[81, 82]. DFA measures how long a data point is influencing upcoming data points in the signal and therefore is a measure of memory within the time series.

The mean square deviations of fluctuations of a signal around a trend line are computed as the time scale of the trend line varies over wide intervals. The dependency between the size of these intervals and the averaged deviation is quantified by the scaling exponent alpha, which corresponds to the slope of a regression line, fitted to a scatter plot of two quantities. A higher value of the scaling factor alpha describes a more deterministic signal, hence the upcoming values are more dependent on the previous one than in signals with a lower scaling factor alpha. A minimal memory as in a totally random signals as white noise has an alpha of 0.5. A scaling exponent alpha <0.5 indicates anticorrelation of the signal.

This method was introduced by Peng et al[81, 82] for the analysis of HRV data. Besides medicine, it has been used to describe fluctuations in various fields, such as meteorology, epidemiology, and stock exchange.

Sample entropy (SampEn)

SampEn quantifies the conditional probability that, with the knowledge of a consecutive number of m data points, within a given tolerance r (usually=0.2*standard deviation), the next data point can be predicted. The fact that the tolerance r is expressed as a fraction of the standard deviation (SD) of the data makes the measure scale-invariant. A higher SampEn value arises from a more irregular (more complex) signal, hence the chance of encountering repeated template sequences in the signal is smaller.
This method was introduced by Richman and Moorman[83] and first used for the analysis of neonatal HRV by Lake et al[58]. They designed the SampEn to reduce the bias of other entropy measures (e.g. approximate entropy) and to achieve closer consensus with theory for datasets with known probabilistic content[58].

3.3.2. Clinical implications of mathematical descriptions of physiological systems

Estimates of long-range correlations provide insight on the effect of disease on feedback control[84, 85]. They have shown to be useful in distinguishing developmental stages in temperature control in young infants[30] and are important indicators for control of breathing in preterm and term infants[11, 86]. Besides that, characterization of fluctuations using DFA has been shown to provide a quantitative basis for objective risk prediction[87, 88], even in the individual patient[89]. That has led to a series of clinical applications of this technique[87, 89-91].
SampEn has been tested as useful marker for early detection of sepsis and sepsis like illnesses in newborn infants[56-59]. Moorman et al could show that diagnosis of sepsis in a NICU setting was faster when HRV analysis including SampEn was taken into account[57]. Lake et al concluded that during a sepsis, repetitive spikes in HRV analysis result in a lower SampEn, thus a more regular signal[58].
Temperature control, minimal handling care, respiratory support and monitoring of vital signs are the key treatments of the immature regulatory systems. Besides that, caffeine treatment is known to decrease AOP in preterm infants and has been shown to have long-lasting benefits, such as reduced rates of cerebral palsy at 18-21 months of age[22, 92, 93]. This beneficial long-term effect, seen with 18-21 months, was not detectable anymore in very low birth weight infants assessed with 5 years[94]. Conclusively, despite the significant improvements in neonatal care in the past years, preterm infants still represent a high-risk population. They are prone to short- and long-term morbidity and mortality like SIDS, neurodevelopmental, cardiorespiratory, and metabolic disorders[4, 6]. Autonomic control in terms of HRV has been shown to be altered for years in former preterm infants, possibly due to changes in the hormonal stress axis (hypothalamic-pituitary-adrenal axis) after stress and painful procedures over the first few months of life, i.e., in a vulnerable phase[61, 95]. Thus, a better understanding of the changes in the regulatory systems and of their interactions would be of great interest.

4. Aim of the PhD

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The aim of science is to seek the simplest explanations of complex facts. We are apt to fall into the error of thinking that the facts are simple because simplicity is the goal of our quest. The guiding motto in the life of every natural philosopher should be, "Seek simplicity and distrust it."

A.N. Whitehead

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Predicting resolution of autonomic dysregulation in very preterm infants still is tainted with a lot of uncertainty. Consequently, there is large variation in PCA at discontinuation of incubator care or withdrawal of continuous positive airway pressure (CPAP) among individual preterm infants both within and between NICUs[96, 97]. Similarly, PCA at cessation of AOP and associated termination of continuous vital sign monitoring varies between 35 to 43 weeks PCA [98]. In the absence of reliable indicators of autonomic stability to guide daily clinical practice, preferred strategies are, not surprisingly, still based on tradition within NICU and personal experience[96].
The aim of this PhD therefor was to investigate whether the immature autonomic system can be characterized using mathematical expressions under clinically relevant conditions in the NICU.

We aimed to characterize dynamics and complexity of body temperature in very preterm and/or very low birth weight infants nursed in incubators using descriptive statistics and nonlinear time series analyses. We hypothesized that dynamics and complexity of body temperature are dependent on demographic factors (maturity at birth, birth weight, and gender), comorbidities of preterm birth, as well as behavioral state and external perturbation during the measurement.

Light exposure is a strong factor influencing the synchronization of sleep-wake processes. However, little is known about the effects of phototherapy on the sleep rhythm of premature infants. We aimed to analyze sleep behavior and the dependency on light deprivation in very preterm infants undergoing phototherapy due to jaundice of the newborn. We hypothesized that sleep in preterm infants would not differ during phototherapy, but that we would see a maturational effect.

We aimed to analyze HRV derived from diaphragmatic surface electromyography (EMG) measurements of very preterm infants during their first days of life. To obtain reliable data we aimed to establish a systematic data cleaning tool for EMG signals acquired under clinically relevant conditions in the NICU. We hypothesized that implementation of systematic data cleaning could significantly influence HRV outcome parameters.
After establishing a reliable data cleaning algorithm for EMG measurements in very preterm infants, we aimed to characterize characteristics and fluctuations in IBI. Our hypothesis was that certain parameters could be reliable means of predicting resolution of autonomic

dysregulation in terms of PCA (i.e., the corrected age) at cessation of AOP, discontinuation of monitoring of vital signs, or pharmacological and respiratory support.

Changes in breathing pattern upon an internal perturbation, as a spontaneous deep inspiration, could give some information about the respiratory systems equilibrium. We aimed to assess whether premature birth per se or its consequences, such as presence and severity of chronic lung disease, are affecting an infant's reaction upon a sigh when compared to healthy controls. We hypothesized that frequency and morphology of sighs, as well as the reaction in breathing pattern upon them differ between preterm infants and healthy controls when measured at a comparable age several weeks after the due date.

Characteristics of temperature regulation; interference of light-deprivation and sleep; fluctuations of heart rate; changes in breathing pattern upon an internal stimulus – all these are expressions of regulatory systems. The aim of this PhD was to study them in preterm infants under clinically relevant conditions and potentially gain insight into the pathophysiology of autonomic dysregulation.

5. Results and Research Papers

5.1. Thermoregulation in very preterm infants

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Heat can never pass from a colder to a warmer body without some other change, connected therewith, occurring at the same time.

- Rudolf Clausius, 1856 -

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State of the paper	Published April 2017: *PLoS ONE* 12(4): e0176670
Contribution of KJ	The work of KJ on this project included performing, processing and analysis of temperature measurements in collaboration with other team members. The outcomes were statistically analyzed and the final manuscript was written by KJ.
Synopsis	Instability of body temperature is a major problem in preterm infants. Early quantification of the dynamics and complexity in body temperature may improve our understanding of autonomic temperature control in this population. We aimed to test the feasibility of characterizing body temperature in preterm/very low birth weight infants during their first days of life. We recorded 3h-time series of body temperature measurements in incubator-nursed preterm infants during their first 5 days of life. Characterizing dynamics and complexity of body temperature in very preterm and/ or very low birth weight infants is safe and feasible under clinically relevant conditions. Anthropometrics and respiratory morbidity explain a substantial amount of inter-individual differences in these outcomes. A simple, observer-independent, and robust index of temperature control, such as the scaling factor alpha, derived by detrended fluctuation analysis, is a promising tool for future longitudinal studies and might help identifying preterm infants who are ready for transfer from incubators to open cots.

PLOS ONE

RESEARCH ARTICLE

Dynamics and complexity of body temperature in preterm infants nursed in incubators

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Abstract

Background

Poor control of body temperature is associated with mortality and major morbidity in preterm infants. We aimed to quantify its dynamics and complexity to evaluate whether indices from fluctuation analyses of temperature time series obtained within the first five days of life are associated with gestational age (GA) and body size at birth, and presence and severity of typical comorbidities of preterm birth.

Methods

We recorded 3h-time series of body temperature using a skin electrode in incubator-nursed preterm infants. We calculated mean and coefficient of variation of body temperature, scaling exponent alpha (T_{alpha}) derived from detrended fluctuation analysis, and sample entropy (T_{SampEn}) of temperature fluctuations. Data were analysed by multilevel multivariable linear regression.

Results

Data of satisfactory technical quality were obtained from 285/357 measurements (80%) in 73/90 infants (81%) with a mean (range) GA of 30.1 (24.0–34.0) weeks. We found a positive association of T_{alpha} with increasing levels of respiratory support after adjusting for GA and birth weight z-score ($p<0.001$; $R^2 = 0.38$).

Conclusion

Dynamics and complexity of body temperature in incubator-nursed preterm infants show considerable associations with GA and respiratory morbidity. T_{alpha} may be a useful marker of autonomic maturity and severity of disease in preterm infants.

Introduction

Poor control of body temperature, i.e., the inability to maintain a normal body temperature by balancing heat loss and heat production in a feedback loop over a wide range of environmental temperatures, is a significant clinical problem in preterm infants and associated with mortality and major morbidity in this population [1–3]. The autonomic nervous system controls several important effector systems of thermoregulation in neonates including brown adipose tissue, vasomotor tone, and sweat glands [4]. In preterm infants assessed during the first few days of life, those systems are characterized by autonomic dysfunction, e.g., by poor vasomotor response to cold stress [5, 6]. It is widely accepted that a stable thermal environment is crucial for preterm infants as lowest mortality and morbidity in infants born before 33 weeks gestational age (GA) have been shown for an admission temperature ranging from 36.5 to 37.2˚C [3]. Thermal stability in newborn infants, defined by the World Health Organization as a state where the core temperature lies between 36.8 and 37.3˚C [7], has beneficial effects on other autonomic processes such as control of breathing and heart rate, and supports growth and sleep regulation [8, 9]. Control of body temperature is particularly important during the physiologically challenging post partum transition and the first few days of life, as environmental conditions and temperatures may change rapidly in this period of time. In order to treat autonomic dysregulation of body temperature, preterm infants are typically nursed in incubators that allow for servo-controlled adjustment of the incubator's air temperature based on the difference between the infant's body temperature and a preset target temperature.

Time series of biological signals such as body temperature often reveal weak statistical long-range correlation properties which are an expression of internal ordering of the time series and a result of internal regulation of the biological system [10]. Temporal dynamics and statistical complexity of time series of fluctuating physiological signals can be quantified using mathematical techniques such as detrended fluctuation analysis (DFA) and sample entropy. Scaling exponent alpha derived from DFA describes the self-similarity (scaling) of a biological signal in a time series across a range of sizes of time windows. Sample entropy reflects the amount of irregularity of a time series by quantifying to what extent a pattern is likely to reoccur in the time series. These methods are established mathematical tools to quantify statistical long-range correlations in time series and have been successfully used for monitoring of disease severity and risk prediction [10–15]. DFA has been successfully used to quantify long-range correlations of body temperature in healthy term infants beyond the neonatal age [16]. In these infants, DFA indicates an association between statistical long-range correlations of body temperature and postnatal age. To the best of our knowledge, abovementioned techniques have not been applied to study temperature dynamics and complexity in preterm infants nursed in incubators, where body temperature is tightly regulated and of utmost clinical importance. In critically ill adults, a decrease in temperature curve complexity i.e., increased statistical regularity in a time series of temperature values, is a significant predictor of mortality [17]. While the predictive value of temperature curve complexity has not been established in preterm infants, recent data suggest that assessing complexity of autonomic dysregulation in terms of heart rate variability using sample entropy, i.e., complexity in an autonomic system closely related to thermoregulation, is a promising approach for predicting sepsis and sepsis-associated mortality in preterm infants [18, 19]. Consequently, analysis of dynamics and complexity of body temperature in preterm infants might reveal objective, clinically relevant information on presence and development of autonomic dysregulation and potential associations with patient characteristics and typical comorbidities of preterm birth.

We aimed to quantify dynamics and complexity of body temperature in very preterm (<32 weeks GA) and/or very low birth weight (<1500 g BW) infants nursed in incubators using

descriptive statistics and time-series analyses. We hypothesized that dynamics and complexity of body temperature quantified as mean (T_{mean}), coefficient of variation (T_{cv}), scaling exponent alpha derived from DFA (T_{alpha}), and sample entropy (T_{SampEn}) of body temperature measured over the first five days of life are associated with demographic characteristics of the infants (GA, body size and body proportions at birth, sex), presence and severity of typical comorbidities of preterm birth, and behavioural state and extent of handling during the measurement.

Methods

Design

We conducted a prospective, observational single center study in the neonatal intensive care unit at the University of Basel Children's Hospital, Switzerland. The study was approved by the local ethics committee and written informed consent was obtained from all parents within the first 24 h after birth and prior to the start of the measurements.

Patients

We included very preterm (<32+0 weeks GA) and/or very low birth weight (<1500 g BW) infants. Exclusion criteria were major congenital malformations, asphyxia, or infants in whom treatment was directed towards palliative care.

Measurements

We recorded time series of body temperature measured over a period of 3 consecutive hours at a sampling frequency of 0.1 Hz in incubator-nursed preterm infants. Measurements were started each morning at 8.30 am on each of the infant's first five days of life. Thus, measurements allowed for collection of >1000 data points and effects of circadian rhythm on outcomes were minimized. Body temperature was measured with a skin electrode (Skin Temperature Probe, Weyer Medical Products, Kuerten Germany; Range: 0–50˚C; max. measurement error +/- 0.1˚C) that was attached to the trunk with an adhesive and positioned between infant and mattress. Depending on the infant's position, the sensor was placed between abdomen and mattress (prone position) or between back and mattress (supine position). This non-invasive setup, i.e., 'the zero heat flux method', has been shown to result in a consistent and reliable approximation of body core temperature [20, 21]. The incubator (Thermocare Vita Weyer GmbH, Kuerten, Germany) was set to servo-control mode using a target body temperature between 36.8 and 37.0˚C. No manual adjustment of air temperature or target body temperature was performed during the 3-h measurements.

Data acquisition

Time series of temperature data were extracted from the RS232 port of the incubator's temperature control unit at a sampling frequency of 0.1 Hz and stored on a mobile computer using HyperTerminal software (Hilgraeve, Inc., Monroe, MI, USA).

Synchronized video recordings (Microsoft 1080 HD Sensor (Life Webcam)) were obtained and manually scored by trained researchers at an identical frequency of 0.1 Hz to determine the infant's behavioural state (awake; quiet sleep; active sleep; assessment based on facial expression, breathing pattern and activity level). This type of analysis was validated in previous studies and allows for assessing sleep without EEG recording in preterm babies [22]. Additionally, positioning of the infant and the extent of handling (open incubator doors in % of recording time and doors-perturbance score, calculated as the cumulative product of time and

number of open incubator doors) were documented. On average, video scoring of a 3-h measurement was completed within 1 h.

Data processing, data analysis and quality control

Temperature data were graphically plotted and visually checked using custom-written scripts developed in Matlab (The MathWorks, Inc., Natick, MA, USA). Measurement time intervals during which the temperature sensor was misplaced or other technical artifacts occured were excluded from the analysis. The amount of excluded data within each measurement was included in the sensitivity analysis of outcomes (for details, see below). We calculated outcome parameters using the statistical software R [23] and the R-package zoo [24]. The time series analysis parameters were calculated using our own implementations in R (occasionally accessing C libraries to reduce run time) of well-known algorithms [25–27]. Many of the implementations are based on the TISEAN package [26].

Outcome parameters

Predefined outcome parameters included descriptive statistics (mean (T_{mean}) and coefficient of variation (T_{cv}) of body temperature) over the measurement period, the scaling exponent alpha (T_{alpha}) as determined by detrended fluctuation analysis (DFA), and measures of sample entropy (T_{SampEn}). T_{alpha} values represent type and degree of correlation of temperature values in the given time series, with higher T_{alpha} values indicating stronger long-range correlations [28], i.e., stronger temperature control. T_{SampEn} reflects complexity, i.e., 'non-regularity' or 'non-predictability' of the temperature time series data. Typically, SampEn values decrease from baseline in association with disease [17].

Detrended fluctuation analysis (DFA)

To estimate the long-range correlation properties in body temperature, we analysed 0.1 Hz-time series of body temperature using DFA. The scaling exponent alpha obtained via DFA was calculated using a geometric window increase with exponent equal to 1.5 and no overlap of windows. The minimal time window size was set to 100 seconds to exclude parts dominated by discretization errors, as the temperature measurements were obtained with an accuracy of only two places after the decimal point. For any given window size, a minimum of 15 windows was required for the calculations. This requirement determined the size of the maximal window size considered, which therefore varied from time series to time series. The data were linearly detrended. The algorithm of DFA used is described in [29] and [26]. DFA plots were visually inspected. Such plots display the logarithm of the root-mean-square errors with respect to the local trend within a given time window versus the logarithm of the corresponding window size. Those plots in which, by visual inspection, a non-linear relationship between the logarithm of the root-mean-square error and the logarithm of the window sizes was suspected were excluded from further analysis. In other words, the scaling exponents derived from such measurements were not included in the regression analysis. In total, 6/285 (2%) of measurements were discarded based on visual inspection of DFA plots.

Sample entropy (T_{SampEn})

The sample entropy was calculated on coarse-grained time series constructed on the basis of collapsing the original values within a window of the size of the scale of interest to one value, namely the average of the measurements over the length of the window. The scale considered (size of window) was 6, which, at a sampling frequency of 0.1 Hz, corresponds to a time

window of 1 minute. The sample entropy was calculated using a comparison length of m = 2 points, and a tolerance of $r = 0.2 * sd$, where sd stands for the standard deviation of all temperature measurements, according to the algorithm described by Richman and Moorman [25] and in the citations therein.

Sensitivity analysis

We performed sensitivity analyses if parts of a measurement had to be excluded due to technical problems (e.g., detached temperature sensor). Therefore, outcomes calculated from all technically acceptable parts of the measurement were compared to those calculated from the single longest, technically acceptable part of the measurement in order to evaluate whether the amount of missing values introduced in the data cleaning process influences results.

Statistical analysis

Aiming at 80% statistical power on the 5% significance level, we aimed to recruit a total of n = 90 infants in order to analyse a minimum of n = 76 preterm infants (expected loss to follow-up 15% due to technical reasons and potential withdrawal of parental consent) allowing for linear regression analysis of at least three continuous independent predictor variables of medium effect size ($f^2 = 0.15$) [30, 31].

Baseline demographics of study participants were extracted from medical records. We performed linear regression analyses to assess associations between outcomes (T_{mean}, T_{cv}, T_{alpha}, T_{SampEn}) and potential predictors. Considered predictors included demographics (GA, BW, BW z-score, relative weight loss over the first five days of life (%), sex); relevant comorbidities associated with preterm birth (early onset sepsis (EOS) (no; suspected (defined as histologically approved chorioamnionitis or CRP>20 mg/mL and antibiotic treatment >5 days), proven (criteria for suspected early onset sepsis plus positive blood cultures)), germinal matrix-intraventricular haemorrhage (maximum grade 1 to 4) documented according to Papile [32]), positioning (prone, supine) during the measurement, phototherapy due to hyperbilirubinaemia, level of respiratory support at study (none, nasal positive pressure support with and without intermittent increase in nasal flow, mechanical ventilation), time to last caffeine dose (hours) at start of the measurement (all infants received caffeine)), and the infant's behavioural state and handling characteristics (see above).

We first used univariable, multilevel modelling to allow for clustering on the individual level given that repeated measurements over the first five days of life were analysed (one 3-h measurement per day in each patient). This step included exploring associations of all considered predictors with outcomes and a p-value < 0.1 was considered to indicate potential relevance of a predictor. We then built multivariable, multilevel linear regression models for each outcome and did stepwise backward elimination of predictors that were not significantly associated with the outcome ($p<0.05$ considered statistically significant). We defined a best model depending on the coefficient of determination of the model (R^2) and compared models using the likelihood ratio test. Models were explored for interaction of predictors and model diagnostics included plotting of residuals against fitted values. We log-transformed outcomes that were not normally distributed (T_{cv}, T_{SampEn}). Statistical analysis was performed using Stata software (StataCorp. 2009. Stata Statistical Software: Release 11. College Station, TX: StataCorp LP).

Results

Between July 2012 and September 2015, a total of 357 temperature measurements, each recorded over a period of 3 consecutive hours at a sampling frequency of 0.1 Hz were conducted in 90 infants. Data of satisfactory technical quality (see methods) were obtained from

285 measurements (79.8%) in 73 infants (81.1%). Fig 1 shows the flow of patients through the phases of the study. Study participants had a mean (range) GA of 30.1 (24.0–34.0) weeks and a mean (range) birth weight (BW) of 1244 (490–1900) g. Baseline characteristics of participants are summarized in Table 1.

Association of outcomes with considered predictors

Results of univariable, multilevel linear regression analyses of all outcomes are summarized in Tables 2 and 3.

Mean temperature (T_{mean}) and coefficient of variation (T_{cv})

There was no association of T_{mean} and T_{cv} with demographic characteristics of study participants. In univariable analysis, T_{mean} was negatively associated with early onset sepsis (EOS) and germinal matrix/intraventricular haemorrhage (IVH), and positively associated with phototherapy and duration of the measurement (Table 2). The best multivariable model included EOS, phototherapy, and duration of the measurement and explained 27% of the variation in T_{mean} between infants ($p<0.001$, $R^2 = 0.27$) (Table 4). T_{cv} was negatively associated with EOS, time awake and time active, and positively associated with respiratory support, phototherapy and duration of sleep cycles (Table 2). The best multivariable regression model included only phototherapy and duration of sleep cycles (Table 4). This model explained 19% of the variability in T_{cv} between infants ($p<0.001$, $R^2 = 0.19$).

Scaling exponent alpha derived from DFA (T_{alpha})

Univariable linear regression analysis suggested a negative association of T_{alpha} with GA and BW, a positive association of T_{alpha} with several comorbidities (EOS, level of respiratory

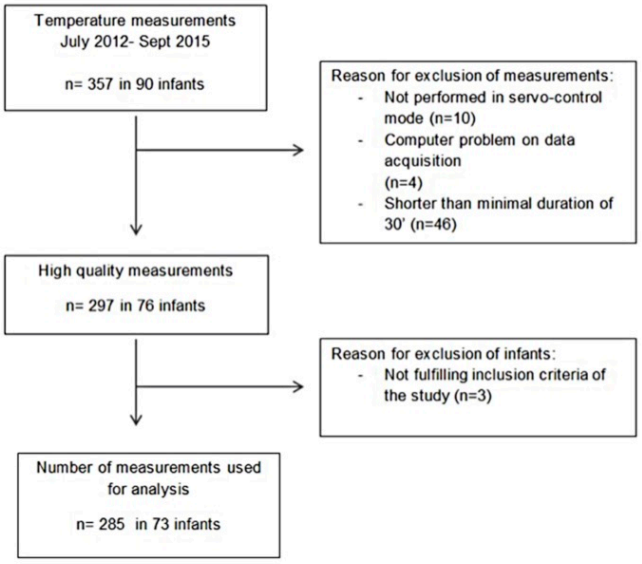

Fig 1. Flow of patients and measurements through the phases of the study.

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support, phototherapy) and a negative association of T_{alpha} with prone position, time to last caffeine dose and time awake (Table 3). The best multivariable, multilevel model confirmed significant negative associations of T_{alpha} with GA and BW z-score and the positive association of T_{alpha} with the level of respiratory support ($p<0.001$, $R^2 = 0.38$) (Table 4). On average, T_{alpha} decreased by 0.03 (95% CI: -0.04 to -0.02; $p<0.001$) per week of GA at birth (Fig 2). A change in the level of respiratory support corresponded to a stepwise increase in T_{alpha} (from no support to continuous positive airway pressure: T_{alpha} +0.13 (95%CI: +0.08 to +0.18; $p<0.001$); from continuous positive airway pressure to endotracheal ventilation: T_{alpha} +0.27 (95% CI: +0.16 to +0.39; $p<0.001$)) (Fig 3). In terms of longitudinal changes of T_{alpha} over the first five days of life, there was a negative association of T_{alpha} with postnatal age of study participants (Fig 4). The mean (SD) difference of T_{alpha} between first and fifth day of life was 0.14 (0.30) ($p<0.001$).

Sample entropy (T_{SampEn})

In univariable regression analysis, T_{SampEn} was positively associated with demographic characteristics (GA and BW) and negatively associated with several comorbidities (EOS, level of respiratory support, phototherapy) and measurement conditions (perturbance score, duration of sleep cycles) (Table 3). Multivariable, multilevel regression established a model including significant predictors GA, BW z-score and phototherapy ($p<0.001$, $R^2 = 0.15\%$) (Table 4): There was a mean increase of T_{SampEn} of 0.07% (95% CI: 0.02 to 0.11%; $p = 0.002$) per week of GA and of 0.1% (95% CI: 0.01 to 0.2%; $p = 0.039$) per unit of BW z-score. Use of phototherapy resulted in a mean decrease of T_{SampEn} of -0.2% (95% CI: -0.4 to -0.02%).

Sensitivity analysis

In 70/285 (25%) of measurements, a reason for partial exclusion of temperature data occurred (e.g., detachment of temperature sensor as verified by synchronized video analysis). The mean

Table 1. Baseline characteristics of study participants.

Variable	
Participants, n (% male)	73 (64)
Gestational age at birth (weeks)	30.05 (2.46)
Birth weight (g)	1244 (337)
Birth weight z-score	-0.94 (1.07)
Weight loss in first week of life (%)	2.35 (4.10)
Maximum level of respiratory support, n measurements (%)	
• None	• 108 (38)
• Continuous positive airway pressure	• 161 (56)
• Endotracheal ventilation	• 16 (6)
*Early onset sepsis, n infants (%)	
• None	• 43 (59)
• Suspected	• 19 (26)
• Proven	• 11 (15)
**Germinal matrix/intraventricular haemorrhage, n infants (%)	
• None	• 63 (86)
• Grade 1	• 5 (7)
• Grade 2	• 0 (0)
• Grade 3	• 4 (6)
• Grade 4	• 1 (1)

Data are presented as mean (standard deviation) unless stated otherwise.

*Defined as no; suspected (CRP>20mg/ml, antibiotic treatment >5 days, histologically proven chorioamnionitis); proven (suspected plus positive blood cultures).

**Maximum grade (1 to 4) documented according to Papile classification [32].

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Table 2. Univariable, multilevel linear regression analyses of mean and coefficient of variation of body temperature.

	T_{mean}				T_{cv} [a]			
Variable	**Coeff beta**	**p-value**	**95% CI**	**R^2 between; overall**	**Coeff beta**	**p-value**	**95% CI**	**R^2 between; overall**
GA (weeks)	0.012	0.183	-0.006 to 0.031	NA	-0.016	0.249	-0.044 to 0.011	NA
Weight (g)	<0.001	0.975	<-0.001 to <0.001	NA	<0.001	0.146	<0.001 to <0.001	NA
BW z-score	-0.034	0.117	-0.076 to 0.008	NA	-0.018	0.587	-0.084 to 0.048	NA
Weight loss (%)	<0.001	0.876	-0.005 to 0.006	NA	0.002	0.796	-0.011 to 0.014	NA
Male gender	-0.012	0.805	-0.219 to 0.032	NA	0.029	0.704	-0.119 to 0.177	NA
EOS	-0.115	0.081	-0.244 to 0.014	7; 1	0.172	0.096	-0.030 to 0.374	6; 2
IVH	-0.123	0.186	-0.306 to <-0.059	NA	0.063	0.673	-0.358 to 0.231	NA
Respiratory support	0.070	0.380	-0.087 to 0.227	NA	0.407	0.011	0.095 to 0.719	11%; 2%
Prone position	0.007	0.860	-0.072 to 0.086	NA	-0.114	0.199	-0.364 to 0.076	8%; 1%
Phototherapy	0.132	<0.001	0.084 to 0.179	8%; 10%	0.250	0.001	0.102 to 0.398	8%; 4%
Time to last caffeine (h)	<-0.001	0.749	-0.005 to 0.004	NA	<-0.001	0.855	-0.011 to 0.009	NA
Duration (min)	-0.001	0.024	-0.002 to <-0.001	7%; 3%	<-0.001	0.824	-0.003 to 0.002	NA
Perturbance	0.082	0.342	-0.087 to 0.250	NA	0.359	0.207	-0.198 to 0.916	NA
Time awake (%)	<-0.001	0.401	-0.002 to 0.001	NA	-0.005	0.017	-0.009 to <-0,001	2%; 3%
Time active (%)	<-0.001	0.521	-0.002 to 0.001	NA	-0.005	0.031	-0.010 to -0.001	6%; 3%
Duration SC (min)	0.001	0.122	<-0.001 to 0.003	NA	0.009	0.001	0.004 to 0.014	16%; 6%

[a]Log-transformed to achieve normal distribution; GA: Gestational age; Weight: Weight at study; BW z-score: Birth weight z-score; Weight loss: % difference of birth weight and weight at study; EOS: Early onset sepsis (none; suspected; proven); IVH: Germinal matrix-intraventricular haemorrhage (none, grade 1–2 vs. grade 3–4); Respiratory support at study (none; continuous positive airway pressure; endotracheal ventilation); Phototherapy: Phototherapy during measurement due to hyperbilirubinaemia; Time to last caffeine: Time from last caffeine dose measured at start of observation (h); Duration: Duration of measurement (min); Perturbance: Perturbance score, i.e., number of open incubator doors x time of open incubator doors during measurement; Time awake: % of measurement spent awake; Time active: % of measurement spent awake or in active sleep; Duration SC: Average duration (min) of sleep cycles (reappearance of active sleep) within one measurement.

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(SD) duration of excluded parts from a 3-h measurement period was 37 (26) min. Table 5 shows a comparison of outcome parameters using all technically acceptable parts vs. only using the single longest continuous, technically acceptable part of the measurements; T_{cv} and T_{SampEn} but not T_{mean} were significantly different (paired t-test). However, results of regression analyses were essentially identical in terms of associations and effect sizes of predictors. Thus, results reported in this manuscript refer to analyses done using all technically acceptable parts of a measurement.

Discussion

We found that dynamics and complexity of body temperature are quantifiable in incubator-nursed preterm infants with a success rate of about 80%. Daily measurements during the first five days of life under servo-controlled conditions indicated that T_{mean} and T_{cv} are independent of GA, BW, and sex and are only modestly associated with typical comorbidities of preterm birth and behavioural state of the infants. Fractal long-range correlations of body temperature characterized by T_{alpha} were significantly associated with demographics (GA, BW z-score) and severity of respiratory disease as measured by the level of respiratory support. The effect size of those associations was considerable ($R^2 = 0.38$) and T_{alpha} decreased over the first few days of life. Complexity of body temperature in terms of T_{SampEn} was only modestly associated with demographics (GA, BW z-score) and presence of phototherapy ($R^2 = 0.15$). The

Table 3. Univariable, multilevel linear regression analyses of scaling exponent alpha and sample entropy of body temperature.

	T_{alpha}				T_{SampEn}[a]			
	Coeff beta	p-value	95% CI	R^2 between; overall	Coeff beta	p-value	95% CI	R^2 between; overall
GA (weeks)	-0.026	<0.001	-0.036 to -0.015	23%; 9%	0.054	0.007	0.015 to 0.094	9%; 3%
Weight (g)	<-0.001	<0.001	<-0.001 to <-0.001	30%; 14%	<0.001	<0.001	<0.001 to <0.001	16%; 5%
BW z-score	-0.017	0.254	-0.046 to 0.012	NA	0.058	0.250	-0.041 to 0.157	NA
Weight loss (%)	-0.002	0.506	-0.006 to 0.003	NA	0.004	0.621	-0.013 to 0.022	NA
Male gender	<-0.001	0.982	-0.067 to 0.065	NA	-0.102	0.349	-0.319 to 0.113	NA
EOS	0.189	<0.001	0.114 to 0.264	29%; 11%	-0.487	0.001	-0.769 to -0.204	15%; 4%
IVH	-0.044	0.500	-0.170 to 0.083	NA	0.399	0.065	-0.025 to 0.822	NA
Respiratory support	0.274	<0.001	0.161 to 0.387	29%; 14%	-0.759	0.001	-1.192 to -0.327	15%; 5%
Prone position	-0.072	0.072	-0.151 to 0.006	9%; 2%	0.211	0.167	-0.089 to 0.510	NA
Phototherapy	0.066	0.011	0.015 to 0.117	12%; 3%	-0.265	0.009	-0.463 to -0.067	2%; 3%
Time to last caffeine (h)	-0.003	0.074	-0.007 to <0.001	6%; 2%	0.009	0.227	-0.005 to 0.023	NA
Duration (min)	<0.001	0.320	<-0.001 to 0.001	NA	<0.001	0.949	-0.003 to 0.004	NA
Perturbance	0.107	0.276	-0.085 to 0.299	NA	-1.035	0.005	-1.765 to -0.307	4%; 4%
Time awake (%)	-0.001	0.074	-0.003 to <0.001	7%; 3%	0.004	0.180	-0.002 to 0.010	NA
Time active (%)	<0.001	0.989	-0.002 to 0.002	NA	0.005	0.210	-0.003 to 0.012	NA
Duration SC (min)	0.003	0.001	0.001 to 0.005	3%; 7%	-0.012	<0.001	-0.018 to -0.005	11%; 8%

Please refer to legend of Table 2.

https://doi.org/10.1371/journal.pone.0176670.t003

main results of this study were not affected by behavioural state and extent of handling of infants and were robust to sensitivity analyses considering amount and fragmentation of temperature data within a 3 h-measurement.

Table 4. Multivariable, multilevel linear regression analyses of all outcomes.

	Coeff beta	p-value	95% CI	R^2 between; overall
T_{mean}				27%; 16%
EOS (proven)	-0.139	0.019	-0.256 to -0.023	
Phototherapy	0.141	<0.001	0.092 to 0.191	
Duration (min)	-0.002	0.001	-0.002 to -0.001	
T_{cv}[a]				19%; 9%
Phototherapy	0.359	0.020	0.056 to 0.661	
Duration SC	0.007	0.013	0.001 to 0.012	
T_{alpha}				38%; 19%
GA	-0.016	0.021	-0.029 to -0.002	
BW z-score	-0.044	<0.001	-0.069 to -0.020	
Respiratory support	0.205	0.003	0.071 to 0.339	
T_{SampEn}[a]				15%; 7%
GA	0.065	0.002	0.023 to 0.106	
BW z-score	0.104	0.039	0.005 to 0.202	
Phototherapy	-0.218	0.031	-0.416 to -0.019	

[a]Log-transformed to achieve normal distribution; GA: Gestational age; BW z-score: Birth weight z-score; EOS: early onset sepsis (none; suspected; proven); respiratory support at study (none; continuous positive airway pressure; endotracheal ventilation); Phototherapy: Phototherapy during measurement due to hyperbilirubinaemia; Duration: Duration of measurement (min); Duration SC: Average duration (min) of sleep cycles (reappearance of active sleep) within one measurement.

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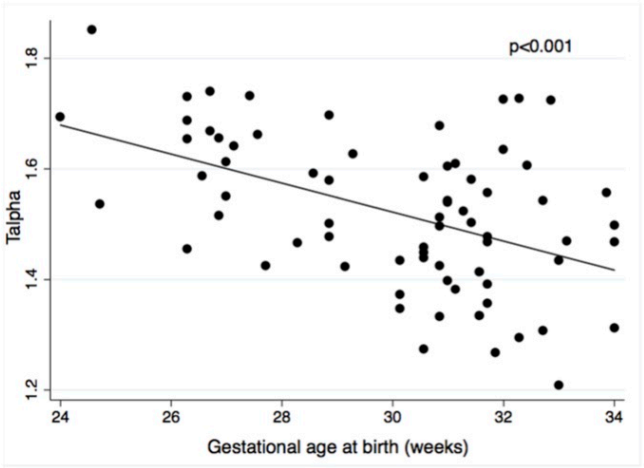

Fig 2. Scaling exponent alpha (T_{alpha}) over gestational age (GA). Average T_{alpha} over the first five days of life derived from detrended fluctuation analysis of temperature time series plotted against GA at birth. Multilevel linear regression analysis demonstrated a significant negative association between T_{alpha} and GA at birth ($p<0.001$, $R^2 = 0.29$).

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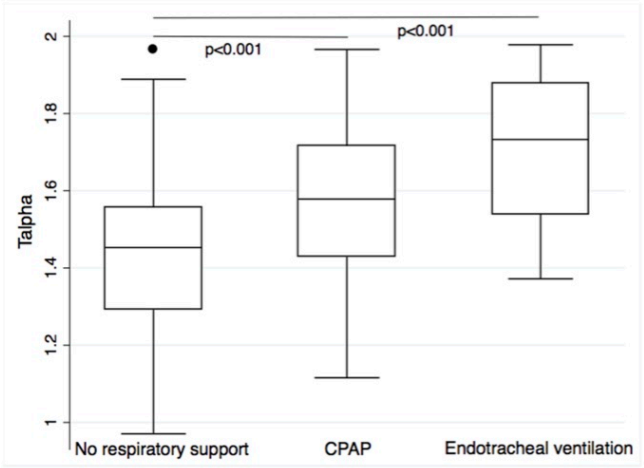

Fig 3. Scaling exponent alpha (T_{alpha}) over respiratory support. T_{alpha} grouped by the level of respiratory support present during a temperature measurement. Multilevel linear regression analysis demonstrated a significant positive association between T_{alpha} and stepwise increase of respiratory support from none to continuous positive airway pressure (CPAP), and from CPAP to endotracheal ventilation ($p < 0.001$, $R^2 = 0.29$).

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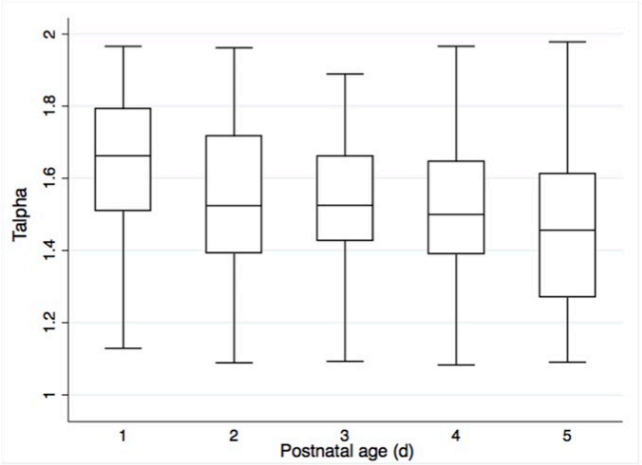

Fig 4. Scaling exponent alpha (T_{alpha}) over postnatal age. T_{alpha} decreased from a mean (SD) of 1.67 (0.18) on day one of life to 1.46 (0.22) on day five of life (p<0.001).

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Comparison of findings with previous literature

Maintaining body temperature within a narrow target range is challenging for preterm infants within the first week of life given their relatively high body surface to weight ratio, large metabolic cost of maintaining temperature balance, and immature temperature control systems [33–35]. Reassuringly, we found that T_{mean} of infants in our study was within the target range of 36.8 to 37.0˚C independent of the degree of prematurity and despite a small but significant effect of comorbidities such as EOS, IVH and use of phototherapy. These results confirm findings from previous studies indicating that servo-controlled temperature care based on skin temperature of preterm infants is safe in the clinical routine setting of a neonatal intensive care unit [21, 33–40].

To our best knowledge, this is the first study to assess fractal long-range correlations of body temperature in preterm infants. Stern et al. previously showed that mean (SD) T_{alpha} obtained from monthly temperature measurements in healthy term infants increased from 1.42 (0.07) at 4 weeks to 1.58 (0.04) at 20 weeks of age [16]. They interpreted this to be due to a maturational effect towards a more deterministic system with increasing age post term. In

Table 5. Sensitivity analysis related to amount and fragmentation of data.

Variable	All acceptable parts	Single longest acceptable part	Mean diff (95%CI); p-value[b]
T_{mean}	37.0 (0.3)	37.0 (0.3)	0.0 (-0.02 to 0.02); p = 0.863
T_{cv}[a]	-66 (57)	-102 (61)	36 (-116 to -81); p<0.001
T_{SampEn}[a]	-1.58 (0.70)	-1.38 (0.76)	-0.20 (-0.35 to -0.06); p = 0.007

Data are presented as mean (standard deviation)

[a]Log-transformed to achieve normal distribution

[b]paired *t*-test; CV: coefficient of variation; T_{SampEn}: Sample entropy; Mean Diff: Difference of outcome comparing all technically acceptable parts vs. only the single longest technically acceptable part.

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contrast, our data showed a decrease in mean (SD) T_{alpha} from 1.67 (0.18) on day one of life to 1.46 (0.22) on day five of life. However, there are important methodological differences between our study and that of Stern et al. including population characteristics and study setting (preterm infants nursed in incubators shortly after birth vs. healthy term infants assessed at home several weeks to months after birth) and data acquisition (different timing, frequency, duration, and sampling rate of temperature measurements) These differences in methodology might explain the discrepancy in the direction of the association between T_{alpha} and postnatal age.

We found lower complexity, i.e., higher T_{alpha} and lower T_{SampEn}, to be associated with the degree of prematurity and growth restriction at birth, and with several comorbidities of preterm infants during their first days of life. These are novel observations suggesting that lower complexity of temperature time series in preterm infants can be interpreted as less deterministic temperature control associated with prematurity, growth restriction, and severity of respiratory disease. In fact, an increase in T_{alpha} was associated with a stepwise increase in the level of respiratory support required during the first few days of life after adjusting for prematurity and intrauterine growth restriction, i.e., increasing loss of complexity reflected the degree of disease in a 'dose-response' relationship. Varela et al. showed that a decrease in temperature curve complexity in 50 critically ill adults with multi organ failure was associated with a higher mortality rate [17]. They found significantly higher T_{alpha} and lower T_{SampEn} in hourly temperature data, summarized as lower complexity, in patients who did not survive their intensive care unit stay after adjusting for age. Hence, and similar to our findings, these authors interpreted the loss of complexity in temperature fluctuations as a marker of severity of disease and, additionally, as an indicator of poor prognosis.

Strengths and limitations of the study

Strengths of this study include assessing a population at high risk of autonomic dysregulation of body temperature in whom thermal balance is of critical importance; using a safe, non-invasive, and reliable method of acquiring temperature time series data including synchronized video recordings to adjust for behavioural state, sleep and handling of the infant; a sample size very close to that projected in pre-emptive power calculations; and sensitivity analyses to test the robustness of results in relation to potential issues due to missing values in time series data. Limitations of our study include the inability to provide exact timing of comorbidities such as IVH, the lack of evaluating the complete temperature feedback loop including air temperature and heat output of the incubator and the fact that we did not collect longitudinal data allowing for assessment of substantial maturational changes in temperature control over weeks to months. Such data would allow for a more complete understanding of the physiological mechanisms underlying temperature fluctuations and provide a setup to study the predictive value of long-range correlations of body temperature in incubator-nursed preterm infants for medium-term outcomes such as growth or resolution of autonomic dysregulation. However, this study provides novel methods and biologically important information on quantitative characterization of temperature regulation in preterm infants in a temperature-wise very controlled environment.

Clinical relevance and future applications

Quantifying the dynamics and complexity of body temperature from time series is a first step towards better understanding of the immature autonomic thermal control system and its interaction with the degree of prematurity and typical morbidities of preterm infants. A key criterion for discharge of preterm infants from the nursery is their ability to maintain body temperature upon transfer from an incubator to an open cot. Arguably, future real-time analysis of temperature time series in a longitudinal fashion might allow for quantitative assessment

of maturation of the temperature control system and provide a rational basis for transfer of infants from an incubator to an open cot. Currently, this decision is primarily based on body weight of infants [41, 42], however, there is little evidence to suggest a specific body weight indicating readiness for transfer, i.e., such transfers are typically a matter of trial and error despite the fact that thermo-neutrality is important for adequate weight gain and has beneficial effects on other autonomic control systems such as breathing and heart rate [9, 43, 44].

Conclusion

Dynamics and complexity of body temperature in preterm infants nursed in incubators can be quantified and show associations of considerable effect size with the degree of maturity at birth, intrauterine growth, and respiratory morbidity. Behavioural state, handling of infants, and missing data have limited impact on fractal temperature dynamics. T_{alpha} is a promising, observer-independent, and robust tool for assessing autonomic maturation and severity of disease in preterm infants. Longitudinal studies are required to evaluate whether T_{alpha} is useful for predicting clinically relevant outcomes and resolution of autonomic dysregulation.

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Author Contributions

Conceptualization: SS UF IP EDE AD KJ.

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Formal analysis: NB EDE SS KJ UF IP.

Funding acquisition: SS UF IP.

Investigation: KJ IP.

Methodology: SS UF IP EDE AD KJ.

Project administration: SS KJ UF.

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Software: NK EDE KJ IP UF SS.

Supervision: SS UF.

Validation: SS UF AD.

Visualization: KJ IP SS AD.

Writing – original draft: KJ.

Writing – review & editing: SS UF EDE NK IP AD.

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5.2. Sleep and phototherapy in premature neonates

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"A well-spent day brings happy sleep."

- Leonardo da Vinci -

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State of the paper	Published 2016: *Sleep Research*. doi: 10.1111/jsr.12408.
Contribution of KJ	KJ performed the measurements and assisted in teaching of sleep stage analysis. The analysis and statistical interpretation of the resulting data was performed by KJ and co-workers. The final manuscript was revised by KJ.
Synopsis	Sleep in infants is important for neurological development, thus, interference with sleep-state regulation might influence this maturation process. It is well known in adults, that light is an important factor in sleep-regulation. Since there is very little data concerning the effect of light-deprivation on sleep in very preterm infants this study was performed. Video recordings of very preterm and/ or very low birth weight infants were performed during their first days of life. Based on breathing patterns, eye- and body movements, behavioral states were defined as being awake, in active sleep or in quiet sleep. Measurements performed during phototherapy, due to neonatal jaundice, where the infants had to wear blindfolds and thus were exposed to light-deprivation, were compared to measurements without phototherapy within the same infants. Our data suggest that the ultradian rhythm of preterm infants seems to be independent of phototherapy (or light-deprivation), supporting the notion that sleep rhythm in this population is mainly driven by their internal clock.

J Sleep Res. (2016) 25, 517–523 Phototherapy in preterm neonates

Immediate effects of phototherapy on sleep in very preterm neonates: an observational study

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Keywords
Light of phototherapy, sleep cycles, very premature newborns

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SUMMARY

Process C (internal clock) and Process S (sleep–wake homeostasis) are the basis of sleep–wake regulation. In the last trimester of pregnancy, foetal heart rate is synchronized with the maternal circadian rhythm. At birth, this interaction fails and an ultradian rhythm appears. Light exposure is a strong factor influencing the synchronization of sleep–wake processes. However, little is known about the effects of phototherapy on the sleep rhythm of premature babies. It was hypothesized that sleep in preterm infants would not differ during phototherapy, but that a maturation effect would be seen. Sleep states were studied in 38 infants born < 32 weeks gestational age and/or < 1 500 g birth weight. Videos of 3 h were taken over the first 5 days of life. Based on breathing and movement patterns, behavioural states were defined as: awake; active sleep; or quiet sleep. Videos with and without phototherapy were compared for amounts of quiet sleep and active states (awake + active sleep). No significant association between phototherapy and amount of quiet sleep was found ($P = 0.083$). Analysis of videos in infants not under phototherapy revealed an increase in time spent awake with increasing gestational age. The current data suggest that the ultradian rhythm of preterm infants seems to be independent of phototherapy, supporting the notion that sleep rhythm in this population is mainly driven by their internal clock.

INTRODUCTION

Sleep, and especially the cycling of sleep states, is vital for neurosensory motor development, as studies in different animal models have shown (Mirmiran *et al.*, 1983; Mirmiran, 1986, 1995; Curzi-Dascalova, 1992; Peirano and Algarin, 2007; Graven and Browne, 2008). The importance of sleep is reflected in the fact that term-born neonates spend about 70% of their time sleeping (Mirmiran *et al.*, 2003).

It is understood that sleep in adults involves two basic processes that must be synchronized with one another. The first is known as Process S, a homeostatic process depending on the rise of sleep propensity during waking and its dissipation during sleep. In proportion to time spent awake (W), slow-wave activity in electroencephalogram (EEG) increases during non-rapid eye movement (NREM) sleep, reflecting neuronal synchronization on the basis of synaptic efficacy (Vyazovskiy *et al.*, 2009), and decreases during the course of the night. The second process is known as Process C, the 'clock' process. This is an internal clock-like mechanism located in the suprachiasmatic nucleus of the hypothalamus. It determines the alternation of high and low sleep propensity, independent of prior sleep and waking. During sleep, an ultradian process alternating the two basic sleep states, NREM and REM sleep, determines sleep cycles. Processes C and S regulate the sleep–wake rhythm (Borbely and Achermann, 1999; Berry, 2012a).

The origin of circadian rhythms can be found at foetal age: the biological clock has been shown to be responsive to maternal entraining signals in the last trimester of pregnancy. Foetal heart rate is at that point synchronized with maternal rest-activity, food intake, heart rate, cortisol, melatonin and

body temperature circadian rhythm. Foetal Process C seems to interplay consistently with maternal Process S as well as endogenous factors mentioned above (Mirmiran and Lunshof, 1996).

Nevertheless, at birth this interaction suddenly fails: a transient ultradian rhythm emerges, dependent only on the internal clock of the newborn. Preterm neonates in particular undergo this dramatic change with early loss of maternal foetal interactions (Groome *et al.*, 1997).

In term-born infants, a circadian rhythm normally appears in multiple steps. First, a circadian rhythm of temperature arises soon after term, at about 1 week old. Then, wake circadian rhythm comes second, at day 45; at the same time increased melatonin concentration is present at sunset, although melatonin is also transmitted by breast milk from the mother. Finally, a sleep circadian rhythm appears at day 56 after term (McGraw *et al.*, 1999). The occurrence of a 24-h sleep–wake rhythm was found at a mean age of 44 weeks for preterm and term infants (Shimada *et al.*, 2003).

Different studies have found ultradian sleep cycles in premature infants as young as 25 weeks post-conceptional age (Scher *et al.*, 2005), although clear discrimination of sleep stages is only visible via EEG after 30 weeks of gestation (Mirmiran *et al.*, 2003). In contrast to adults, active sleep (AS) represents the most important part of sleep for infants, particularly in terms of cerebral development.

Different authors have pointed out similarities between foetal and preterm infants' sleep, questioning to what extent the environment impacts the newborn's sleep patterns (de Vries *et al.*, 1987; Mirmiran *et al.*, 2003; Peirano and Algarin, 2007). For example, Mirmiran *et al.* (2003) stated that their findings in terms of the time course of quiet sleep (QS) and wake development in preterm infants were very similar to intrauterine findings of other authors at about 30 weeks gestational age (GA).

In human premature infants, studies of the impact of difference of light exposure (constant dimmed light or cycled light) on sleep structure did not show significant differences (Mirmiran and Ariagno, 2000). Shimada *et al.* (2003) studied 90 term and preterm infants, and also did not observe any changes in the entrainment of circadian rhythmicity due to different lighting conditions produced by phototherapy. Nevertheless, this study cohort did not differentiate between premature and term patterns.

McMillen *et al.* (1991) compared premature infants who spent several weeks in a hospital nursery after birth with term-born infants. Premature infants were not exposed to environmental time cues from the beginning like term-born infants. The circadian day–night rhythm appeared after a similar time of exposure but at an earlier postconceptional age compared with term-born infants. This was interpreted as a possibly stronger effect of external zeitgebers to the emergence of a circadian sleep–wake rhythm than previously expected. This was confirmed by a recent study (Guyer *et al.*, 2015): premature babies were either exposed to cycled light (in group 1) or to dim light (in group 2) conditions during their neonatal hospitalization. Sleeping and activity variables at corrected 5, 11 and 25 weeks post-term showed that the entrainment of the circadian sleep–wake rhythm of preterm infants appeared earlier than in term-born infants (Guyer *et al.*, 2015).

Overall maturation of sleep states initially seems to be a result of brain maturation and a little later also an effect of external factors. Acquired knowledge about sleep promotion could possibly lead to better neonatal care, and may 1 day produce better long-term outcomes in premature infants.

The current hypothesis was that sleep in preterm infants would not differ during phototherapy based on experiences with circadian rhythmicity, but that a maturation effect would be seen in subjects with higher GA.

MATERIALS AND METHODS

Material and subjects

This study is part of an ongoing observational study examining the development of autonomic control in preterm infants (Swiss National Science Foundation No. 141206). Children were recruited between June 2012 and August 2013. The study included neonates with a birth weight < 1 500 g and/or GA < 32 weeks at birth, whose parents had given written informed consent. The ethics committee of Basel (Ethik Kommission beider Basel, EKBB) approved this study, which was conducted according to the principles of the Declaration of Helsinki (EKBB-Nr.: 37/12). Exclusion criteria were defined as follows:

-lack of written informed parental consent;
-perinatal asphyxia, defined as either arterial cord pH < 7.0, or base deficit > 12 mmol/l or serum lactate > 5 mmol/l during the 1st hour of life;
-infant in extremis, independent of cause; and
-major congenital malformations, including neuromuscular disorders, thoracic malformations and major cardiac malformations.

Withdrawal criteria were defined as the development of one of the above exclusion criteria. Demographic data of study participants can be found in Table 1.

Study design

This is an observational study. Subjects neither received nor were withheld any special care. Treatments such as phototherapy or intravenous caffeine were prescribed following hospital guidelines. Phototherapy for treatment of hyperbilirubinemia was prescribed dependent on fixed thresholds based on the infants' GA and postnatal age. Caffeine was administered routinely to all participants every 24 h.

Method

Measurements were standardized to be performed in the morning (start at 08:00 hours), and were repeated every day

Table 1 Demographic data of the study participants [n = 38 (♂ = 20, ♀ = 18)]

Parameter	*Mean*	*Minimum*	*Maximum*	*Standard deviation*
Gestational age (weeks/days)	29/0	24/0	32/6	2/4
Birth weight (g)	1097	420	1650	317.5
z-score birth weight	−0.97	−3.13	0.7	0.90
Length (cm)	36.8	28.0	43.0	4.2
Head circumference (cm)	26.6	20.0	29.7	2.6

Infants with intraventricular haemorrhage n = 6: I°, n = 4; II°, n = 1; III°, n = 1.

from the 1st to the 5th day of life unless emergency interventions prohibited that. Videos of a duration of 3 h [mean of 185 min (SD: 11 min)] were taken through the incubators' roofs and/or doors. Initially, the camera was filming from above for examination of body movements and respiration. After a pilot phase, subjects were filmed with an additional camera focusing on the face of the infant for better examination of the eyes. In addition to standard clinical monitoring of heart rate [electrocardiogram (ECG)] and pulse oximetry, electromyography electrodes were placed on the newborn's lower back in order to register respiration and an additional ECG trace. To protect the skin from irritation, a small amount of sunflower oil was administered before fixation of the electrodes. Two temperature probes – one attached to the trunk on the infant's depending body part for central temperature recording, and one fixed on the infant's foot – were attached as part of the standard monitoring in the neonatal intensive care unit (NICU). For synchronization of recordings, the two low-light cameras [Microsoft 1080 HD Sensor (Life Webcam)] were connected to a laptop and started filming simultaneously using Polybench (Inbiolab, Groningen, the Netherlands). In sunny conditions, a curtain was installed to shield the incubator from direct sunlight, in order to reduce reflections and limit alterations of light conditions. Recording time included minimal routine clinical care (e.g. feeding and nappy changes). Any such interference with sleep was documented. Invasive or longer procedures or interventions were not performed during recording time.

Videos were analysed in 10-s intervals with vlc media player (version 2.0.8, Free Software Foundation, Boston, MA, USA) at two–four times the normal speed. Three medical students in their 9th semester analysed the videos. A paediatric sleep specialist trained and supervised the students using a standardized analysis and assessment protocol. Unclear situations were resolved by discussion with the paediatric sleep specialist.

Video analysis

Behavioural states were divided into W, AS and QS using the following adaptation of the criteria from Anders, Emde and Parmelee (Berry, 2012b). This type of analysis was validated in previous studies and allows for assessing sleep without EEG recording in premature babies (Mirmiran *et al.*, 2003; Sung *et al.*, 2009).

W: eyes open, irregular respiration, crying, calm or high motor activity.AS: eyes closed, rapid eye movements, irregular respiration, frequent and fluid movements, grimacing, sucking.QS: eyes closed, no eye movements, slow, deep, regular respiration, little motor activity except for myoclonic jerks.

From the sleep structure point of view, from premature and term age until the age of 3 months after term, babies fall asleep in AS, the predecessor of REM sleep, then go to QS, the predecessor of NREM sleep. This phase is followed by another rather low-voltage AS or a short wake state, and completes a full ultradian sleep cycle. AS is also characterized by REMs, irregular breathing and higher motor activity, whereas QS has no REMs, a more regular breathing pattern and less body movements. Intermediate sleep (IS) is defined as not fitting into one of the above sleep state definitions (Mirmiran *et al.*, 2003; Berry, 2012b). In premature and term babies up to 3 months old, sleep starts with AS and continues with QS. After 3 months old, sleep onset is in light and deep NREM sleep (former QS) and then changes to REM sleep. This chronological order is maintained until adult age.

At premature age, transitions not completely fulfilling the criteria for AS and QS sleep are called IS (Berry, 2012b). In moments of doubt the student assigned one of the above behavioural states based on the respiration's regularity, thus, IS was not accounted for. Because eyes are not visible during phototherapy, wake states could not be differentiated from AS and were classified as active state, except in the case of obvious crying. A change in behavioural state was documented when it lasted longer than 1 min. Sleep states were documented in 10-s intervals in an excel file, along with the number of open incubator doors, duration of feeding interventions, and a short description of interventions and interactions (e.g. visit of mother). For each measurement, the total time spent in each behavioural state, their respective percentage, as well as the average length of sleep cycles per measurement were calculated. Sleep cycles were defined as the reappearance of active state. Only cycles longer than 5 min were considered, as shorter cycles were interpreted as transitional phases.

Table 2 Details of all measurements, the measurements without and with phototherapy

	All measurements (n = 160)		*Without phototherapy (n = 122)*		*With phototherapy (n = 38)*	
Parameter	*Median (IQR) (min)*	*% of total 3-h sleep time*	*Median (IQR) (min)*	*% of total 3-h sleep time*	*Median (IQR) (min)*	*% of total 3-h sleep time*
Time spent W			18 (3–49)	10 (2–26)		
Time spent in AS			83 (46–110)	46 (26–60)		
Time spent in QS	67 (41–91)	36 (23–48)	68 (41–92)	36 (23–49)	55 (34–76)	34 (22–44)
Duration of sleep cycle	33 (24–44)		31 (22–43)		40 (27–41)	

Sleep cycles are defined as the reappearance of active state or AS greater 5 min.
AS, active sleep; IQR, inter-quartile range; QS, quiet sleep; W, awake.

Statistical analysis

In order to test the current hypothesis, the mean sleep cycle length was compared with the percentage of time spent in QS between measurements with and without phototherapy as primary outcomes. To test a possible maturational effect, mean sleep cycle length and percentages of different sleep states were analysed as secondary outcomes. For this, a subgroup analysis of videos without phototherapy was performed, a necessity to score wake states.

Statistical analysis was performed using JMP® pro (version 11.0 64-bit Copyright© 2013, SAS Institute, Cary, NC, USA) and Stata 13 (StataCorp 2013, Stata Statistical Software: Release 13; Stata Corp LP, College Station, TX, USA). Multilevel, univariable linear regression analysis was performed to investigate the influence of phototherapy on sleep by looking for associations between percentages of QS as well as sleep cycle duration and phototherapy. Multilevel analysis was chosen to account for differences in the number of measurements per subject. GA, birth weight, birth weight *z*-scores and sex were considered as possible predictors. After determination of all significant predictors in univariable regression analysis ($P < 0.01$), a multivariable model containing all these variables was built, followed by stepwise backward elimination to find the final best model for every outcome parameter. The significance level alpha of the final model was set at $P < 0.05$. Besides that, each infant was used as its own control by comparing the results from the first measurement with and the first measurement without phototherapy using paired *t*-test.

For the secondary analysis, the study focused on measurements without phototherapy, including only videos filmed from two angles, allowing to more confidently score wake states. Here, W, AS, QS and sleep cycle length were analysed using the same approach in building a multivariable model as for the primary outcomes.

RESULTS

Demographic data

Thirty-eight neonates who met the inclusion criteria were studied. Overall, 160 of the planned 215 videos were analysed, thereof 38 with and 122 without phototherapy; 55 videos were not included due to corruption during file compression (35), withdrawal of consent (15) and secondary exclusion due to pronounced growth retardation in one mature subject (5).

The current population was heterogeneous in terms of GA, birth weight, respiratory support and co-morbidities. Demographic data of study participants can be found in Table 1.

Out of 38 observed infants, 24 infants had at least one measurement performed during phototherapy and one measurement performed without phototherapy. The GA of study participants did not differ between measurements performed during phototherapy [mean (SD) GA: 28.54 (2.58) weeks] and measurements performed without phototherapy [mean (SD) GA: 29.20 (2.54) weeks].

For details of the measurements without phototherapy and all measurements, see Table 2.

The median number of sleep cycles was 4.0 for all measurements ($n = 160$), and for the measurements without phototherapy ($n = 122$) with an inter-quartile range (IQR) of 3–6. For the measurements without phototherapy, the median number of sleep cycles was 3.8 with an IQR of 3–5.

Phototherapy and sleep

The percentage of QS did not significantly differ between days with and days without phototherapy ($P = 0.083$; Fig. 1). There was no significant influence on the percentage of time spent in QS by the following predictors, when all measurements were considered: GA ($P = 0.111$); birth weight ($P = 0.073$); birth weight *z*-scores ($P = 0.975$); and sex ($P = 0.871$).

Also, no differences in sleep cycle duration during phototherapy were found ($P = 0.405$). Neither GA ($P = 0.856$) nor birth weight ($P = 0.876$), birth weight *z*-score ($P = 0.176$) or sex ($P = 0.444$) had any significant influence on sleep cycle duration.

When looking at possible differences introduced by phototherapy within each infant by paired *t*-test, no differences in the percentage of QS or duration of sleep cycles were found. The mean (SD) difference of QS (%) was 2.6 (25.0)% (95% CI −8.0 to 13.2; $P = 0.614$). The mean (SD) difference of

sleep cycle length was 6.7 (23.0) min (95% CI −3.5 to 16.9; $P = 0.185$). A detailed overview of results from univariable regression analysis is shown in Table 3.

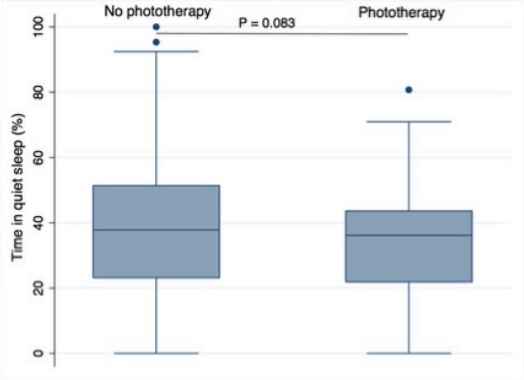

Figure 1. Box-plot comparing the percentage of time spent in QS in measurements with phototherapy (n= 38 measurements performed in 24 infants) and without phototherapy (n= 122 measurements performed in 38 infants)

Table 3 Univariate multilevel linear regression analysis

	Coefficient	P-*value*	R^2 *overall;* R^2 *between (%)*
QS (%)			
GA (weeks)	−1.299	0.111	NA
Birth weight (g)	−0.011	0.073	NA
Birth weight *z*-score	−0.072	0.975	NA
Male gender	−0.684	0.871	NA
Phototherapy	−6.502	0.083	NA
Duration sleep cycle (min)			
GA (weeks)	−1.839	0.856	NA
Birth weight (g)	0.001	0.876	NA
Birth weight *z*-score	3.694	0.176	NA
Male gender	3.827	0.444	NA
Phototherapy	3.553	0.405	NA
W (%)			
GA (weeks)	1.309	0.026*	12; 22
Birth weight (g)	0.004	0.439	NA
Birth weight *z*-score	−2.271	0.158	NA
Male gender	−7.052	0.035*	6; 17
AS (%)			
GA (weeks)	−1.067	0.233	NA
Birth weight (g)	< 0.001	0.978	NA
Birth weight *z*-score	3.604	0.126	NA
Male gender	8.891	0.046*	8; 18

For QS and duration of sleep cycle, all analysed measurements ($n = 160$) in all infants ($n = 38$) are displayed. For W and AS, measurements without phototherapy ($n = 54$) in infants during the first week of life (main study; $n = 19$) are displayed.
AS, active sleep; GA, gestational age at birth in weeks; QS, quiet sleep; W, awake.
*P-value < 0.05; in case of significant association; overall R^2 is displayed.

Secondary results

A subgroup analysis was conducted of 54 videos from 19 infants without phototherapy taken in the post-pilot phase. In this analysis, a significant increase in the relative time spent in W in infants with higher GA was found (Coef. 1.309; 95% CI 0.153–2.465; $P = 0.026$; R^2 between = 22.2%; R^2 overall = 11.7%; Fig. 2). Besides that, significantly lower values were found for time spent in W in male than in female study participants (Coef. −7.052; 95% CI −13.625 to −0.480; $P = 0.035$). The amount of W was not influenced by birth weight ($P = 0.439$) or birth weight *z*-scores ($P = 0.158$). A multivariable model of time spent in W including GA and sex resulted in a R^2 between = 48.5%; R^2 overall = 29.4%.

The duration of sleep cycles was unchanged by GA ($P = 0.273$), birth weight ($P = 0.506$) or sex ($P = 0.873$), but showed a significant increase with higher birth weight *z*-scores (Coef. 10.738; 95% CI 3.586–17.891; $P = 0.003$; R^2 between = 22.9%; R^2 overall = 14.5%). These birth weight *z*-scores had no effect on any of the behavioural states, but showed a strong random correlation with GA ($P < 0.001$; $R^2 = 41.5\%$).

Quiet sleep showed no significant correlation with any of the tested predictors: GA ($P = 0.758$); birth weight ($P = 0.401$); birth weight *z*-scores ($P = 0.692$); and sex ($P = 0.413$).

Active sleep showed a significant but weak positive correlation with male sex (Coef. 8.981; 95% CI 0.180–17.783; $P = 0.046$). GA birth weight and birth weight *z*-scores had no influence on AS.

Inter-rater reliability was assessed using 11 measurements scored by two students independently. It showed a moderate agreement with a Cohen's kappa of 0.56 for sleep state observation and an excellent agreement with a kappa of 0.90 for scoring of nursing measures. Despite the moderate agreement level, 38% of the detected events of disagreement (range 22–59.5%) involved maximum three or less frames in a row, which means only 30 s or less. Therefore, about 38%

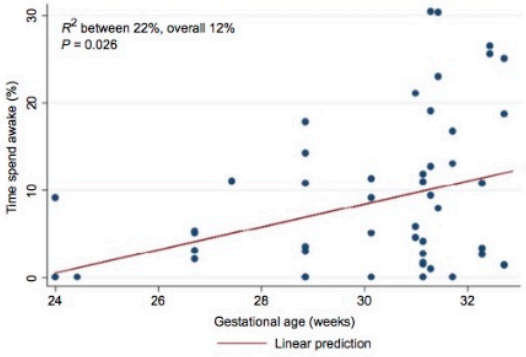

Figure 2. Scatter plot showing the increase of the percentage of time spent awake with increasing gestational age. (post pilot phase, only infants without phototherapy included)

of the detected disagreement did not affect consistently statistical analysis.

DISCUSSION

The results of this study confirm the first hypothesis suggesting that sleep in very premature infants is not altered during phototherapy. Also, sleep cycle duration did not significantly differ with or without phototherapy. The amount of QS and active states, including AS and W, did not change under light influence, or lack of light changes.

This suggests that the ultradian rhythm of premature babies depends exclusively on Process C, the internal clock in the suprachiasmatic nuclei. Neither continuous intensive light exposure (blue-green light) nor a lack of light changes can alter this rhythm. These results are in line with other sleep studies (Mirmiran *et al.*, 2003; Shimada *et al.*, 2003).

Cycled light exposure has been described nevertheless to be associated with a greater rate of weight gain, shorter time to reach full enteral feeds, shorter duration of ventilation and shorter duration of phototherapy (Miller *et al.*, 1995). Other parameters, like length of stay in hospital, weight gain and crying after discharge also seem to benefit from a clear day–night cycle in the NICU according to a recent Cochrane review from Morag and Ohlsson (2013) and Guyer *et al.* (2012). Looking at the current results, these effects in premature infants seem to be rather secondary to a more peaceful atmosphere influencing the handling of the nursing staff, and not due to a light-driven day and night rhythm.

Nevertheless, the entrainment of circadian sleep–wake rhythm was shown to be earlier in former premature infants compared with term-born infants (McMillen *et al.*, 1991; Guyer *et al.*, 2015). Although no difference was found between cycled light or dim light exposure during the neonatal period at the hospital in premature infants, a possible influence of time cues on earlier maturation of the 24-h sleep–wake rhythm has to be supposed (Guyer *et al.*, 2015).

The role of melatonin production during phototherapy has been studied in term and preterm newborns: Jaldo-Alba *et al.* (1993) used phototherapy as a model for light deprivation, as patients need to wear a blindfold for protection of the retina. They found that in term-born infants, melatonin increases significantly during phototherapy, proving that at this age the necessary mechanisms are already working in the first 72 h of life. This was also tested in preterm infants at 33–36 weeks GA. The premature subjects did produce melatonin themselves, but melatonin levels did not increase due to light deprivation during phototherapy (Mantagos *et al.*, 1996).

In line with the current results, the absence of light on the retina seems not to stimulate melatonin production and indirectly not to have any influence on Process C at premature age.

A subgroup analysis focusing on videos with the best visibility of the eyes showed that infants spent significantly more time awake with increasing GA. This effect was collinear with birth weight and respiratory support in univariable analysis, but not in multivariable analysis including GA. Other studies described similar findings (Mirmiran *et al.*, 2003), suggesting that behavioural states are primarily determined by brain maturation. A strong correlation between sleep cycle length and birth weight *z*-scores was found. Also, an even stronger association between birth weight *z*-scores and GA was found. Because per definition of *z*-scores this association is a random effect of the population, the association between sleep cycle duration and birth weight *z*-scores is most probably random. Similarly, the association of AS and sex seems to be best explained as an effect of the heterogeneity within the subgroup whereof two-thirds were male.

A novelty of this study is the extreme prematurity of study participants that, hitherto, has rarely been looked at in the literature concerning sleep states. There are also very few studies that observed sleep states during an otherwise unchanged clinical environment (Mirmiran *et al.*, 2003; Sung *et al.*, 2009).

A strength of this study is the relatively large number of data points resulting from a large amount of video material. A very safe, purely observational approach was used, reflecting clinically relevant conditions in an unaltered NICU setting. However, there are factors limiting the explanatory power of this observational study. Firstly, the current population was diverse in terms of GA, birth weight and co-morbidities. Secondly, there was an inconsistency in video quality due to the subjects' movements or routine nursing measures. As most of the children were on respiratory support, breathing patterns were most likely influenced by this therapy; however, none of the study participants was under any sedative therapy during the measurements. EEG recordings were not possible because they would have been considered too invasive in the most immature infants during their first week of life. However, the importance of EEG information is debatable, as sleep stages are only distinguishable in EEG recordings after 30–32 weeks of GA. Observational sleep stage scoring has been used for several sleep studies in premature and term babies (Berry, 2012b). Curzi-Dascalova *et al.* (1993) found clearly distinguishable sleep states at 27 weeks GA in a group of preterm neonates ranging from 27 to 34 weeks GA. According to the findings of Birnholz (1981), REMs are present *in utero* as early as 24 weeks of GA, and appear more frequently after 30 weeks of GA. So, the idea of differentiating sleep states even at 24 weeks of GA, as the current observations suggest, is conceivable. In general, the first appearance and the developmental time course of sleep states are still a matter of debate (Peirano and Algarin, 2007), and probably subject to a wide range of physiological variability.

As already mentioned, wake states could not be differentiated from AS during phototherapy, resulting in a higher degree of uncertainty in the current results. Furthermore, this study did not account for IS, an obvious phenomena when only scored through video observation.

CONCLUSION

In conclusion, this study suggests that sleep of very premature infants is not altered during phototherapy. This supports the findings of other studies that sleep at a premature age is probably a product of brain maturation rather than external influences. After a circadian rhythm synchronized to the mother's cycles at foetal age, ultradian rhythms of premature infants are transitorily free-run and depend exclusively on Process C, the internal clock, until entrainment to day–night rhythm. This entrainment has been reported to happen a little earlier in very preterm infants compared with term-born infants. Neither phototherapy nor other light seems to change the premature infant's ultradian rhythm.

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AUTHOR CONTRIBUTIONS

MC scored the sleep states and wrote the paper; KJ planned the study, recruited the patients, made the recording installation, performed the statistics and corrected the paper; AG scored the sleep states; IP planned the pilot study and made the recording installation; ED-E helped with statistics; UF supervised the study; SS supervised the recording installations and corrected the paper; AD was mainly responsible for the study, made the study design, supervised the recording installation, defined the methods, taught the students to score, discussed all unclear cases, supervised the statistics, wrote and corrected the manuscript.

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5.3. Data cleaning for study purpose

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"Quality means doing it right when no one is looking."

- Henry Ford -

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State of the paper	Published 2017: *Physiological Measurements* 39 (2018) 015004 (13pp)
Contribution of KJ	KJ performed the measurements, and collaborated in the development of the data cleaning algorithm. The visual control of the cleaning process, as well as the statistical interpretation of the results was performed by KJ. The final manuscript was written by KJ in collaboration with other team members.
Synopsis	Variability in physiological systems, such as heart rate variability (HRV) is getting more attention in clinical studies. Collection and analysis of data to derive HRV is prone to factors that could influence the outcome. We hypothesized that depending on the cleaning, HRV outcomes in preterm infants would differ significantly. We developed a four step cleaning algorithm for systematic pre-processing of electromyography (EMG) measurements, to obtain HRV outcomes. The algorithm included synchronized video recordings for motion detection and assessment of sleep stages, a threshold based cleaning, comparison of different event (QRS complex) detection algorithm, and visual inspection. Finally, we compared the dependency of the outcomes on the performed signal cleaning steps. EMG measurements, performed in very preterm/ very low birth weight infants under clinically relevant conditions, contain noisy parts that have to be identified and excluded before assessment of HRV. Resulting HRV outcomes could else be biased and lead to false assumptions.

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Surface electromyography for analysis of heart rate variability in preterm infants

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Keywords: signal processing, electromyography, preterm infants, heart rate variability

Abstract

Objective: Characterizing heart rate variability (HRV) in neonates has gained increased attention and is helpful in quantifying maturation and risk of sepsis in preterm infants. Raw data used to derive HRV in a clinical setting commonly contain noise from motion artifacts. Thoracic surface electromyography (sEMG) potentially allows for pre-emptive removal of motion artifacts and subsequent detection of interbeat interval (IBI) of heart rate to calculate HRV. We tested the feasibility of sEMG in preterm infants to exclude noisy raw data and to derive IBI for HRV analysis. We hypothesized that a stepwise quality control algorithm can identify motion artifacts which influence IBI values, their distribution in the time domain, and outcomes of nonlinear time series analysis. *Approach*: This is a prospective observational study in preterm infants <6 days of age. We used 100 sEMG measurements from 24 infants to develop a semi-automatic quality control algorithm including synchronized video recording, threshold-based sEMG envelope curve, optimized QRS-complex detection, and final targeted visual inspection of raw data. *Main results*: Analysis of HRV from sEMG data in preterm infants is feasible. A stepwise algorithm to exclude motion artifacts and improve QRS detection significantly influenced data quality (34% of raw data excluded), distribution of IBI values in the time domain, and nonlinear time series analysis. The majority of unsuitable data (94%) were excluded by automated steps of the algorithm. *Significance*: Thoracic sEMG is a promising method to assess motion artifacts and calculate HRV in preterm neonates.

Introduction

Characterization of heart rate variability (HRV) in neonates by time series analysis of data from cardiorespiratory monitoring has gained increased attention over the last several years. Monitoring of HRV is useful to quantify maturational changes in neonates and to identify infants at risk of neonatal sepsis (Griffin *et al* 2007, Moorman *et al* 2011, Fyfe *et al* 2015, Lucchini *et al* 2016). Given the interdependency of the cardiac and respiratory systems, simultaneous monitoring of heart rate and respiratory rate is routinely performed in preterm infants (Di Fiore 2004). Chest impedance measurement is the standard of care for monitoring of heart rate and respiratory rate in the neonatal intensive care unit (NICU) (Di Fiore 2004).

HRV is expressed as the beat-to-beat variation in the interbeat interval (IBI). The distribution, complexity and fractal dynamics of a sequence of IBIs can be characterized with nonlinear time series techniques such as scaling exponent alpha (ScalExp) derived from detrended fluctuation analysis (DFA) (Peng *et al* 1993) or sample entropy analysis (SampEn) (Lake *et al* 2002). These techniques quantify long-range correlations and complexity of time series of IBIs and are useful for prediction of critical events in neonates including intraventricular hemorrhage and sepsis (Lake *et al* 2002, Tuzcu *et al* 2009).

Time series of IBIs in neonates commonly contain outliers that can be caused for example by spontaneous movements, touching by parents, handling of infants during nursing or medical interventions, or true cardiac arrhythmias (Kemper *et al* 2007). Eliminating noisy data through quality control procedures is important

to derive valid HRV estimates. This is of particular relevance when studying preterm infants as raw data are obtained in the busy setting of a NICU where motion artifacts and repetitive handling of infants are unavoidable. Additionally, data gaps, random spikes, or changes in local signal characteristics may influence the statistical properties of biological signals (Chen *et al* 2002). Thus, standardized sampling and quality control of data is required to assess robustness of different HRV outcomes in the light of noisy data. Further, the effect of motion artifacts on the statistical characteristics of IBIs (e.g. standard deviation) might differ from that on nonlinear time series analysis outcomes such as ScalExp or SampEn (Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology 1996, Lake *et al* 2002).

In general, to make use of time series analysis of IBIs in a busy clinical setting, a compromise between ideal data integrity and extensive resource allocation for data quality control measures needs to be found: manual filtering and quality control of long time series of IBIs can be extremely time-consuming; on the other hand, IBIs derived from commercial chest impedance monitoring systems often do not provide raw data of heart and diaphragmatic muscle activity. Thus, the extent of motion artifacts and frequency and distribution of outliers, and their impact on calculation of IBIs cannot readily be assessed. Consequently, many studies relying on IBIs obtained in a clinical setting do not describe their data filtering and processing procedures.

Thoracic surface electromyography (sEMG) has previously been used to measure heart rate and respiratory rate in preterm infants (Kraaijenga *et al* 2015). Whether sEMG can be used to identify motion artifacts and to measure IBIs for HRV analysis of preterm infants in a NICU and how filtering and quality control of such data could be ascertained has not been reported previously.

In the current study, we tested the feasibility of sEMG measurements in very preterm and/or very low birth weight infants during their first week of life to derive IBIs for HRV analysis. We aimed to evaluate a stepwise data quality control algorithm to discriminate between periods of good data quality and those with noisy data. We further aimed to assess whether data gaps introduced by removing noisy data affect HRV outcome parameters. We hypothesized that a systematic data quality control algorithm of sEMG measurements allows for identification of motion artifacts and has a significant effect on IBI values, their distribution in the time domain, and outcomes of nonlinear time series analysis as determined by ScalExp and SampEn.

Material and methods

Study design

We conducted a prospective, single centre observational study in the NICU of the University of Basel Children's Hospital (UKBB), Basel, Switzerland. The study was approved by the Ethics Committee of Northwestern and Central Switzerland and written informed consent of the parents was obtained prior to inclusion of study participants. Inclusion criteria were as follows: Preterm infants less than 24 h of chronological age; gestational age (GA) of <32 completed weeks and/or birth weight <1500 g. Exclusion criteria included major congenital malformation, asphyxia, or infants in whom treatment was directed towards palliative care.

Study population

Between January 2013 and September 2015, we performed 330 sEMG measurements in 78 preterm infants. Out of these, 19 measurements could not be used for further analysis due to truly irregular heart beat (repetitive supraventricular extra systole; $n = 9$) or displacement of electrodes ($n = 10$). Thus, a total of 311 measurements from 78 infants (33 female, 45 male) were conducted. Mean (SD) gestational age and birth weight of participants was 30.3 (2.4) weeks and 1277 (341) g, respectively. Mean (SD) duration of measurements was 169 (34) min. No adverse events related to the measurements were reported.

In the current methodological report, we describe the development and performance of a quality control algorithm and filtering procedure based on a representative subsample of 100 measurements from 24 infants. Characteristics of this subsample are outlined below. This representative subsample was chosen to limit cost and computer processing time of developing quality control and filtering algorithms (on average, 75 min to perform quality control algorithm, assess effects of each step of the algorithm, and evaluate the effect of gaps in measurement traces). The quality control algorithm and filtering procedures described in the current report were then applied to all 311 measurements from 78 infants to assess the predictive value of time series analysis on clinically relevant outcomes. The full clinical results assessing the associations of HRV with indicators of morbidity of infants and the prognostic utility of HRV on clinical outcomes will be published elsewhere.

Selection and characteristics of representative subsample

We selected 100 representative measurements based on demographic characteristics (gestational age, birth weight, sex) and the level of respiratory support (none, continuous positive airway pressure, endotracheal ventilation) during the measurement. Measurements were obtained from 24 infants (14 male) with a mean (SD, range) gestational age and birth weight of 30.8 (2.9, 24.7–37.0) weeks and 1275 (346, 670–1876) g. Out of the 100

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Table 1. Characteristics of study population and selected measurements.

Value	Study population	Selected measurements	p-value
GA (weeks)	30.3 (2.4)	30.8 (2.9)	0.40
Birth weight (g)	1277 (341)	1275 (346)	0.98
Sex (male (%))	45/78 (58%)	14/24 (58%)	0.96
Respiratory support (measurements (%))			
—None	−129/311 (41%)	−43/100 (43%)	0.79
—CPAP	−169/311 (55%)	−53/100 (53%)	0.82
—Endotracheal ventilation	−13/311 (4%)	−4/100 (4%)	0.94

Note: Continuous variables are displayed as mean (SD); *p*-value derived from unpaired t test or chi-square test as appropriate. GA, gestational age; CPAP, continuous positive airway pressure.

measurements, 43 were done under no respiratory support, 53 under continuous positive airway pressure, and four during endotracheal ventilation. Distribution of demographic characteristics and respiratory support levels were comparable between subsample and total cohort (table 1).

Measurements

Measurements were conducted during the infant's first five days of life. On each of those days, a sEMG measurement was started at 8.30 am and lasted for 3 h. All infants were nursed in incubators, wearing a nappy only. Two sEMG electrodes (standard silver/silver chloride sEMG electrode patches; MultiBioSensors Inc., El Paso, Texas, USA) were placed bilaterally on the caudal, medioclavicular end of the rib cage. A reference electrode was placed higher up on the thoracic wall (figure 1). sEMG raw signal was captured at a sampling frequency of 500 Hz using commercially available hardware and software (Polybench, Inbiolab BV, Roden, NL) and data were exported as csv-files. Simultaneous video recordings of the infants were obtained during the measurements (LifeCam, Microsoft Corporation, Redmond, Washington, USA). Video raw data was recorded at 15 Hz. Synchronization of sEMG and video data was performed online within Polybench software.

sEMG data quality control algorithm

We developed a four-step quality control algorithm to reliably detect QRS complexes for the calculation of IBIs. Figure 2 shows a flow diagram outlining the sequence of steps involved in the data quality control procedure. The four steps were as follows.

Step one: motion detection software for identification of large movement artifacts

We analyzed videos using custom written software (Python scripts augmented with mathematical functions from numpy, signal processing routines implemented in scipy, and graphical utilities from matplotlib). The software was designed to identify phases of spontaneous movement in preterm infants: Colored movies were analyzed as sequences of grey-scale images featuring a sampling frequency of 1 Hz. First, the region of interest (ROI, i.e. the study participant) was determined for each measurement based on variance analysis of grey-scale pixel values over the whole movie. Thresholding the resulting variance map by means of an experience-based threshold, revealed a ROI in the center of the movies. The average grey-value change over all pixels in the ROI was calculated and plotted (blue line in figure 3(a)). Sequences featuring an average grey-value change above a certain time-dependent threshold were considered to be motion-prone (bottom line in figure 3(c)). Time-dependent thresholds were calculated based on a rolling average over windows of 800 s (black line in figure 3(a)). We marked sequences indicating spontaneous movement (figure 3(a), yellow arrows) or handling of an infant (figure 3(a), red stars) as potentially unsuitable for IBI analysis.

Step two: root mean square (RMS) of raw sEMG signal after extraction of QRS complexes

The Polybench software of the sEMG device reported the RMS of the sEMG measurement in addition to the raw sEMG signal. The RMS signal is the remaining signal after detection and removal of the QRS complex, according to the gating technique described by O'Brien *et al* (1983) using a running average to fill the gaps and applying a thresholding t[13] to eliminate suspicious sequences:

$$t = \min\{\mu(f) + 0.6\,\sigma(f),\ 2\mu(f)\}.$$

Here f is the RMS signal reported by the measurement device, μ is the mean function and σ is the standard deviation. In order to increase stability we defined border regions (4 s) around time periods where the RMS curve was above threshold t. These sequences were then marked as potentially unsuitable for IBI analysis.

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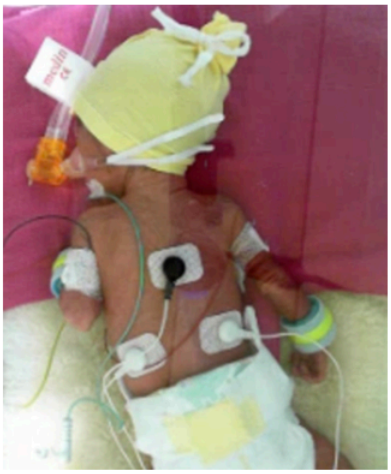

Figure 1. Example of measurement setting to illustrate electrode positions.

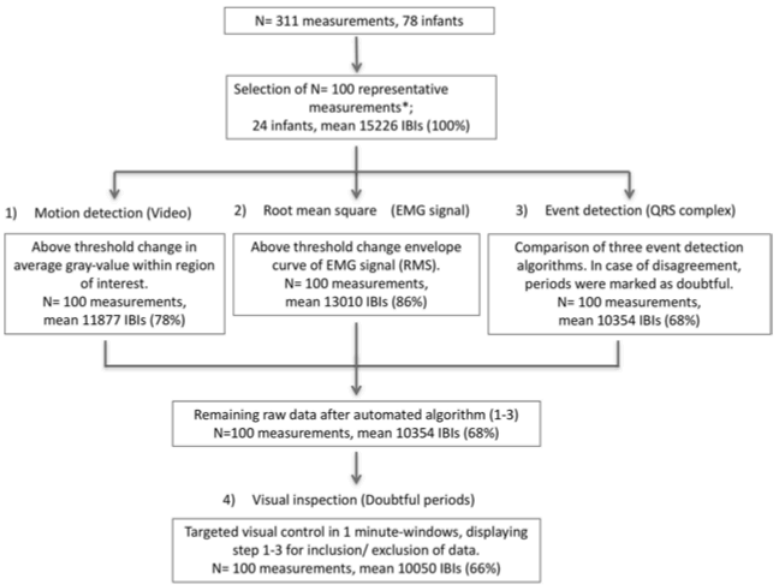

Figure 2. Schematic overview of quality control algorithm including decision rules for exclusion of data points. * Measurements were selected as representative with respect to gestational age, birth weight, sex, and the level of respiratory support during the measurement. EMG, electromyography; IBIs, interbeat intervals.

Step three: comparison of three different QRS detection algorithms

Both the built-in default QRS detection algorithm of the sEMG device and the well-established QRS detection algorithm by Friesen *et al* (1990) did not perform as expected for detecting heart beat sequences in sEMG raw data featuring spontaneous movement in these small infants. Thus, we additionally developed a custom algorithm for reliable peak detection within the scope of the present study. As we observed a wide variety of peak shapes, a straightforward, template-based search algorithm was not applicable; the variety of shapes made the application of one globally optimized threshold impossible. Therefore, we developed a three-stage peak-detection algorithm as follows: (i) per-measurement threshold optimization, (ii) peak location followed by (iii) peak location fine-adjustments. Generally, thresholding the baseline-shift corrected raw signal with a threshold set too high forfeits the majority of actual peaks. On the other hand, if the threshold is set too low, a very large number of

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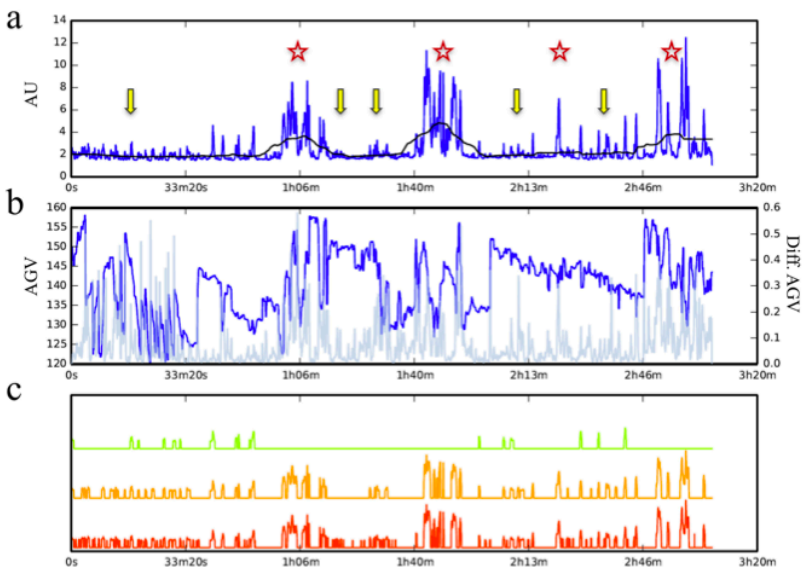

Figure 3. Motion detection software interface after focusing video on the infant. (a) *Y*-axis: AU: artificial unit, representing the cumulative gray value difference of all pixels in the region of interest (ROI) between two consecutive frames. 0 indicates no difference between all grey values of all pixels when comparing frame (n) with frame ($n + 1$), a larger number implies a significant change of the scenery from one frame to the next. Yellow arrow indicates that infant is moving; red star indicates handling of the infant. Blue line represents grey level difference over the duration of the movie; black line represents time dependent, moving average difference value of grey level over window size of 800 s. Significant movement of the infant is indicated when black line moves above blue line and the resulting signal is displayed as the bottom line in panel (c). (b) Left *Y*-axis: AGV: average grey value of a frame, 0 represents black, 255 stands for white. Right *Y*-axis: diff AGV: difference between the average grey value of two consecutive frames within the ROI. 0 implies no change in the average grey value within the ROI, a large change implies a larger change in average grey value usually caused by a new object entering the scenery. Filtering of false-positive events. Blue line represents average grey value of video frames over duration of the video; gray line represents derivative (slope) of solid blue line, based on second order differential quotient. If absolute slope value is larger than mean (slope)—1/3 × SD (slope), frames are removed and marked as non-moving sequence. The resulting signal is displayed as middle line in panel (c). (c) To remove short sequences that were labelled as frames containing movement, sequences where summed difference grey values were smaller than a predefined threshold were removed. Top line in figure 1(c) represents the resulting signal allowing for discrimination of sequences with versus without infant movement.

false-positive events is incurred. Ideally, an optimal threshold is applied to each measurement. Consequently, we applied various thresholds to each measurement and calculated the global beat frequency based on each threshold. For each measurement, we documented a specific, threshold dependent stability regimen, in which the measured overall frequency did not, or only minimally change. In order to make sure that segments of raw signal considered to be a 'peak' were consistently detected throughout a 3 h measurement, the third step of the custom algorithm searched for a consistent shape (single maximum or minimum as determined by above mentioned threshold optimization), repeatedly present along the whole measurement trace. The precise location of the consistent shape around the previously found peak locations ensures a reliable QRS-detection used for further analysis. This three-stage peak-detection algorithm was applied to all recorded datasets and results were then compared with the two QRS detection algorithms mentioned above (O'Brien *et al* 1983, Friesen *et al* 1990). We observed that for sequences with a high noise-level caused by movement of the study participants, agreement of the three QRS-detection algorithms instantly was disrupted. Nevertheless, some windows within those segments showed a remarkably consistent heart beat frequency independent of the engaged peak detection method. Thus, sequences with major disagreement between the different QRS-detection algorithms were software-labelled as potentially unsuitable for IBI analysis. Major disagreement between the three detection algorithms was assumed if the interval of detected QRS-complexes deviated by >100 Hz (>0.2 × sampling frequency of 500 Hz). In case of such a disagreement, a minimal window of 8 s (4 s before and 4 s after start of disagreement) was labelled.

Automated inclusion and exclusion of raw data after steps one to three

The algorithm automatically *included* raw data for IBI analysis if (a) all of the above mentioned steps indicated data of acceptable quality or if (b) QRS detection methods were in complete agreement and either motion detection software or RMS curve indicated raw data of acceptable quality. Further, the algorithm automatically *excluded* raw data if the data in all of the above mentioned steps were marked as potentially unsuitable for IBI analysis.

In cases where motion detection software and/or RMS curve indicated raw data of acceptable quality but QRS detection methods disagreed, the raw data were labelled for targeted visual inspection and manual data quality control as outlined below.

Step four: targeted visual inspection of windows with questionable data quality

A custom-made software tool (see figure 4) was developed to enable efficient visual control of and fast-forwarding between sequences indicating major disagreement between QRS detection algorithms. The tool also displayed results of motion detection analysis (step one) and RMS envelope curve assessment (step two) as outlined above. An inbuilt jump function displayed windows of 1 min duration whenever results of motion detection analysis (step one) or RMS envelope curve (step two) suggested valid data but QRS detection algorithms disagreed (step three). In these instances, trained study personnel manually excluded data if QRS-complexes detected by the implemented threshold technique (figure 4(c)) did not correspond to those that were visually detectable in the raw signal (figure 4(a)).

IBI outlier identification and elimination

The above data quality control algorithm of the sEMG signal was followed by systematic identification and elimination of IBI outliers as recommended by Kemper *et al* (2007). As suggested for the age group of infants, each IBI above 2 s was marked as an outlier. Further, sudden changes in IBI >1 s in the fourth IBI within a sequence of seven IBIs were identified as outliers using a moving window algorithm. Finally, all outliers were interpolated and replaced by the mean of the two neighbouring IBI values.

IBI calculation

Once QRS complexes were defined and IBI outliers were identified and eliminated, normal to normal beat intervals (NN), defined as all intervals between two consecutive beats resulting from sinus node depolarization were analysed as recommended (Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology 1996). Distances between two successive QRS complexes (IBI) were calculated in the time domain. In case of segmentation of the EMG trace due to the quality control algorithm mentioned above, the IBI between the last QRS complex of the period preceding and the first interval of the period following the excluded segment were discarded.

Outcome parameters of HRV

Analysis of the signal including time series analysis was performed in Matlab (The Math Works, Inc., Natick, Massachusetts, United States). We thus calculated the following standard IBI outcomes in the time domain: Mean, SD (SDNN), coefficient of variation (CVNN), square root of the mean of squared successive differences (RMSSD), skewness, ScalExp derived from DFA, and SampEn (Peng *et al* 1995a, 1995b, Task Force of the European Society of Cardiology and The North American Society of Pacing and Electrophysiology 1996, Richman and Moorman 2000, Yum *et al* 2001, Lake *et al* 2002, Kleiger *et al* 2005, Nakamura *et al* 2005). ScalExp reflects the self-similarity (scaling) of a biological signal in a time series across a range of sizes of time windows and is a measure of memory in a system. SampEn describes the degree of randomness of a signal in a time series with higher values of SampEn reflecting a less predictable signal.

Influence of individual steps in the quality control algorithm on HRV outcomes

We calculated the fraction (%) of usable IBI data points after applying the different steps of the data quality control process as outlined in figure 2. We further assessed the effect of individual steps in the quality control algorithm on all assessed HRV outcomes using methods for outlier identification and elimination as described above.

Reliability of quality control algorithm

In order to detect whether the quality control algorithm allowed for reliable detection of IBI data, a trained team member extracted IBIs from 10 representative sEMG measurements using the above mentioned stepwise algorithm. Subsequently, the same researcher extracted IBIs from the same 3 h files by complete manual data cleaning based only on visual inspection of the entire measurement trace using a window size of 1 min.

Effect of gaps in sEMG traces and number of IBIs on time series analysis parameters

It is important to realize that the above mentioned quality control algorithm introduces gaps in a continuous signal trace. In order to evaluate whether the amount of data fragmentation introduced by the signal cleaning process influences outcomes of DFA and SampEn, IBIs calculated using all technically acceptable segments of the sEMG measurement were compared to those calculated from the single longest technically acceptable consecutive series of data points using the four step quality control algorithm. The minimal number of consecutive IBI data

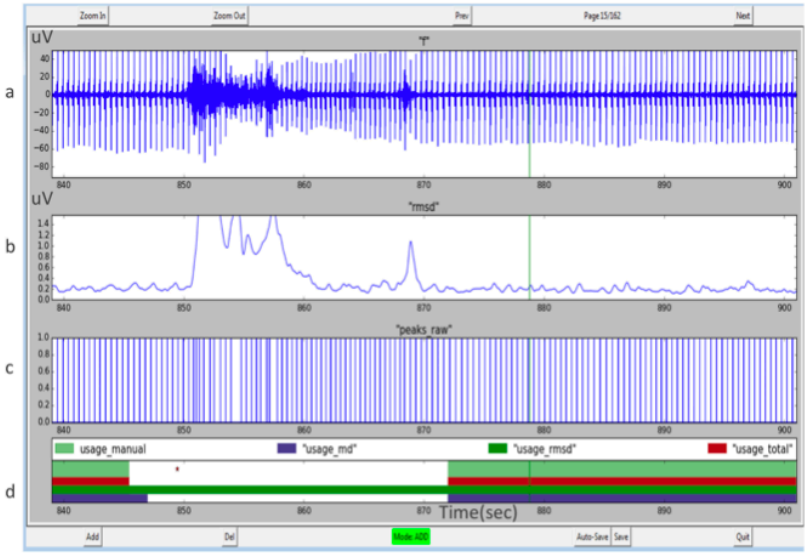

Figure 4. Custom-made visualization software tool to perform step four in the cleaning algorithm. (a) Raw signal of diaphragmatic electromyography measurement. (b) Envelope curve of the raw signal after extraction and interpolation of QRS-complexes (RMS). (c) Vertical lines indicate QRS complexes as detected by the implemented threshold technique. (d) Light green bar on top indicates sequences of acceptable signal quality based on visual control of detected QRS complexes; red bar indicates consistency of QRS detection as determined by three different QRS detection algorithms (O'Brien *et al* (1983), Friesen *et al* (1990); built-in threshold technique, refer to methods); dark green bar indicates sequences of acceptable signal quality based on the RMS curve derived from the measurement device (Polybench, NL); purple bar on bottom indicates video sequences free of movement artifacts based on motion detection software tool of synchronized video recordings. * Period of signal acquisition requiring visual inspection due to disagreement between the three QRS-detection algorithms. Inclusion/exclusion of questionable data was performed based on results of all quality control steps. Final decision on data exclusion was based on agreement between visually detectable QRS-complexes in the raw signal and those defined by the implemented threshold technique (c).

points considered to be acceptable for time series analysis by DFA was set to 360 as recommended previously (Stern *et al* 2009).

Statistical analysis

All HRV outcomes were visually assessed for normal distribution of data. Comparison of the effect of using different steps of the cleaning algorithms on IBI and time series analysis was done by paired Student's t-test. The influence of calculating IBIs from all technically acceptable segments versus using only those obtained from the single longest, technically acceptable segment of a measurement on ScalExp and SampEn was assessed by linear regression and Bland–Altman analysis. Associations between number of IBI data points and ScalExp or SampEn were examined by linear regression analysis. Statistical analyses were performed using Stata software (StataCorp. 2009. Stata Statistical Software: Release 11. College Station, TX: StataCorp LP). A p-value < 0.05 was considered to be statistically significant.

Results

Performance of quality control algorithm and outlier identification/elimination

Figure 2 shows an overview of the quality control algorithm including decision rules and the consequences on the amount of raw data available for calculation of HRV outcomes. On average, the quality control algorithm excluded 34% of raw data, i.e. 66% of the IBI values were judged to be suitable for HRV analysis. Subsequent outlier identification resulted in a mean (SD) of 0.5% (0.8%) of IBI outliers, which were replaced by interpolating the mean value of the two neighbouring IBI values.

Influence of steps of quality control algorithm on HRV data

HRV outcomes are summarized in table 2. Steps one, two and three of the quality control algorithm (motion detection software, RMS of sEMG signal and agreement of QRS detection algorithms) on average (SD) labelled 14 (11) to 32 (11) % of data points as being not suitable for HRV analysis. Step four (visual inspection) lead to

Table 2. Outcome parameters derived after different steps in the quality control algorithm.

Outcome variables	Raw data	Motion detection (video)	RMS curve	Four step algorithm	Mean difference[c] (95% CI)	*p*-value[c]
Mean IBI	0.415 (0.033)	0.414 (0.033)	0.414 (0.033)	0.415 (0.033)	<0.001 (10^{-4}–10^{-3})	<0.001
SDNN IBI	0.028 (0.011)	0.029 (0.011)	0.029 (0.011)	0.029 (0.011)	0.001 (0.001–0.002)	<0.001
CVNN IBI	0.068 (0.027)	0.069 (0.026)	0.070 (0.026)	0.072 (0.026)	0.003 (0.002–0.005)	<0.001
RMSSD IBI[a]	−4.379 (0.612)	−4.151 (0.471)	−4.190 (0.532)	−3.967 (0.417)	0.412 (0.342–0.481)	<0.001
Skewness IBI[a]	0.735 (1.428)	1.036 (1.088)	1.008 (1.402)	1.128 (1.191)	0.450 (0.238–0.661)	<0.001
ScalExp, all	0.994 (0.093)	0.982 (0.076)	0.978 (0.085)	0.961 (0.074)	0.033 (0.047–0.020)	<0.001
ScalExp single[b]	N/A	1.091 (0.197)	1.091 (0.197)	1.091 (0.197)	NA	NA
SampEn, all	0.453 (0.222)	0.440 (0.211)	0.434 (0.219)	0.425 (0.219)	0.028 (0.010–0.047)	0.003
SampEn, single	N/A	0.394 (0.249)	0.389 (0.255)	0.379 (0.259)	NA	NA
Usable IBIs (%)	100 (0)	78.1 (7.9)	86.4 (10.5)	65.8 (10.8)	34.2 (32.0–36.3)	<0.001

[a] Data log transformed to achieve normal distribution.
[b] Identical parts were used for analysis.
[c] Raw data versus four step algorithm, values are displayed as absolute differences.
Values are displayed as mean (SD) unless specified otherwise.
IBI, interbeat interval; SDNN, standard deviation of normal to normal (NN) IBI; CVNN, coefficient of variation of IBI; RMSDD, square root of the mean of squared successive differences in IBI; ScalExp, scaling exponent alpha from detrended fluctuation analysis—all, calculated using all acceptable parts of measurement;—single, calculated using only the single longest, acceptable part of measurement; SampEn, sample entropy—all, calculated using all acceptable parts of measurement;—single, calculated using only the single longest, acceptable part of measurement.

further exclusion of 1.9 (0.6) % of IBI data points resulting in a total of 65.8 (10.8) % usable data points after completion of the algorithm.

Significant but small differences in mean IBI, SDNN IBI, CVNN IBI and considerable differences in RMSSD IBI and skewness IBI were noted after applying quality control steps. Interestingly, we found only small differences in ScalExp and SampEn due to quality control steps (table 2).

Reliability of quality control algorithm

Table 3 details comparison of IBI data points from the four-step quality control algorithm versus visual inspection of the entire measurement trace in a subset of 10 representative sEMG measurements. Visual inspection of entire files versus using the four step algorithm resulted in a mean (SD) increase of 18.8% (10.3%) of included data points of good quality and a mean (SD) increase of 1.7% (1.8%) of excluded data points of poor quality. Mean (SD) time required for visual inspection of entire files was 50.4 (13.4) min per 3 h measurement versus 12.4 (7.3) min per 3 h measurement when using the four step algorithm, i.e. using the four step algorithm reduced analysis time by 76%.

Effect of gaps in sEMG traces and number of IBIs on time series analysis parameters

There was a significant effect of using IBIs from all technically acceptable segments of the sEMG measurement versus only those from the single longest, technically acceptable consecutive series of data points in the sEMG measurement on ScalExp: The mean difference in ScalExp was −0.134 (95% CI: −0.174 to −0.095; $p < 0.001$). Bland–Altman analysis indicated poor agreement with proportional bias of the two methods (figures 5(a) and (b)). The single longest, technically acceptable consecutive series of data points included a mean (range) of 1221 (373–4562) IBIs. Thus, all measurements fulfilled the preset criterion of a minimum of 360 data points for DFA analysis.

The mean difference in SampEn of IBIs derived from all technically acceptable segments of the sEMG measurement versus only those from the single longest, technically acceptable consecutive series of data points was 0.047 (95% CI: 0.028 to 0.067; $p < 0.001$). Bland–Altman analysis indicated acceptable agreement between the two methods (figures 5(c) and (d)).

Linear regression analyses indicated no significant association between ScalExp or SampEn and the number of IBI data points obtained from the single longest, technically acceptable segment of the measurement after applying the four step algorithm (figure 6).

Discussion

To the best of our knowledge, this is the first study to use thoracic sEMG measurements for assessment of HRV in humans. We found that thoracic sEMG measurements for analysis of HRV in preterm infants are feasible. Using a stepwise data quality control algorithm to exclude motion artifacts and improve QRS detection had significant

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Table 3. Amount of usable data from 10 individual time series comparing semi-automatic four step algorithm with visual inspection of entire measurement.

	Usable IBIs, four step algorithm	Usable IBIs, complete visual inspection	Difference in usable IBIs, complete visual—four step
Trace	No (%)	No (%)	No (%)
1	10272/15250 (67.36)	14061/15250 (92.20)	3789 (24.84)
2	9007/15490 (58.15)	12324/15490 (79.56)	3317 (21.41)
3	9952/14992 (66.38)	12962/14992 (86.46)	3010 (20.08)
4	11942/15734 (75.90)	14194/15734 (90.21)	2252 (14.31)
5	10789/15603 (69.15)	13916/15603 (89.19)	3127 (20.04)
6	9934/15321 (64.84)	12197/15321 (79.61)	2263 (14.77)
7	9128/14779 (61.76)	13054/14779 (88.33)	3926 (26.57)
8	12299/15016 (81.91)	14340/15016 (95.5)	2041 (14.31)
9	12190/15197 (80.21)	14939/15197 (98.30)	2749 (18.09)
10	10923/15142 (72.14)	13699/15142 (90.47)	2776 (18.33)

Note: Data representative in terms of gestational age and level of respiratory support. IBIs, interbeat interval. The number of usable IBIs was significantly lower after application of the four step algorithm compared to complete visual inspection (t-test; mean difference: 2925 IBIs, $p < 0.001$).

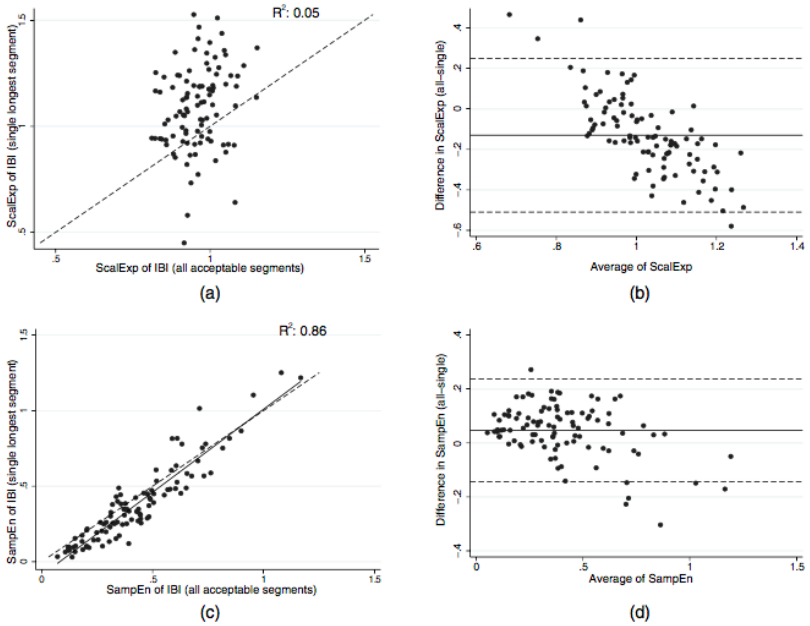

Figure 5. Comparison of scaling exponent alpha derived from detrended fluctuation analysis (ScalExp) and sample entropy (SampEn) of interbeat intervals calculated using all acceptable segments of a measurement versus using only the single longest, technically acceptable segment of a measurement. (a) Absolute values of ScalExp (all acceptable segments versus single longest, acceptable segment). Dashed line represents line of identity. There was no significant association between ScalExp, all acceptable segments and ScalExp, single longest, acceptable segment (R^2 0.05). (b) Bland–Altman plot of the difference in ScalExp (all acceptable segments—single longest, acceptable segment) plotted against the average ScalExp. The solid line indicates mean difference while the dashed line indicates 95% limits of agreement (mean difference ±1.96 SD). (c) Absolute values of SampEn (all acceptable segments versus single longest, acceptable segment). Dashed line represents line of identity, solid line represents line of linear regression (R^2 0.86). (d) Bland–Altman plot of the difference in SampEn (all acceptable segments—single longest, acceptable segment) plotted against the average SampEn. The solid line indicates mean difference while the dashed line indicates 95% limits of agreement (mean difference ±1.96 SD).

impact on sEMG data quality in terms of exclusion of unsuitable IBI data points, distribution of IBI values in the time domain, and calculation of ScalExp and SampEn. Visual inspection of a subset of entire measurement files compared to the stepwise quality control algorithm yielded an additional 18.8% of data points suitable for HRV analysis but lead to exclusion of only 1.7% of unsuitable data points. In our opinion, the fourfold decrease in analysis when applying the semi-automated algorithm instead of inspecting entire measurement files justifies

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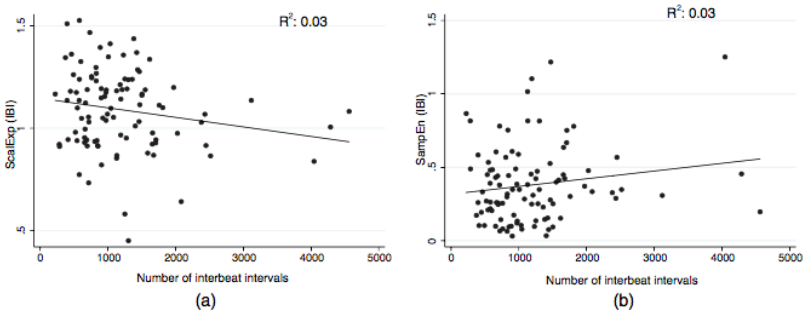

Figure 6. Scatter plots of (a) scaling exponent alpha derived from detrended fluctuation analysis (ScalExp) and (b) sample entropy (SampEn) of interbeat interval (IBI) over the total number of IBIs within the single longest, technically acceptable segment of a measurement after applying the four step algorithm. There was no significant association of ScalExp (R^2 0.03) or SampEn (R^2 0.03) and the number of IBIs within a measurement.

a reduction of about 19% of potentially suitable raw data. Fragmentation of data within a 3 h measurement had a significant but relatively small effect on both ScalExp and SampEn.

Interpretation and methodological considerations

Electrocardiogram (ECG) is the standard method for recording electrical activity of the heart in order to calculate IBI and perform HRV analysis. While ECG is explicitly designed to assess the heart muscle, thoracic sEMG measures a summary signal of electrical activity of the heart, diaphragm, and skeletal muscles. Arguably, specific assessment of the heart is preferable when performing HRV analysis; on the other hand, detecting movement of the limbs or trunk by sEMG may be very helpful when studying HRV in preterm infants, as measurements in these infants inevitably contain noisy data due to motion artifacts, potentially influencing IBI data quality and requiring exclusion of unsuitable raw data (Kemper *et al* 2007). In contrast to the combination of ECG and motion detection sensors, the sEMG not only detects body movements/accelerations but also measures tonic contractions of the thoracic muscles and/or the diaphragm. Those are common in preterm infants and might not be detected by motion detection sensors. Although some of the excluded raw data were potentially usable based on visual inspection of the entire 3 h measurement files, only 1.7% of IBI values were not excluded by the stepwise algorithm indicating that the quality of data produced by the stepwise algorithm is high when compared to the reference standard of complete visual inspection of all raw data. This interpretation is supported by the fact that subsequent outlier detection based on methods reported by Kemper *et al* (2007) resulted in little additional exclusion of unsuitable raw data (on average, 0.5%). Complete visual inspection of entire time series is extremely time-consuming and probably unrealistic when repeatedly analysing time series of several hours of data from large groups of patients; thus, we believe the stepwise algorithm to produce an appropriate trade-off between obtaining data of high quality within reasonable time and loss of potentially usable raw data (on average, 18.8%). The vast majority of noisy raw data were excluded automatically. Only 1.9% of additional raw data were excluded manually during step four, i.e. by targeted visual inspection of segments marked by software due to disagreement between QRS detection algorithms. Video-based motion detection software and the RMS (envelope curve) of the sEMG signal were particularly effective in excluding noisy raw data. Therefore, further development of the algorithm towards a fully automated quality control system is desirable and will likely result in additional savings in analysis time.

The data quality control algorithm had a significant but predominantly small effect on mean, SDNN, and CVNN of IBI values (table 2). Nonetheless, even if those differences were small when pooling data from the study cohort, the resulting effect may influence interpretation of the data on an individual level. There was a considerable effect on RMSSD and skewness of IBI, suggesting that removing noisy raw data is particularly relevant for distributive indices of IBI reflecting the shape of the IBI histogram. Although the stepwise algorithm only had a small effect on mean values of ScalExp and SampEn, there was a considerable difference in ScalExp values calculated from all acceptable segments and from the single longest, technically acceptable segment of the measurement (figure 5). Inherently, removing noisy raw data produces gaps in time series of signals. As fractions of data are cut out during the cleaning and remaining segments of data are stitched together, the cutting procedure introduces non-stationarities due to discontinuities in the signals (Chen *et al* 2002). Surprisingly, signals exhibiting long-range correlations, i.e. for $0.5 < \text{ScalExp} < 1.5$, ScalExp is hardly affected by such cutting procedures and is independent of segment size even when up to 50% of the data are removed assuming the long-range correlation properties of the signal are constant (Chen *et al* 2002). Thus, the observed differences in ScalExp in our data set

are not likely to be explained by the amount of missing data given that on average, 34% of data were cut out by the quality control algorithm. A potential explanation, however, relates to the rapid sleep cycling of preterm infants: typically, motion artifacts occur more often during active rather than quiet sleep and infants are more likely to be handled by staff and parents when awake or in active sleep. Given that (a) sleep stage affects long-range correlation properties of heart rate (Bunde *et al* 2000) and (b) preterm neonates in the first week of life pass through three to six sleep cycles within just 3 h (Cremer *et al* 2016), the discrepancy between ScalExp from all acceptable segments versus the single longest, technically acceptable segment is most likely due to changes in local signal behaviour resulting from greater variation in sleep stages in the former compared to the latter segments. If this is the case, ScalExp of the single longest segment captures long-range correlations within a relatively short period of time and is sensitive to local properties of the infant's state during that time period. On the other hand, ScalExp of all acceptable segments together would give a more robust measure of long-range correlations of the infant, averaged over several non-stationary periods (e.g. transitions between sleep-wake phases) and perhaps be more representative of an individual subject. Depending on the aim of a given study, we recommend to take this into account as ignoring this fact could lead to altered results. By contrast, SampEn was much less influenced by fragmentation of data; although there was a numeric difference in SampEn values calculated from all segments versus those from the single longest segment of the measurement, agreement between methods was acceptable as indicated on Bland–Altman plot (figures 5(b) and (d)).

Comparison with previous literature

While there are no validation studies comparing HRV from sEMG with that obtained from ECG recordings, Kraaijenga *et al* recently demonstrated that deriving heart rate from sEMG in preterm infants on non-invasive respiratory support is feasible and repeatable (Kraaijenga *et al* 2015). Reassuringly, these authors showed that heart rate and respiratory rate obtained from sEMG measurements were in good agreement with those from chest impedance monitoring using surface ECG electrodes. Although duration of measurement in their study was considerably shorter than in ours (six windows of 1 min duration were extracted from an observation period of 1 h) and only average values of heart rate and respiratory were assessed, it is encouraging to note that sEMG provided heart rate values comparable to those acquired by a monitoring system considered to be standard of care in the NICU. There are no reference ranges for HRV outcomes in preterm infants measured within the first week of life, however, distribution of IBI values and SampEn calculated in our study after applying the quality control algorithm (table 2) are within the range of values documented in cohorts of preterm infants within the first few weeks of life as reported by Lake *et al* (2002) and Griffin and Moorman (2001), Griffin *et al* (2003). Additionally, the four-step algorithm resulted in values of ScalExp similar to those reported by Nakamura *et al* who studied preterm infants within the first several months of life (average ScalExp, 0.99–1.08) (Nakamura *et al* 2005). Thus, using the quality control algorithm appears to result in physiologically acceptable IBI values and HRV outcomes.

We observed that an extremely precise, multi-stage peak detection algorithm is required for reliable QRS detection and processing and thus developed such an algorithm within the scope of the current study. Some of the observed QRS peak shapes featured two maxima. Such data would not be processed properly by simple thresholding or template-based techniques. Consequently, recommended or commercially available event detection algorithms for ECG data (O'Brien *et al* 1983, Friesen *et al* 1990) are not suitable for analysing sEMG data in this study population.

Outlier identification has been shown to be crucial to reliably perform HRV analyses (Kemper *et al* 2007). The use of SDNN to define a threshold has been shown to identify more IBI outliers in infants, whereas a threshold defined by exceeding a certain percentage of the signal has been shown to improve outlier identification in children that are moving (Kemper *et al* 2007). Unsurprisingly, not only the general method but also the specific procedure of setting a threshold (e.g. size of observed time window, height of IBI threshold) influences the rate of outliers. Given that further management of identified outliers has little effect on data quality of raw IBI values (Kemper *et al* 2007), we decided to interpolate outliers with the mean of the two neighbouring IBIs as suggested by Kemper *et al* (2007).

Strengths and limitations

The strengths of this study include the development of a method to simultaneously assess HRV, motion artifacts, and skeletal muscle activity in preterm infants within a single, non-invasive device. Further, repeated measurements were conducted at the same time of the day and at identical points in the nursing/feeding cycle to reduce measurement error and information bias due to potential circadian influences or systematic differences in daily clinical routine. Also, the stepwise quality control algorithm offers a good compromise between resource allocation for data acquisition versus yield and quality of raw data. The limitations include the lack of comparison with the reference standard of ECG; we did not compare sEMG to ECG (or photoplethysmography from pulse oximeters) as the clinical monitors in our NICU did not have serial ports to extract ECG or photoplethysmogram

and we did have ethical concerns about skin damage from additional ECG electrodes for research purposes given the highly vulnerable skin of extremely preterm neonates in their first week of life. A direct validation against ECG would have been ideal although alternatively, photoplethysmography would have been an interesting option as it has been shown to be useful in assessing maternal heart rate variability (Goncalves *et al* 2016). However, the sEMG in use was originally developed in direct comparison with ECG and the gating technique reported by O'Brien *et al* (1983) allows for to isolation and visualisation of QRS complexes as illustrated by Kraaijenga *et al* (2015). Since we did not make a head-to-head comparison between our new technology and ECG, and since the current work demonstrates the feasibility of using sEMG instead of ECG, future studies are required to directly compare ECG with sEMG for HRV analysis. Another limitation is that—despite establishing a semi-automatic procedure for quality control of raw data, the algorithm still requires a fair amount of postprocessing and substantial computing power, although the former could be drastically reduced by continuing development of the algorithm towards a fully automated system. Finally, ScalExp was sensitive to fragmentation of IBI sequences resulting from motion artifacts and data processing.

Implications and future outlook

In the current study, we aimed at developing methods to use thoracic sEMG to extract heart beat data for analysis of HRV in very preterm infants. Potentially, sEMG could also be used to provide a detailed assessment of control of breathing in this population. Respiratory rate from sEMG is in good agreement with that derived from chest impedance as outlined above (Kraaijenga *et al* 2015); additionally, sEMG has been successfully used to complement mechanical lung function measurements (i.e. tidal breathing and lung volume analyses) in preterm infants (Hutten *et al* 2010). Extracting breath-to-breath respiratory rate from sEMG would allow researchers to evaluate both respiratory pattern/control of breathing and HRV data in preterm infants and reduce the impact of motion artifacts on those concurrently acquired signals. Such an approach potentially offers unique opportunities to simultaneously monitor longitudinal changes in cardiorespiratory maturation, presence and progression of disease, and the possibility to assess cardiorespiratory interactions.

Conclusion

Analysis of HRV from thoracic sEMG measurements in preterm infants hospitalized in the NICU is feasible. The proposed semi-automatic algorithm significantly improves data quality of HRV outcomes including distribution of IBI in the time domain and time series analysis based on ScalExp and SampEn. The algorithm results in fourfold reduction in postprocessing time compared to visual inspection of entire data files. Complete automation of the algorithm appears worthwhile and will potentially lead to further savings in analysis time.

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5.4. Autonomic dysregulation in preterm infants

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"It is in man's heart that the life of nature's spectacle exists; to see it, one must feel it."

- Jean-Jacques Rousseau -

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State of the paper	Approved by all co-authors; submitted December 2017
Contribution of KJ	The measurements were performed by KJ and other team members. The development of codes (Matlab, Mathworks) to assess heart rate variability was done by KJ in collaboration with the co-authors. Quality control and statistical analysis was performed by KJ and other team members. The final manuscript will be written by KJ.
Synopsis	A better understanding of immature regulation processes in preterm infants, such as control of heart rate, would be very desirable. In this population, repeated episodes of apnea and bradycardia require a long-term intensive care monitoring, weeks to months of pharmacological therapy and respiratory support. We aimed to test if heart rate variability (HRV) within the first few days of life in preterm infants exhibit nonlinear dynamics that can be characterized using tools as detrended fluctuation analysis or sample entropy. Further we aimed to test if such measures could help to predict an infant's autonomic development in terms of postconceptional age (PCA) at cessation of pharmacological treatment (caffeine), and PCA at successful termination of respiratory support. We assessed HRV from electromyography (EMG) measurements in very preterm/ very low birth weight infants during their first days of life under clinically relevant conditions. Sample entropy of HRV significantly improved the predictive value of regression models aiming to predict PCA at termination of caffeine treatment and duration of respiratory support after adjusting for gestational age and intra-uterine growth.

Heart rate variability after birth predicts subsequent cardiorespiratory stability in preterm infants

Short title: Heart rate variability in preterm infants

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Keywords

Prematurity; autonomic dysfunction; maturation; heart rate variability; prediction

Abstract

Background Preterm infants show cardiorespiratory instability in the form of apnea and bradycardia. This requires cardiorespiratory monitoring, caffeine therapy, and respiratory support over months as prolonged hypoxemic episodes are associated with neurodevelopmental impairment. Cardiorespiratory stability is a prerequisite for discharge from the neonatal intensive care unit (NICU) but very difficult to predict. We aimed to assess whether characterizing heart rate variability (HRV) within the first five days of life has prognostic utility for subsequent cardiorespiratory stability of preterm infants required for discharge from the NICU.

Methods We conducted a prospective cohort study using a previously validated surface diaphragmatic electromyography (sEMG) method to calculate interbeat interval (IBI) time series. We characterized HRV by time series parameters including sample entropy (SampEn) and scaling exponent alpha (ScalExp) obtained from daily 3-h measurements. Data were analysed by multivariable, multilevel linear regression.

Results We obtained acceptable raw data from 309/330 (94%) sEMG measurements in 76/90 (85%) infants born at a mean (range) of 30.2 (24.7-34.0) weeks gestation. We found a significant negative association of SampEn with duration of respiratory support in the NICU ($R^2 = 0.53$, $p < 0.001$) and corrected age at discontinuation of caffeine therapy ($R^2 = 0.35$, $p < 0.001$) after adjusting for sex, gestational age, birth weight z-score and sepsis.

Conclusion Baseline SampEn calculated over the first five days of life carries prognostic utility for an estimation of subsequent respiratory support and pre-discharge cardiorespiratory stability in preterm infants, both important for planning of treatment and utilisation of health care resources.

Introduction

Preterm birth affects about 10% of all infants born worldwide and is associated with a range of adverse cardiovascular, respiratory, and central nervous system outcomes which may be attributed to altered development of these systems[1]. During the first few months of life, the autonomic nervous system of preterm infants is characterized by autonomic dysregulation and undergoes a critical maturational transition[2]. Typically, autonomic dysregulation manifests as apnea and bradycardia of prematurity (AOP). AOP, defined as a cessation of breathing lasting for at least 20 seconds or one of less than 20 seconds that is associated with bradycardia and/or cyanosis, affects at least 80% of very preterm infants[3] (i.e., those born before 32 weeks of gestational age, (GA)). AOP may lead to bradycardia and hypoxemia requiring immediate resuscitation[4], necessitates continuous long-term monitoring of vital signs, caffeine therapy, and respiratory support over weeks to months. Moreover, prolonged hypoxemic episodes during the first two to three months of life of preterm infants are associated with late death and a considerably increased risk of neurodevelopmental impairment at 18 months of age[5]. Thus, AOP represents a considerable burden of disease and has a significant impact on resource allocation in neonatal intensive care units (NICUs) across the globe[6-8]. Indeed, in infants born before 26 weeks GA, each additional day of monitoring cost approximately US$ 40,000 to 130,000 per quality-adjusted life year saved[8]. Notably, cardiorespiratory stability is a crucial prerequisite for discharge of preterm infants from the NICU[9].

Predicting cardiorespiratory stability in preterm infants is notoriously difficult. Postconceptional age (PCA) at cessation of AOP varies considerably with a range of about 35 to 43 weeks PCA[10]. Thus, there is great uncertainty and substantial variability in deciding if and when infants are considered stable enough to withdraw from respiratory support, caffeine therapy, and cardiorespiratory monitoring. However, those decisions have critical influence on safety considerations, allocation of health care resources, and discharge planning[11, 12].

Assessing heart rate variability (HRV) in neonates by time series analysis of data from cardiorespiratory monitoring is useful to quantify maturational changes in neonates and to predict critical events such as neonatal sepsis[13, 14]._We hypothesized that HRV assessed over the first few days of life can be used to predict medium-term cardiorespiratory stability and hence guide decisions related to discharge from the NICU.

To this end, we performed a prospective cohort study using thoracic surface electromyography (sEMG) measurements for analysis of HRV in very preterm infants during their first five days of life. After application of a systematic data quality control algorithm[15], we calculated interbeat interval (IBI) values, their distribution, and several indexes derived from nonlinear time series analysis including sample entropy (SampEn)[16] and scaling exponent alpha (ScalExp) from detrended fluctuation analysis (DFA)[17, 18]. Given that sleep stage potentially influences HRV[19], we additionally performed sleep stage analysis using synchronized video recordings as described previously[20].

We aimed to investigate whether HRV characteristics of very preterm infants measured during their first five days of life (exposure) predict cardiorespiratory stability in terms of (i) total duration of respiratory support in the NICU (primary outcome), (ii) PCA at cessation of AOP, discontinuation of caffeine therapy, and stopping of continuous electrocardiography (ECG) monitoring in the NICU (secondary outcomes) after adjusting for known demographic and clinical determinants of cardiorespiratory stability.

Methods

Study design

We conducted a prospective, single-center, observational cohort study in the tertiary level NICU of the University of Basel Children's Hospital (UKBB), Basel, Switzerland. The study was approved by the Ethics Committee of Northwestern and Central Switzerland, conducted according to the principles of The Declaration of Helsinki, and written informed consent of the parents was obtained prior to inclusion of study participants. Study participants were not given or withheld any special care related to the conduct of this study. Inclusion criteria were as follows: Preterm infants less than 24 h of chronological age; GA < 32 completed weeks and/or birth weight <1500 g. Exclusion criteria included major congenital malformation, asphyxia, or infants in whom treatment was directed towards palliative care. Withdrawal criteria included loss of parental consent or surgical intervention during the first five days of life.

Measurements

sEMG measurements for acquisition of HRV data and synchronized video recordings for sleep staging were performed as reported in recent validation studies[15, 20, 21, 22]. Briefly, daily sEMG measurements were conducted during the infant's first five days of life, starting at 8.30 am and lasting for three hours. Two sEMG electrodes (standard silver/silver chloride sEMG electrode patches; MultiBioSensors Inc., El Paso, Texas, USA) were placed bilaterally on the caudal, medioclavicular end of the rib cage. A reference electrode was placed higher up on the thoracic wall. sEMG raw signal was captured at a sampling frequency of 500 Hz using commercially available software (Polybench, Inbiolab BV, Roden, NL). Time-synchronized video recordings of the infant's face and body were obtained at 15 Hz (LifeCam, Microsoft Corporation, Redmond, Washington, USA). Video based sleep stages (awake, active sleep,

quiet sleep) were scored at 10s intervals as described previously[20] and based on recommendations[22] and validation studies on sleep staging in preterm infants without the use of electroencephalography[23].

Quality control and data processing

Extensive, systematic quality control of raw sEMG data was performed as described previously[15]. Briefly, synchronized video recordings were analyzed using custom software to detect and label sequences with large body movements and periods in which the infant was touched by parents or staff. Secondly, we used a threshold- based root mean square (RMS) envelope curve of the raw sEMG signal to mark periods containing less obvious movement artifacts or periods with tonic thoracic muscle activity. Thirdly, we assessed agreement of three QRS-detection algorithms to reliably detect QRS complexes. Both the built-in default QRS detection algorithm of the sEMG device and the well-established QRS detection algorithm by Friesen et al.[24] did not perform adequately in these small infants. Thus, we additionally developed a custom algorithm for reliable peak detection as reported previously[15]. This algorithm was applied to all datasets and results were then compared with the two QRS detection algorithms mentioned above[24, 25]. Sequences with disagreement between the different QRS-detection algorithms were software-labelled for subsequent targeted visual quality control. Disagreement between the three QRS detection algorithms was assumed if the interval of detected QRS-complexes deviated by > 100 Hz (> 0.2 x sampling frequency of 500 Hz). In case of such disagreement, a minimal window of 8 seconds (4 seconds before and 4 seconds after start of disagreement) was labelled for visual inspection. Finally, software-labelled periods of potentially noisy data were visually checked and manually assigned by trained study personnel to be included or excluded in the analysis based on results of motion detection analysis, RMS envelope curve assessment, and QRS detection

agreement as outlined above. Raw data passing these quality control steps were extracted for calculation of IBI using Matlab (The MathWorks, Inc., Natick, Massachusetts, United States). Based on recommendations for IBI outlier identification in infants[26], outliers included all IBIs > 2 s and those that varied by more than 1 s from the two neighbouring IBIs. Identified outliers were interpolated with the two neighbouring IBI values.

HRV characteristics considered to influence outcomes

We calculated the following HRV characteristics on normal to normal IBIs (NN) taking into consideration all intervals between two consecutive heart beats resulting from sinus node depolarization[27]: Mean (IBI_{Mean}), SD (IBI_{SDNN}), coefficient of variation (IBI_{CV}), square root of the mean of squared successive differences (IBI_{RMSS}), and skewness ($IBI_{Skewness}$)[27, 28]. SampEn was calculated according to the algorithm described by Richman and Moorman and in the citations therein[29]. SampEn is a measure of randomness of a time series. It quantifies the conditional probability that, with the knowledge of a consecutive number of m data points within a given tolerance r (usually r = 0.2*standard deviation), the next data point can be predicted. To estimate long-range correlations of IBIs, we calculated ScalExp derived from DFA as reported previously[17, 18, 30, 31]. ScalExp describes the self-similarity (scaling) of the fluctuations of a biological signal in a time series across a range of sizes of time windows and thus reflects the long-range correlations (memory) of the signal.

Demographic and clinical factors considered to influence outcomes

Factors potentially influencing clinical outcomes included demographic characteristics of study participants (GA, birth weight (BW), BW z-score, sex), prenatal corticosteroid

treatment, relevant comorbidities associated with preterm birth including early onset sepsis (EOS) and late onset sepsis (LOS) (no; suspected EOS: defined as histologically approved chorioamnionitis or C-reactive protein (CRP) > 20 mg/L and antibiotic treatment > 5 days initiated within first 72 h after birth; suspected LOS: CRP > 20 mg/L and antibiotic treatment > 5 days initiated beyond 72 h after birth, proven sepsis (criteria for suspected sepsis plus positive blood cultures)), necrotizing enterocolitis (≥ grade II according to Bell[32]), maximum grade of germinal matrix-intraventricular hemorrhage (IVH, grade I to IV, documented according to Papile[33]), cystic periventricular leukomalacia, phototherapy due to hyperbilirubinemia, level of respiratory support at study (none, nasal continuous airway pressure with and without intermittent increase in flow, endotracheal ventilation), presence of bronchopulmonary dysplasia (defined as supplemental oxygen requirement at 36 weeks PCA to achieve preductal oxygen saturation of 90%), mean body temperature (T_{mean}) during a measurement, weight loss over the first five days of life (%), time to last caffeine dose (hours) at start of measurement, and the infant's behavioural characteristics (positioning (prone, supine); sleep stage (see above; for regression analysis, an awake-score was calculated as follows: Time spent awake (%) * 3 + time spent in active sleep (%) * 2 + time spent in quiet sleep (%)); extent of handling (product of number and time (%) of open incubator doors (doors perturbance-score))).

Clinical Outcomes

Primary outcome was the total duration of respiratory support in the NICU. Respiratory support was defined as a composite of endotracheal ventilation, nasal continuous positive airway pressure and high-flow nasal cannula therapy with and without supplemental oxygen. Respiratory support was managed at the discretion of the attending neonatologist and

withdrawal was based on a set of criteria summarized in standard operating procedures (acceptable work of breathing (no grunting and no deep recessions to achieve stable blood gases), supplemental oxygen < 30% to achieve preductal oxygen saturation of 87-95%, no manual stimulation for apnea > 20 s or bradycardia < 100/min for at least 24 h in non-intubated infants). Secondary outcomes included PCA at cessation of apnea (defined as cessation of breathing lasting for at least 20 seconds or one lasting for less than 20 seconds that is associated with bradycardia and/or cyanosis[3]), PCA at discontinuation of caffeine therapy (all infants received caffeine citrate therapy from day one of life based on clinical standard operating procedure; caffeine therapy was routinely stopped 72 h after last manual stimulation for AOP), and PCA at stopping of routine ECG monitoring in the NICU (72 h after last bradycardia and ≥ 5 days after stopping of caffeine therapy).

Statistical analysis

Aiming at a statistical power of 80% on the 5% significance level, we anticipated to recruit a total of n = 90 infants in order to analyze a minimum of n = 76 preterm infants (expected loss to follow-up 15% due to technical reasons, inherent mortality of extremely preterm infants, and potential withdrawal of parental consent), allowing for linear regression analysis of at least three continuous independent predictor variables of medium effect size ($f2 = 0.15$)[34, 35].

We extracted demographics, clinical factors, and clinical outcomes from medical records. HRV characteristics were calculated several months after extraction of this data, i.e., assessors of clinical outcomes were unaware of HRV characteristics. We performed linear regression analysis to assess associations between HRV characteristics, demographical, and clinical factors with outcomes (see lists of considered predictors and outcomes above). We first used univariable, multilevel modelling to allow for clustering on the individual level given that

repeated measurements over the first five days of life were analysed. This step included exploring associations of all considered predictors with outcomes. A p-value < 0.1 was considered to indicate potential relevance of a predictor. We then built multivariable, multilevel linear regression models for each outcome followed by stepwise backward elimination of predictors that were not significantly associated with the outcome ($p<0.05$ considered statistically significant). We defined a best model depending on the coefficient of determination of the model (R^2) and compared models using likelihood ratio tests. Models were explored for interaction of predictors and model diagnostics included plotting of residuals against fitted values. We log-transformed outcomes that were not normally distributed (duration of respiratory support). Statistical analysis was performed using Stata software (StataCorp. 2009. Stata Statistical Software: Release 11. College Station, TX: StataCorp LP).

Results

Between January 2013 and September 2015, 330 sEMG measurements were performed in 90 infants. Acceptable raw data were obtained from 309/330 (94%) measurements in 76/90 (85%) preterm infants. Twenty-one measurements were not suitable for analysis due to truly irregular beat (repeated supraventricular extrasystole, n=9), displacement of sEMG electrodes (n = 10), and 50 Hz interference (n = 2). Mean (range) GA was 30.2 (24.7 to 34.0) weeks and mean (range) birth weight was 1274 (420 to 1900) g. Fig 1 shows the flow of the study. Table 1 lists the HRV characteristics, demographic and clinical factors, and clinical outcomes of study participants. Results of univariable, multilevel linear regression analyses of all outcomes are summarized in Table 1 of the Online Data Supplement. Detailed results of multivariable, multilevel linear regression analyses are shown in Table 2 and are summarized below. Fig 2 shows the values of SampEn and ScalExp calculated over the first five days of life.

Primary outcome

Multivariable, multilevel modelling established a significant negative association of duration of respiratory support in the NICU with SampEn after adjusting for sex, GA, and birth weight z-score ($R^2 = 0.53$, $p < 0.001$; see Table 2 and Figure 3). Adding SampEn to a model including sex, GA, and birth weight z-score improved the predictive value of the model from 49% to 53% ($p = 0.04$, likelihood ratio test). ScalExp however did not add predictive value to this model.

Secondary outcomes

PCA at discontinuation of caffeine therapy was negatively associated with SampEn after adjusting for sex, GA, birth weight z-score, and EOS ($R^2 = 0.35$, $p < 0.001$; see Table 2 and

Figure 4). Adding SampEn to a model with these demographic and clinical factors resulted in a 6% increase in the predictive value of the model from 29% to 35% ($p = 0.01$, likelihood ratio test). ScalExp did not add predictive value to this model.

After adjusting for relevant demographic and clinical factors, PCA at last apnea and PCA at cessation of ECG monitoring were not associated with HRV characteristics after birth. The best multivariable models showed that PCAs at last apnea and at cessation of ECG monitoring were positively associated with male sex, EOS, and IVH, and negatively associated with GA and birth weight z-score (PCA at last apnea, $R^2 = 0.42$, $p<0.001$; PCA at cessation of ECG monitoring, $R^2 = 0.35$, $p <0.001$; see Table 2).

Discussion

We found that SampEn calculated over the first few days of life significantly improves prediction of subsequent cardiorespiratory stability in preterm infants after adjusting for sex, the degree of prematurity, intrauterine growth, and comorbidities such as sepsis or IVH. Results were robust towards the influence of contextual factors including sleep stage, infant positioning, and extent of external handling. Distributive indices of IBI in the time domain (IBI_{Mean}, IBI_{SDNN}, IBI_{CV}, IBI_{RMSSD}, $IBI_{Skewness}$) and ScalExp derived from DFA did not contribute to the predictive value of regression models after adjusting for relevant demographic and clinical factors.

To our best knowledge, this is the first study to show that low baseline SampEn of IBI calculated within the first five days of life is useful for predicting cardiorespiratory stability of preterm infants over a time course of weeks to months. Since this prediction was made very early in life, it may significantly contribute to planning of treatment and allocation of health care resources.

Comparison with previous literature

Low HRV has long been established as an indicator of poor prognosis in adults after acute events such as myocardial infarction[36, 37]. Also, low SampEn repeatedly has been shown to be an early marker of incipient sepsis in preterm neonates hospitalized in the NICU[16, 38, 39]. In a landmark study, Moorman et al. reported in their large, multi-center, randomized trial (n=3003) that real-time display of HRV characteristics to clinicians in the NICU resulted in reduced sepsis-related mortality in preterm infants[40]. In addition to such studies aiming at detecting serious, subacute events through changes in SampEn prior to clinical deterioration of patients, our findings indicate that baseline SampEn calculated daily within the first five

days of life improves medium-term prediction of cardiorespiratory stability over weeks to months, e.g., duration of respiratory support or PCA at discontinuation of caffeine therapy. This suggests that SampEn confers prognostic value towards autonomic control in the absence of incipient events and after adjusting for known predictors of outcome such as degree of prematurity at birth, birth weight z-score, and significant comorbidities (IVH, sepsis) occurring between early measurement of HRV and outcome assessment several months after birth. Fairchild et al. demonstrated that altered heart rate characteristics within 28 days after birth were associated with abnormal brain imaging in the NICU and neurodevelopmental impairment at 1 year of age in extremely low birth weight infants[41]. These results were adjusted for the degree of prematurity at birth and sepsis during NICU stay. The authors hypothesized that abnormal HRV in the first few weeks of life might reflect inflammatory processes such as sepsis or other systemic inflammatory conditions causing secondary brain injury (and abnormal brain imaging) but could also directly indicate brain injury or neurological dysfunction in preterm infants. They concluded that it remains unclear whether HRV during early life independently influences neurodevelopmental outcomes. Similarly, although we had adjusted our analysis for known and statistically significant predictors of cardiorespiratory outcomes, we cannot rule out the possibility that SampEn at least partially is associated with clinical outcomes as a surrogate measure of other, unmeasured factors.

Strengths and limitations of the study

A strength of this study is the prospective assessment of HRV with systematic data quality control in a population of infants at high risk of autonomic dysregulation in whom cardiorespiratory stability is difficult to predict and of clinical importance. We reached a very high success rate of sEMG measurements (94%) and further considered various factors, such as adjustment for sleep stages and comorbidities potentially influencing both HRV within the

first days of life and study outcomes during prolonged NICU stay of the infants. We performed extensive quality control of sEMG raw data in order to detect and exclude motion artifacts. A limitation of our study is the lack of real-time analysis of HRV at the bedside. We analysed data offline, i.e., the current setup would not yet allow clinicians to incorporate SampEn into their decision making in the NICU. However, as shown previously[15], the data quality control procedure could potentially be fully automated and thus bring this setup an important step closer to real-time analysis. We assessed HRV using sEMG instead of the typically used ECG traces. As an advantage, this allows simultaneous detection of motion artifacts, on the other hand, our results might not be directly comparable to findings from HRV analysis by ECG. Lastly, given that filtering of sEMG raw data predominantly affects periods of active sleep and awake periods[15], we may have missed true changes in HRV occurring predominantly during those periods and we do not know whether this affects the results of our study.

Interpretation and significance

Low SampEn represents a composite of regularity of heart rate and presence of spikes, e.g., transient decelerations, in a time series of IBIs[16]. The pathophysiological mechanism causing changes in SampEn is unknown. In the context of incipient events, changes in SampEn have been attributed to the effect of inflammatory cytokines[39]. Our observation that daily baseline measurements of SampEn early after birth predict medium-term cardiorespiratory stability is novel and suggests that SampEn represents an integrative marker of individual stability of the autonomic nervous system in preterm infants. In other words and as contemplated by Lake et al., high SampEn, representing some level of natural variability, might be a general indicator of health in this population[16]. We reject the interpretation that the predictive value of SampEn on PCA at discontinuation of caffeine therapy is predominantly due to an interaction of

SampEn with EOS given that model estimates for SampEn were very similar in infants with and without EOS and an interaction term was not significant.

The fact that ScalExp from the first five days of life did not add any predictive value to models with known risk factors for cardiorespiratory stability could be due to methodological or physiological factors. From a methodological point of view, time series analysis can be very sensitive to loss of data[26, 42]. We have previously shown that distributive indices of IBI (IBI_{Mean}, IBI_{SDNN}, IBI_{CV}, $IBI_{Skewness}$) and ScalExp are more sensitive to loss of raw data and gaps in the data record than SampEn[15]. This might explain why only SampEn conferred prognostic utility among considered HRV indices in the current study. SampEn has been shown to be remarkably unaffected both analytically and experimentally by loss of up to 40% of raw data[16]. As reported previously, we performed extensive data quality control in order to reduce the effect of motion artifacts and handling of infants on IBI data records[15], periods which are clearly detectable by sEMG measurements in contrast to conventional ECG measurements. The quality control procedure resulted in an overall loss of 34% of raw IBI data and had only minimal effect on SampEn which is consistent with the literature[16]. Further, HRV has been shown to vary with transitions from being awake to being asleep and between different sleep stages[19]. Therefore, we considered adjusting for changes in sleep stage to be of particular importance as neonates do not have a circadian sleeping rhythm but follow a much shorter, internal clock driven, ultradian sleep/wake rhythm, potentially leading to changes in HRV during measurements of several hours. Results from a methodological study on sleep staging in a subgroup of our cohort indicated that the duration of a sleep cycle (reappearance of active sleep) on average was about 22 minutes[20]. In the current study, sleep stage and extent of infant handling during data acquisition did not significantly influence outcomes after adjusting for demographic and clinical factors. Lastly, we had employed an unconventional method of acquiring HRV data using thoracic sEMG. This approach has the disadvantage of

requiring additional chest electrodes compared to deriving IBI from ECG electrodes used in clinical routine. On the other hand, sEMG allowed us to quantify and exclude motion artifacts which are inevitable when studying preterm infants in the NICU[15].

Clinical relevance

We demonstrated feasibility of sEMG measurements for calculating HRV in a clinical NICU setting and found that the method could potentially be fully automated[15]. Our findings suggest that SampEn is a promising physiological marker to monitor health and development of preterm infants over relatively long time periods. In terms of prognostic power, the effect size of SampEn in the overall models was moderate; e.g., SampEn increased the coefficient of determination from 0.49 to 0.53 in the model assessing duration of respiratory support. This suggests that GA at birth, intrauterine growth (birth weight z-score) and sex are still the major determinants of outcome. Further, these values show that the overall model only explains slightly more than half of the amount of variation in the outcome. While this is substantial, there are obviously other factors influencing the outcome that are not included in the model. Prognostically, the first week of life clearly represents a critical period of time and it is encouraging to note that baseline SampEn obtained at such an early stage of life is predictive of medium-term outcomes. This is of particular importance as planning of resource allocation (staffing, equipment) and of discharge home require considerable time. Arguably, repeated measurements of SampEn over weeks to months may add further information to adapt the initial prediction of cardiorespiratory stability derived from early measurements after birth.

Our cohort included infants from 24 weeks GA who were at high baseline risk of complications and prolonged NICU stay due to cardiorespiratory instability. However, most infants were relatively healthy: none had bronchopulmonary dysplasia at 36 weeks PCA which is an important factor influencing duration of respiratory support; also, there was only

one infant with necrotizing enterocolitis and one case of periventricular leukomalacia. Thus, we do not know whether our findings can be extrapolated to infants with such complications. In general, it is conceivable that in the future, real-time display of SampEn (or more complex HRV indices) might assist clinicians not only in prognosis but also resource allocation and decision-making as both respiratory support and caffeine therapy are critical determinants of discharge from NICU.

Conclusion

Baseline SampEn calculated over the first five days of life improves prediction of subsequent cardiorespiratory stability over weeks to months in preterm infants. Characterizing HRV in these infants confers promising prognostic utility independently of subacute events at an extremely early stage of hospitalization. Further studies on the predictive value of SampEn with repeated measurements over prolonged periods of time are warranted.

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Disclosures

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Figure Legends

Legend Figure 1: Abbreviations: CV, coefficient of variation; ECG, electrocardiography; HRV, heart rate variability; IBI, interbeat interval; PCA, postconceptual age; RMSSD, route mean square analysis; SDNN, standard deviation of normal to normal (NN) IBIs; sEMG, surface electromyography; SampEn, sample entropy; ScalExp, scaling exponent alpha derived by detrended fluctuation analysis.

Legend Figure 2: Abbreviations: SampEn, sample entropy; ScalExp, scaling exponent alpha derived by detrended fluctuation analysis. SampEn (a) and ScalExp (b) did not significantly change over the first five days of life.

Legend Figure 3: Duration of respiratory support (h) (log- transformed) over sample entropy of interbeat interval. There was a significant negative association of sample entropy with duration of respiratory support. Each data point reflects average values over the first five days of life (one data point per infant).

Legend Figure 4: Postconceptual age at cessation of caffeine therapy (w) over sample entropy of interbeat interval. There was a significant negative association of sample entropy with postconceptual age at cessation of caffeine therapy. Each data point reflects average values over the first five days of life (one data point per infant).

Tables

Table 1. HRV characteristics, demographic and clinical factors, and clinical outcomes of study participants (n=76)

HRV characteristics	
IBI_{Mean}, s	0.41 (0.03)
IBI_{SDNN}, s	0.03 (0.01)
IBI_{CV}, %	6.3 (2.1)
IBI_{RMSSD}	0.02 (0.01)
$IBI_{Skewness}$	4.76 (4.39)
SampEn	0.37 (0.22)
ScalExp	1.11 (0.19)
Demographic factors	
Male sex	44 (57.9)
Gestational age, w	30.2 (2.2)
Birth weight, g	1274 (344)
Birth weight z-score	-0.90 (1.12)
Clinical factors	
Early onset sepsis	
none	50 (65.8)
suspected	22 (28.9)
proven	4 (5.3)
Late onset sepsis	
none	70 (92.1)
proven	6 (7.9)
Necrotizing enterocolitis	
none	75 (98.7)
stage III	1 (1.3)
Intraventricular hemorrhage	7 (9.2)

Cystic periventricular leukomalacia	1 (1.3)
Bronchopulmonary dysplasia	0 (0)
Prenatal corticosteroids	
none	8 (10.5)
incomplete	5 (6.6)
complete	63 (82.9)
Clinical outcomes	
Duration of respiratory support, h	422.2 (31.1–883.1) [#]
Postconceptional age at last apnea, w	36.4 (2.1)
Postconceptional age at discontinuation of caffeine therapy, w	35.0 (1.5)
Postconceptional age at cessation of ECG monitoring, w	36.1 (2.2)

Legend Table 1: Continuous variables are displayed as mean (standard deviation) unless specified otherwise; categorical variables are displayed as count (%); [#]median (interquartile range). Abbreviations: ECG, electrocardiography; IBI_{Mean}, mean of interbeat interval (IBI); IBI_{SDNN}, standard deviation of IBI; IBI_{CV}, coefficient of variation of IBI; $IBI_{Skewness}$, skewness of IBI; SampEn, sample entropy, ScalExp, scaling exponent alpha.

Table 2. Multivariable, multilevel linear regression models for all outcomes

Variable	Coefficient ß	95% CI	P-value	R^2
Outcome: Duration of respiratory support[*]				0.53
Sample entropy	-0.808	-1.589 to -0.028	0.042	
Male sex	0.681	0.400 to 0.962	<0.001	
Gestational age, w	-0.497	-0.567 to -0.427	<0.001	
Birth weight z-score	-1.315	-0.261 to -0.010	0.035	
Outcome: Postconceptional age at last apnea				0.42
Male sex	0.476	0.066 to 0.889	0.024	
Gestational age, w	-0.411	-0.525 to 0.298	<0.001	
Birth weight z-score	-0.815	-1.021 to -0.608	<0.001	
Early onset sepsis suspected proven	 1.002 2.431	 0.031 to 1.008 1.363 to 3.499	 0.042 <0.001	
Intraventricular hemorrhage	0.352	0.016 to 0.688	0.040	
Postconceptional age at discontinuation of caffeine therapy				0.35
Sample entropy	-0.805	-1.524 to -0.086	0.028	
Male sex	0.809	0.497 to 1.114	<0.001	
Gestational age, w	-0.240	-0.340 to -0.149	<0.001	
Birth weight z-score	-0.003	-0.037 to 0.849	0.974	
Early onset sepsis suspected proven	 0.443 1.327	 0.037 to 0.849 0.621 to 2.033	 0.033 <0.001	
Postconceptional age at cessation of ECG monitoring				0.35
Male sex	0.818	0.380 to 1.255	<0.001	
Gestational age, w	-0.229	-0.350 to -1.075	<0.001	
Birth weight z-score	-0.799	-1.018 to -0.586	<0.001	
Early onset sepsis suspected proven	 0.131 2.937	 -0.444 to 0.706 1.768 to 4.105	 0.655 <0.001	
Intraventricular hemorrhage[#]	0.752	0.423 to 1.028	0.045	

Legend Table 2: Final models of multivariable, multilevel linear regression analyses. Abbreviations: ECG, electrocardiography; R^2, coefficient of determination of multivariable, multilevel model; [*]data log-transformed to achieve normal distribution; [#]binary coded (grades 0 to 1 vs. grades 2 to 4; based on Papile classification, see text).

Figures

Figure 1: Study flow sheet

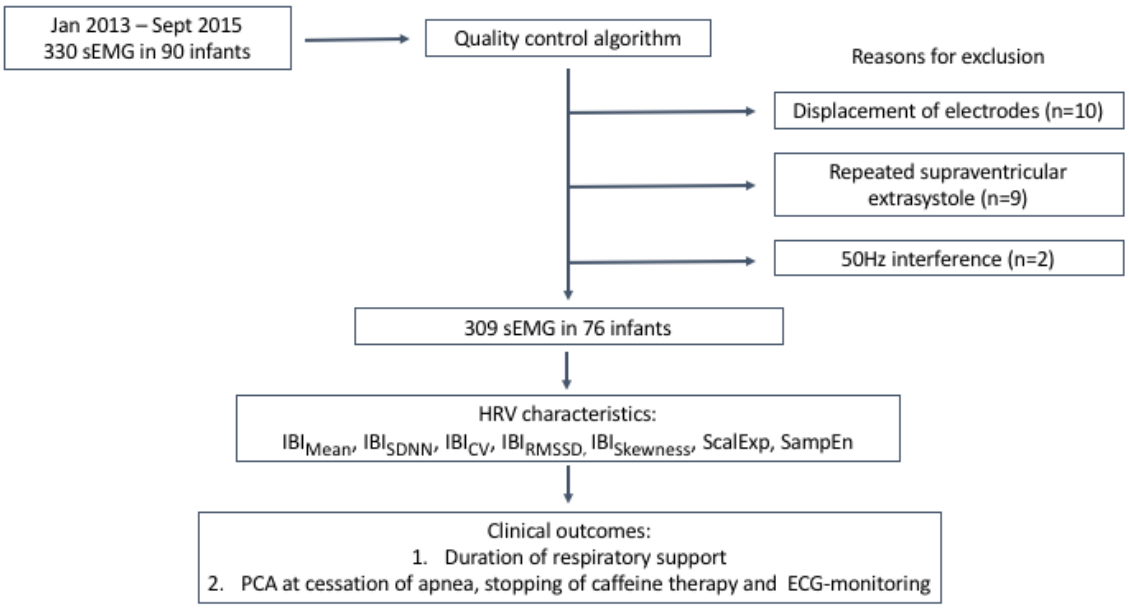
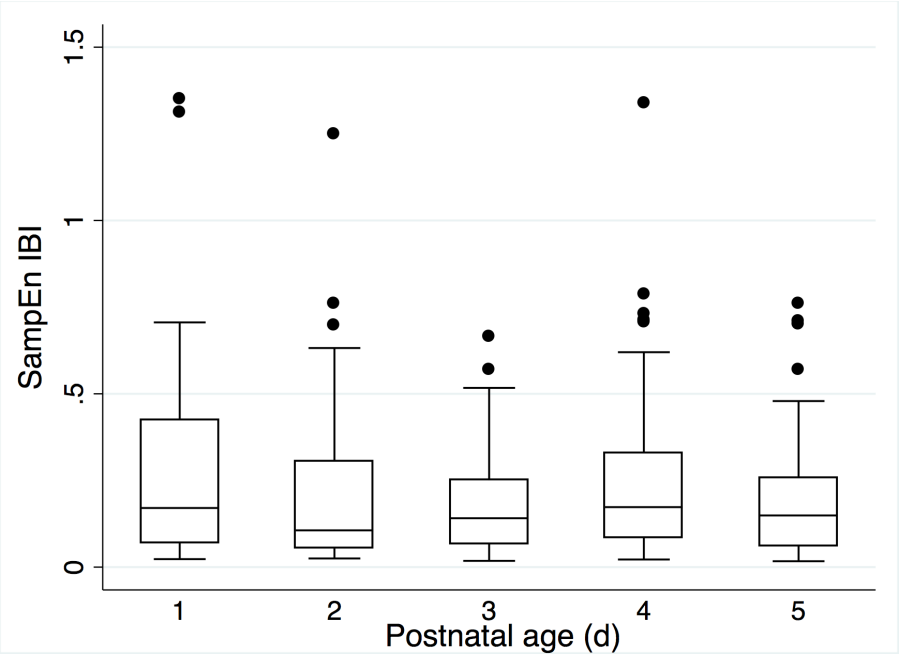


Figure 2: Sample entropy and scaling exponent over the first five days of life

a



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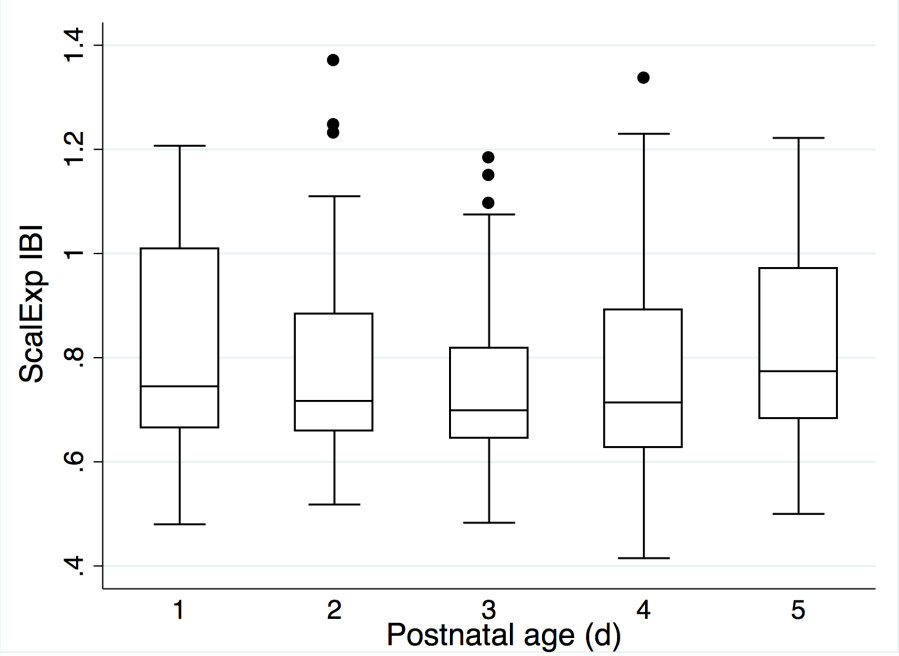


Figure 3: Predictive effect of sample entropy on duration of respiratory support

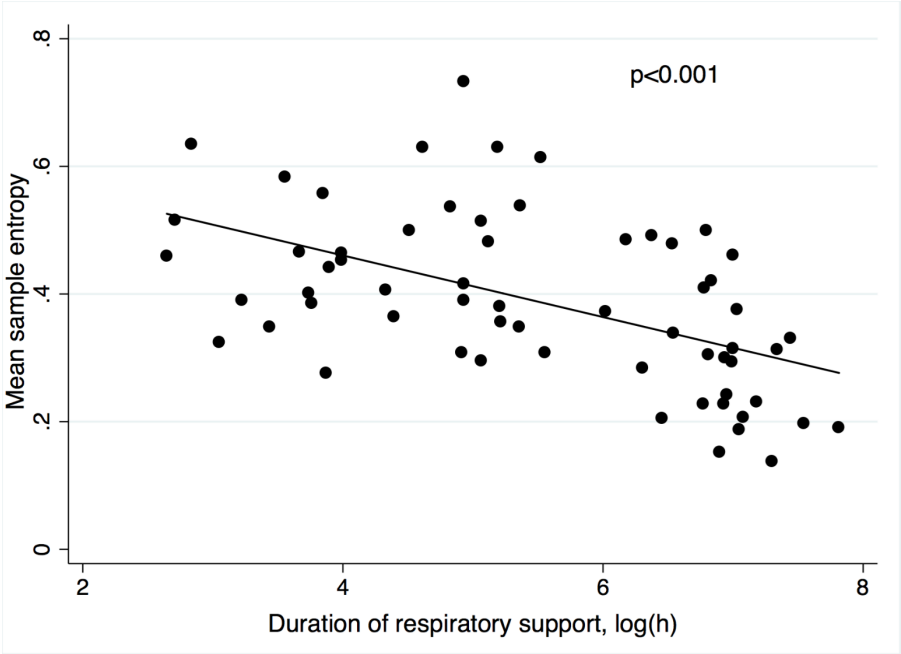
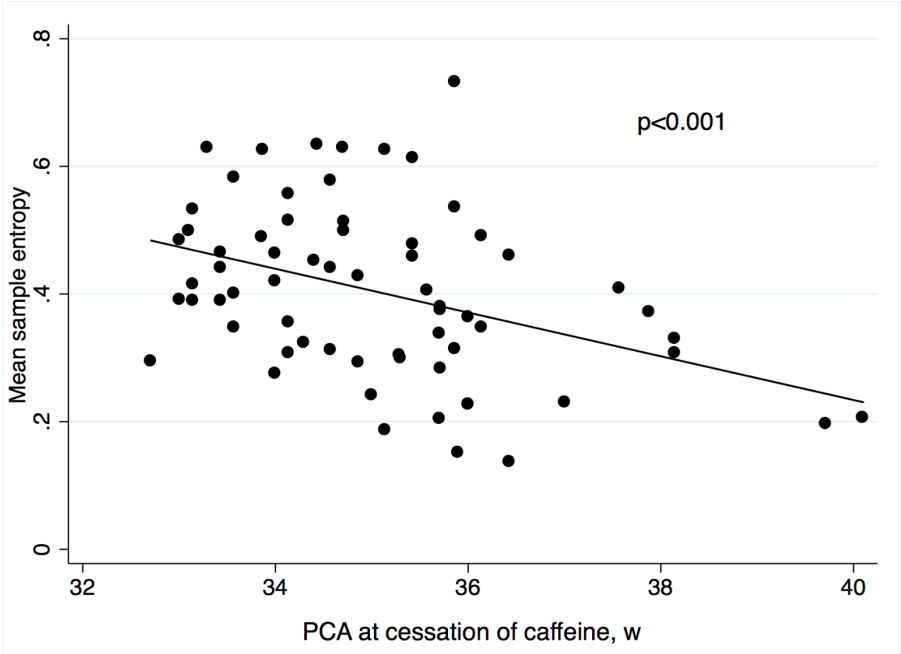


Figure 4: Predictive effect of sample entropy on cessation of caffeine therapy



SUPPLEMENTAL MATERIAL

Online Data Supplement (ODS)

Heart rate variability after birth predicts subsequent cardiorespiratory stability in preterm infants

Table 1. ODS: Results of univariable, multilevel regression analyses

Variable	Coefficient ß	95% CI	P-value	R^2
Outcome: Duration of respiratory support[*]				
IBI_{Mean}, s	-14.127	-20.377 to -7.877	<0.001	0.08
$IBI_{Skewness}$, s	0.121	0.079 to 0.163	<0.001	0.12
SampEn	-2.850	-3.832 to -1.869	<0.001	0.12
ScalExp	1.359	0.199 to 2.519	0.022	0.02
Male sex	0.564	0.167 to 0.961	0.006	0.03
Gestational age, w	-0.490	-0.553 to -0.426	<0.001	0.34
Birth weight, g	-0.002	-0.003 to -0.002	<0.001	0.34
Birth weight z-score	-0.094	-0.273 to 0.085	<0.001	0.07
Early onset sepsis (proven)	3.370	1.866 to 4.873	<0.001	0.10
Late onset sepsis (proven)	3.253	1.812 to 4.694	<0.001	0.06
Outcome: Postconceptional age at last apnea				
IBI_{Mean}, s	-8.738	-17.121 to -0.354	0.041	0.02
$IBI_{Skewness}$, s	0.097	0.040 to 0.154	0.001	0.04
SampEn	-1.612	-2.966 to -0.284	0.020	0.02
Male sex	.0796	0.282 to 1.310	0.003	0.04
Gestational age, w	-0.318	-0.431 to -0.204	<0.001	0.11
Birth weight, g	-0.003	-0.004 to -0.002	<0.001	0.21
Birth weight z-score	-0.554	-0.783 to -0.326	<0.001	0.08
Early onset sepsis (proven)	2.331	1.245 to 3.416	<0.001	0.10
Intraventricular hemorrhage	1.651	0.494 to 2.809	0.005	0.03
Outcome: Postconceptional age at discontinuation of caffeine therapy				
IBI_{Mean}, s	-7.051	-13.180 to -0.922	0.024	0.02
$IBI_{Skewness}$, s	0.067	0.026 to ,.108	0.002	0.04
SampEn	-2.367	-3.334 to -1.398	<0.001	0.09
ScalExp	1.111	0.042 to 2.179	0.042	0.02
Male sex	0.835	0.47 to 1.200	<0.001	0.08
Gestational age, w	-0.275	-0.359 to -0.191	<0.001	0.14
Birth weight, g	-0.001	-0.002 to -0.001	<0.001	0.11
Birth weight z-score	-0.080	-0.258 to 0.099	0.380	<0.001
Early onset sepsis (proven)	1.773	1.095 to 2.452	<0.001	0.14

Outcome: Postconceptional age at cessation of ECG monitoring				
IBI_{CV}, %	10.401	-0.669 to 21.483	0.065	0.02
$IBI_{Skewness}$, s	0.055	-0.002 to 0.113	0.061	0.01
SampEn	-1.577	-2.915 to -0.240	0.021	0.02
Male sex	1.055	0.548 to 1.562	<0.001	0.06
Gestational age, w	-0.136	-0.254 to 0.018	0.024	0.02
Birth weight, g	-0.002	-0.003 to -0.001	<0.001	0.10
Birth weight z-score	-0.619	-0.843 to -0.396	<0.001	0.10
Early onset sepsis (proven)	1.946	0.853 to 3.039	0.001	0.07
Intraventricular hemorrhage[#]	1.568	0.379 to 2.757	0.010	0.02

Legend Table 2. ODS: The table shows predictors significantly associated with outcomes in univariable regression analysis. Abbreviations: ECG, electrocardiography; IBI, interbeat interval; IBI_{Mean}, mean of IBI; $IBI_{Skewness}$, skewness of IBI; $IBI_{CV,}$ coefficient of variation of IBI; R^2, coefficient of determination of univariable, multilevel model; SampEn, sample entropy; ScalExp, scaling exponent alpha derived from detrended fluctuation analysis; [*]data log-transformed to achieve normal distribution; [#]binary coded (grades 0 to 1 vs. grades 2 to 4; based on Papile classification, see text of main manuscript).

5.5. Sighs in preterm infants

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"The unprepared mind cannot see the outstretched hand of opportunity."

- Alexander Fleming -

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State of the paper	Published in *Physiological Reports* (new online journal of *The Physiological Society* and *The American Physiological Society*), November 2015 *The Editor's Choice feature of the month* (December 2015).
Contribution of KJ	The work of KJ on this project included quality control of existing lung function measurements, selection of suitable measurements for sigh analysis, and sigh analysis *per se* with a custom-made code using the statistical software R. The code was written by KJ in collaboration with other team members. The outcomes were statistically analyzed and the final manuscript was written by KJ.
Synopsis	Large tidal breaths at least double the average tidal volumes, also known as sighs, have been associated with various physiological and pathophysiological mechanisms. The effect of sighs depends on subject characteristics such as age: Sighs can lead to hypoventilation and apnea in infants but might induce higher minute ventilation in adults. As sighs are associated with apneas in infancy, we were interested whether there is a difference in the reaction upon a sigh between infants born premature and term-born healthy controls. A better understanding of the differences in both control of breathing and lung function could be useful as potential marker for respiratory sequelae in preterm infants. We could show that sigh-induced changes in breathing pattern differ between stable preterm infants with and without bronchopulmonary dysplasia and term healthy controls when measured during quiet sleep at equivalent postconceptional age shortly after term. Whether or not sigh characteristics are useful as predictive marker of later respiratory morbidity should ideally be investigated in future longitudinal studies.

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ORIGINAL RESEARCH

Sigh-induced changes of breathing pattern in preterm infants

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Keywords
Bronchopulmonary dysplasia, control of breathing, preterm infant, sigh, variability.

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Abstract

Sighs are thought to play an important role in control of breathing. It is unclear how sighs are triggered, and whether preterm birth and lung disease influence breathing pattern prior to and after a sigh in infants. To assess whether frequency, morphology, size, and short-term variability in tidal volume (V_T) before, during, and after a sigh are influenced by gestational age at birth and lung disease (bronchopulmonary dysplasia, BPD) in former preterm infants and healthy term controls measured at equivalent postconceptional age (PCA). We performed tidal breathing measurements in 143 infants during quiet natural sleep at a mean (SD) PCA of 44.8 (1.3) weeks. A total of 233 sighs were analyzed using multilevel, multivariable regression. Sigh frequency in preterm infants increased with the degree of prematurity and severity of BPD, but was not different from that of term controls when normalized to respiratory rate. After a sigh, V_T decreased remarkably in all infants (paired *t*-test: $P < 0.001$). There was no major effect of prematurity or BPD on various indices of sigh morphology and changes in V_T prior to or after a sigh. Short-term variability in V_T modestly increased with maturity at birth and infants with BPD showed an earlier return to baseline variability in V_T following a sigh. In early infancy, sigh-induced changes in breathing pattern are moderately influenced by prematurity and BPD in preterm infants. The major determinants of sigh-related breathing pattern in these infants remain to be investigated, ideally using a longitudinal study design.

Introduction

Sighs, that is, large tidal breaths at least double the average tidal volume (V_T) of the preceding breaths, have been associated with various physiological and pathophysiological mechanisms. Sighs play an important role in the plastoelastic stretching of lung tissue and breathing muscles, which may result in improvement of lung compliance, reduction in airway resistance and recruitment of lung volume (Davis and Moscato 1994). Additionally, the effect of sighs depends on subject characteristics such as age: Sighs can lead to hypoventilation and apnea in infants, but might induce higher minute ventilation in adults. It is, however, unknown whether maturation of respiratory control systems or biomechanical lung development is the primary cause of these results (Qureshi et al. 2009).

Baldwin et al. described decreased short-range breath-to-breath memory prior to and increased variability in V_T, and minute ventilation immediately following a sigh in healthy term infants, measured 5 weeks after their expected date of delivery during quiet sleep (Baldwin

et al. 2004). These findings were interpreted as additional evidence that the ability to sigh may play an important role in control of breathing. The authors hypothesized that the reaction to a sigh might differ between term and preterm infants due to differences in both control of breathing and lung function, and that it could be a potential marker for respiratory sequelae in preterm infants. The role of sighs in preterm infants is poorly understood, particularly in those who have bronchopulmonary dysplasia (BPD) (Qureshi et al. 2009); BPD is a chronic, developmental lung disease characterized by altered breathing pattern, poor lung function, and impaired lung growth, affecting at least 12,000 preterm infants in the United States each year (Jobe and Bancalari 2001; Baldwin et al. 2006; Hulskamp et al. 2009; Latzin et al. 2009b; Van Marter 2009).

We thus hypothesized that the frequency, morphology, and short-term variability in tidal breathing differ between healthy term infants and preterm infants with BPD when measured at the same corrected age. We further hypothesized that differences in those outcomes are mainly influenced by the degree of prematurity at birth and severity of BPD. Thus, we aimed to assess whether frequency, morphology, and short-term variability of V_T before, during, and after a sigh are influenced by gestational age (GA) at birth, and presence and severity of BPD in former preterm and healthy term control infants.

Methods

Study design

This is a retrospective analysis of data obtained from a prospective birth cohort study conducted in Bern, Switzerland (BILD cohort study) (Latzin et al. 2009a). Infants had participated in tidal breathing measurements according to European Respiratory Society (ERS) standards (Bates et al. 2000) from September 2002 to December 2009. The study was approved by the Bernese Ethics Committee and written informed consent was obtained for each subject prior to the measurement.

Patients

We studied former preterm infants ($n = 57$) born at <37 weeks GA and healthy term control infants ($n = 86$). Preterm infants were assessed for presence of BPD based on their duration of supplemental oxygen requirement at 36 weeks GA. Mild, moderate, and severe BPD was defined based on the National Institutes of Child Health Consensus definition (Jobe and Bancalari 2001). Figure 1 shows a flow chart of patients through the phases of the study.

Measurements

Detailed measurement set up has been published previously (Fuchs et al. 2012). Briefly, measurements were conducted at a mean (range) postconceptional age (PCA) of 44.8 (41.7–51.9) weeks in infants without any respiratory infections. Tidal breathing measurements lasted up to 10 min and were conducted following international guidelines for lung function testing in infancy (Bates et al. 2000). Measurements were conducted with the infant in supine position, during behaviorally defined, quiet unsedated sleep (Prechtl 1974). A face mask was placed on the infant's mouth and nose during regular tidal breathing (size 1, Homedica, Cham, Switzerland). The mask was connected to an infrared CO_2 analyzer and an ultrasonic flowmeter (Spiroson Exhalyzer D, EcoMedics AG, Duernten, Switzerland) as described previously (Latzin et al. 2009b). A bypass flow of 14 L/min was applied. End-tidal CO_2 was monitored for the entire measurement period and did not increase. Signals were 12-bit analog-to-digital converted and sampled at a frequency of 200 Hz using a commercially available data acquisition and analysis package (WBreath version 3.7.6.0, Firmware v3.06, NDD Medizintechnik AG, Zürich, Switzerland).

Data processing

After BTPS correction and correction for flow offset, we visually examined all tidal breathing measurements for sighs. We aimed to analyze a minimum of 50 baseline breaths before and after the sigh, respectively. Breath traces were processed in the statistical software R (R Core Team [2013]. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.), and sighs were automatically identified by software script according to the following criteria: (1) Sigh $V_T > 2$ standard deviations (SD) of average V_T of preceding breaths (Thach and Taeusch 1976; Davis and Moscato 1994); (2) Minimal distance of 10 breaths to preceding or following sigh; (3) Availability of at least 5 breaths before and 10 breaths after each sigh. If those criteria were not fulfilled, measurements were excluded from analysis.

Data analysis

The following parameters were then analyzed for all measurements containing at least one sigh that met inclusion criteria:

Sigh frequency

All measurements lasting at least 10 min were used for the analysis of sigh frequency. Respiratory rate (mean

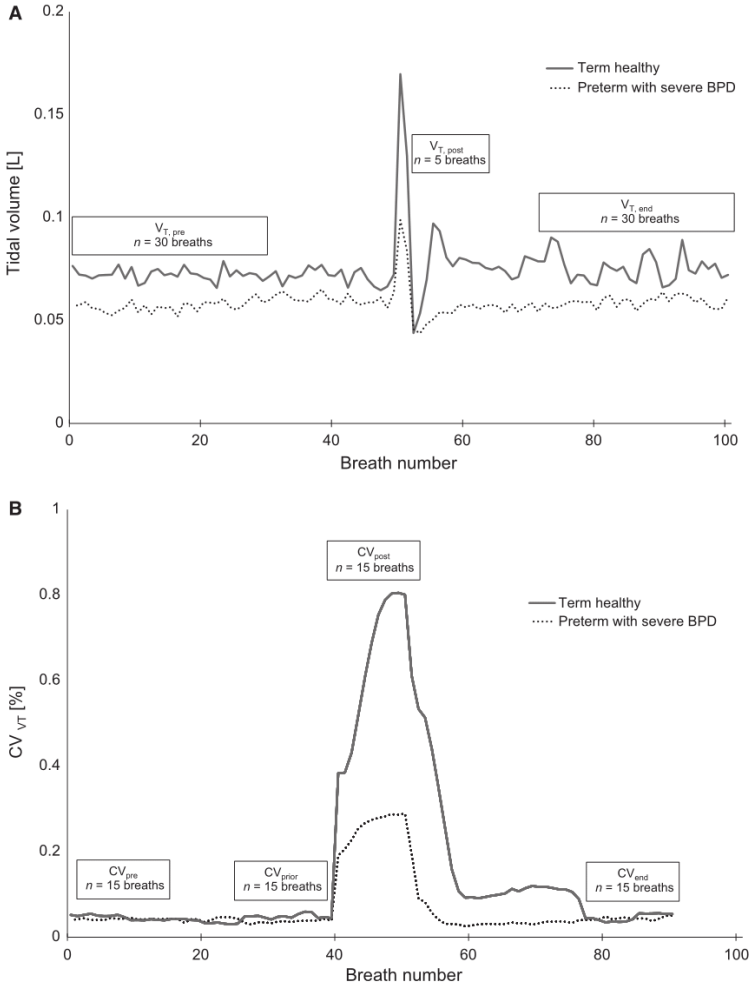

Figure 1. Representative tidal breathing traces of V_T (A) and coefficient of variation in V_T (CV_{VT}) (B) for an infant with severe bronchopulmonary dysplasia (BPD) and a term healthy control infant. $V_{T,pre}$, mean tidal volume over the first 30 breaths of the measurement; $V_{T,post}$, mean tidal volume over the first five breaths after the sigh; $V_{T,end}$, mean tidal volume over the last 30 breaths of the measurement; CV_{pre}, CV_{post}, and CV_{end}, coefficient of variation in $V_{T,pre}$, $V_{T,post}$, and $V_{T,end}$, respectively; CV_{prior}, coefficient ofvariation in V_T over 15 breaths preceding a sigh.

over 10 min), number of sighs within 10 min, and extrapolated value of sighs expected in 1000 breaths were defined.

Sigh morphology

Maximal inspiration ($V_{I,max}$) and maximal expiration ($V_{E,max}$) during a sigh (absolute values and values corrected for V_T at baseline), and the ratio of $V_{E,max}/V_{I,max}$ were calculated to describe the morphology of a sigh.

Changes in V_T

The first 30 breaths of the tidal breathing measurement were used as baseline before the sigh ($V_{T,pre}$), the last 30 breaths as baseline after the sigh ($V_{T,end}$). The five breaths just after the sigh were defined as immediate postsigh period ($V_{T,post}$). We then calculated the difference in mean $V_{T,pre} - V_{T,post}$ as a measure of changes in V_T upon a sigh ($V_{T,diff}$). We further calculated the number of breaths that deviated from $V_{T,pre}$ in excess of 2 SD within the periods 15 breaths before and 15 breaths after a sigh,

and discriminated between the number of high ($V_{T,high}$) and low ($V_{T,low}$) values.

Short-term variability in V_T

Short-term variability in V_T was determined using a moving window algorithm, in which the coefficient of variation (CV) of V_T (CV_{VT}) was obtained from windows of 11 breaths. The window was shifted by one breath four times in four predefined regions of the tidal breathing measurement (baseline at the beginning, just prior to the sigh, immediately after a sigh, baseline at the end of the measurement). This approach resulted in four consecutive windows per region. Baseline CV_{VT} was calculated at the beginning (CV_{pre}) and at the end (CV_{end}) of a measurement. Also, CV_{VT} was calculated just prior to the sigh (CV_{prior}) and immediately after a sigh (CV_{post}). CV_{diff} ($CV_{post} - CV_{pre}$) describes the change from baseline to postsigh period. We further calculated changes in CV_{VT} (CV_{post_slope}) by subtracting the value of the second moving window just after the sigh from the first one just after the sigh, normalized for individual CV_{pre}. Sample tidal breathing traces including a graphical overview on outcomes related to changes in V_T and variability in V_T are displayed in Figure 1A and B, respectively.

Statistical analysis

Our main outcome parameters, as described above, were number of sighs (sigh frequency); $V_{I,max}$ and $V_{E,max}$ (sigh morphology); size of tidal volume before and after a sigh (changes in V_T), and coefficient of variation in V_T (Short-term variability in V_T).

We performed linear regression analyses to assess associations between sigh characteristics (frequency, morphology, changes in V_T, variability in V_T) and potential predicting factors. Considered predictors included degree of prematurity (GA), BPD (expressed as number of days with supplemental oxygen, i.e., as a continuous variable), body size at test (weight), intrauterine growth restriction (birth weight *z*-score), gender, and maternal smoking during pregnancy. We used multilevel modeling to allow clustering on the individual level given that some measurements incorporated more than one sigh. Model building included exploring associations of all considered predictors with sigh characteristics in univariable regression analysis where $P < 0.1$ was considered to indicate potential relevance of a predictor. We then built multilevel, multivariable linear regression models for each outcome and did stepwise backward elimination of predictors that were not significantly associated with the outcome ($P < 0.05$ considered statistically significant). Lastly, we defined a best model depending on the coefficient of determination of the model (R^2). Statistical analysis was done using Stata software (StataCorp. 2009. Stata Statistical Software: Release 11. College Station, TX: StataCorp LP).

Results

We screened data from 399 infants. Out of these, 143 (35.8%) showed at least one sigh during their tidal breathing measurement and were included in this study. A total of 233 sighs met inclusion criteria and were used for further analysis (Fig. 2). Demographic data and tidal breathing outcomes are summarized in Tables 1 and 2, respectively. Multilevel models are detailed in Table 3.

Sigh frequency

Respiratory rate was positively associated with BPD ($P = 0.008$) and negatively associated with GA ($P = 0.001$), body weight ($P = 0.076$), and birth weight *z*-score ($P = 0.004$). Eighty-six out of 223 term infants (39%), 35 out of 87 preterm infants without BPD (40%), and 22 out of 89 preterm infants with BPD (25%) had at least one sigh during their tidal breathing measurement. The number of sighs over a 10 min measurement period was associated with BPD ($P = 0.059$), but not GA ($P = 0.12$) (Table 3). On normalizing sigh frequency to sighs per 1000 breaths, no significant associations between sigh frequency and GA, BPD or any other predictor variable were found.

Sigh morphology

In univariable analyses, maximal inspiratory volume of the sigh ($V_{I,max}$) was not associated with any predictor when corrected for baseline V_T. In contrast, maximal expiratory volume of the sigh ($V_{E,max}$) was positively associated with BPD ($P = 0.038$) and negatively with maternal smoking ($P = 0.006$) after correcting for V_T at baseline. Additionally, the ratio of $V_{E,max}/V_{I,max}$ was negatively associated with GA ($P < 0.001$) and maternal smoking ($P = 0.078$), and positively associated with BPD ($P < 0.001$). In multivariable analysis, $V_{E,max}/V_{I,max}$ remained weakly associated with BPD after adjusting for maternal smoking ($R^2 = 0.09$) (Table 3).

Changes in V_T

In univariable analyses, both $V_{T,pre}$ and $V_{T,end}$ were positively associated with GA, body weight, and male sex, but negatively associated with BPD. In contrast, $V_{T,post}$ was neither associated with GA nor with BPD. The only significant predictors of $V_{T,post}$ were body weight

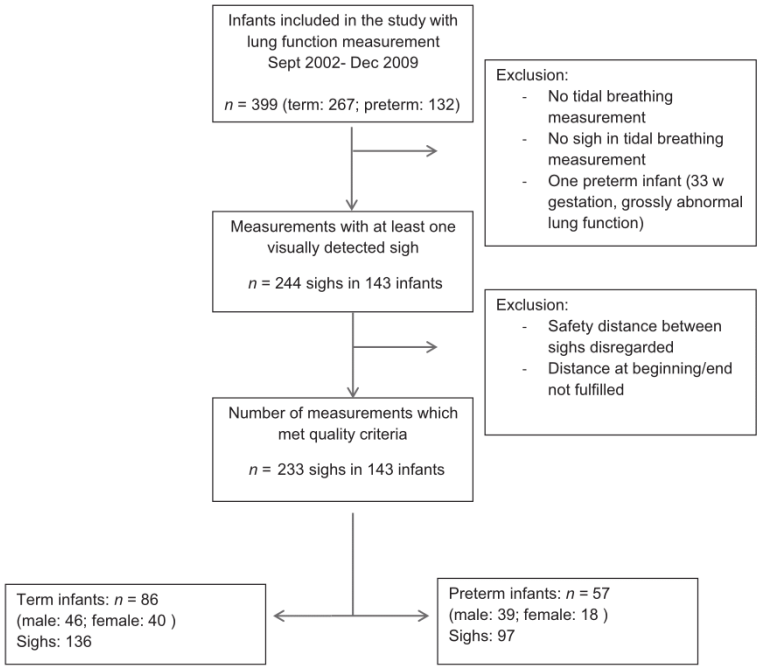

Figure 2. Flow of patients through the phases of the study.

Table 1. Demographic characteristics of study participants

	Term healthy (n = 86)	Preterm without BPD (n = 35)	Preterm with BPD (n = 22)
Sex, male (% male)	46 (53%)	23 (66%)	16 (73%)
Gestational age (w)	39.4 (37.0, 41.9)	30.6 (24.1, 35.6)	27.4 (24.3, 31.6)
Birth weight (kg)	3.3 (2.5, 4.9)	1.5 (0.5, 3.0)	1.0 (0.4, 2.6)
Birth weight z-score	−0.12 (−2.07, 2.75)	−0.60 (−3.58, 2.29)	−0.48 (−4.03, 3.46)
Study weight (kg)	4.3 (3.2, 6.4)	4.3 (2.7, 6.8)	3.4 (2.6, 5.6)
Postconceptional age (w)	44.3 (41.7, 48.0)	44.9 (43.4, 47.0)	44.7 (43.3, 51.9)
Infants with sighs/measured infants (%)	86/223 (39)	35/94 (37)	22/82 (27)
Number of observed sighs	136	52	45

Values are described as mean (range). BPD, bronchopulmonary dysplasia.

($P < 0.001$) and sex ($P = 0.007$). $V_{T,diff}$ was positively associated with GA ($P = 0.023$), body weight ($P = 0.022$), and birth weight z-score ($P = 0.062$), and negatively associated with BPD ($P = 0.024$). $V_{T,post}$ was smaller than $V_{T,pre}$ (paired t-test, $P < 0.001$). $V_{T,high}$ after the sigh was negatively associated with BPD ($P = 0.028$). There were no associations between major deviation from $V_{T,pre}$ ($V_{T,high}$, $V_{T,low}$) and GA or BPD.

Multivariable modeling established a positive association of both $V_{T,pre}$ ($R^2 = 0.40$) and $V_{T,end}$ ($R^2 = 0.40$) with GA, but not BPD after adjusting for body weight and sex (Table 3). We found no multivariable model for $V_{T,diff}$.

Short-term variability in V_T

In univariable analyses, CV_{pre} showed a weak positive association with GA ($P = 0.045$), but not BPD and a negative association with male sex ($P < 0.001$). CV_{prior} was not associated with any tested predictor. CV_{post} was positively associated with GA ($P < 0.001$) and negatively associated with BPD ($P < 0.001$), male sex ($P = 0.043$), and

Table 2. Tidal breathing outcomes

	Term healthy $n = 86$	Preterm without BPD $n = 35$	Preterm with BPD $n = 22$
Respiratory rate	42.60 (7.84)	48.32 (12.45)	49.92 (13.01)
Sighs/10 min	1.52 (0.65)	1.41 (0.67)	2.00 (1.08)
Sighs/1000 breaths	3.67 (1.69)	3.16 (1.77)	4.28 (2.40)
$V_{I,max}/V_{T,pre}$	3.07 (0.99)	2.92 (0.65)	2.76 (0.63)
$V_{E,max}/V_{T,pre}$	2.32 (0.68)	2.37 (0.62)	2.50 (0.45)
$V_{E,max}/V_{I,max}$	0.79 (0.18)	0.84 (0.21)	0.93 (0.16)
$V_{T,pre}$ (mL)	32.95 (5.67)	32.17 (7.60)	27.31 (4.96)
$V_{T,post}$ (mL)	23.18 (7.74)	23.04 (6.58)	20.53 (6.25)
$V_{T,diff}$ (mL)	9.76 (7.28)	9.01 (6.92)	6.78 (4.93)
VT_{high} after sigh (breaths)	4.69 (4.84)	5.02 (5.36)	3.39 (4.55)
$V_{T,end}$ (mL)	32.71 (5.61)	30.71 (7.37)	26.48 (4.96)
CV_{pre}	0.08 (0.06)	0.06 (0.03)	0.07 (0.06)
CV_{prior}	0.09 (0.10)	0.08 (0.07)	0.08 (0.06)
CV_{post}	0.37 (0.16)	0.32 (0.12)	0.27 (0.15)
CV_{diff}	0.30 (0.16)	0.26 (0.12)	0.20 (0.16)
CV_{post_slope}	0.96 (0.92)	1.58 (1.80)	2.58 (2.26)
CV_{end}	0.10 (0.07)	0.09 (0.08)	0.07 (0.03)

Values are given as mean (SD).

maternal smoking in pregnancy ($P = 0.085$). CV_{end} showed a positive association with GA ($P = 0.041$) and birth weight *z*-score ($P < 0.001$), and a negative association with BPD ($P = 0.043$) and male sex ($P = 0.003$). CV_{diff} as a marker of changes in CV of V_T from baseline to immediately after a sigh was positively associated with GA ($P = 0.010$), and negatively associated with BPD ($P = 0.015$) and maternal smoking ($P = 0.022$). CV_{post_slope} as a marker of how fast variability in V_T after a sigh returns down to baseline, was negatively associated with GA ($P < 0.001$) and positively associated with BPD ($P < 0.001$; $R^2 = 0.15$).

Multivariable analysis (Table 3) established a positive association of CV_{post} with GA, but not BPD after adjusting for sex and maternal smoking ($R^2 = 0.16$). Similarly, CV_{end} was significantly associated with GA, but not BPD after adjusting for sex and birth weight *z*-score ($R^2 = 0.16$). CV_{diff} was associated with GA after adjusting for maternal smoking in pregnancy ($R^2 = 0.07$). Figure 3 shows CV_{pre}, CV_{prior}, CV_{post}, and CV_{end} for the subgroups of healthy term infants, preterm infants without BPD, and preterm infants with BPD.

Discussion

We found that sigh-induced changes in breathing pattern differ modestly between stable preterm infants with and without BPD and term healthy controls when measured during quiet sleep at equivalent PCA shortly after term. Sigh frequency in preterm infants increased with the degree of prematurity at birth and severity of BPD. Sigh frequency of preterm infants was not different from that of term healthy controls when normalized to respiratory rate. There was no major effect of prematurity or BPD on various indices of sigh morphology indicating that former preterm infants were able to recruit similar amounts of V_T during a sigh despite their known restrictive lung disease (Choukroun et al. 2013; Schmalisch et al. 2013). Changes in V_T immediately prior to or after a sigh did not substantially differ between preterm and term infants. Although infants were studied at equivalent PCA, short-term variability in V_T modestly increased with maturity at birth and infants with BPD showed an earlier return to baseline variability in V_T following a sigh.

Comparison with previous literature

To the best of our knowledge, this is the first study examining sigh-related breathing pattern during quiet sleep in former preterm and term healthy control infants measured at equivalent PCA shortly after term corrected age. Qureshi et al. (2009) compared sigh-related tidal breathing of 10 term and 10 preterm infants (mean PCA of 33–34 weeks) within the first 3 weeks of life to that of 10 healthy adults. They found a higher frequency of sighs in infants compared to adults but no significant difference in sigh frequency, sigh morphology, and changes in V_T between preterm and term infants. Sigh frequency normalized to respiratory rate, variability in V_T, and the influence of concomitant factors on outcomes was not

Table 3. Results of multilevel, multivariable regression analyses on sigh frequency, sigh morphology, tidal volume and variability in tidal volume

	Multivariable models			
	Coefficient	CI 95%	*P*-value	R^2
Respiratory rate				
Gestational age (w)	−0.5433	−0.8995, −0.18722	0.003	0.15
Birth weight *z*-score	−2.4207	−4.2435, −0.5980	0.010	
Sigh frequency				
Sighs/10 min				
BPD	0.0047	−0.0002, 0.0096	0.059	0.03
Sigh morphology				
$V_{E,max}/V_{T,pre}$				
BPD	0.0036	0.0004, 0.0068	0.028	0.06
Maternal smoking	−0.4936	−0.8383, −0.1489	0.005	
$V_{E,max}/V_{I,max}$				
BPD	0.0016	0.0008, 0.0023	<0.001	0.09
Maternal smoking	−0.0791	−0.1606, 0.0023	0.057	
Changes in V_T				
$V_{T,pre}$				
Gestational age (w)	0.0003	0.0001, 0.0004	0.001	0.40
Weight (kg)	0.0048	0.0037, 0.0061	<0.001	
Sex, male	0.0016	−0.0001, 0.0034	0.058	
$V_{T,post}$				
Weight (kg)	0.0034	0.0018, 0.0050	<0.001	0.12
Sex, male	0.0023	0.0002, 0 .0045	0.036	
$V_{T,diff}$				
BPD	−0.0000	−0.0001, −0.0000	0.024	0.04
VT_{high} after sigh (breaths)				
BPD	−0.0297	−0.0515, −0.0079	0.008	0.03
$V_{T,end}$				
Gestational age (w)	0.0003	0.0017, 0.0005	<0.001	0.40
Weight (kg)	0.0045	0.0032, 0.0057	<0.001	
Sex, male	0.0020	0.0002, 0.0037	0.027	
Short-term variability in V_T				
CV_{pre}				
Gestational age (w)	0.0008	−0.0004, 0.0020	0.190	0.05
Sex, male	−0.0930	−0.1521, −0.0338	0.002	
CV_{post}				
Gestational age (w)	0.0073	0.0039, 0.0107	<0.001	0.16
Sex, male	−0.0394	−0.0786, −0.0002	0.043	
Maternal smoking	−0.0692	−0.1350, −0.0035	0.041	
CV_{diff}				
Gestational age (w)	0.0055	0.0013, 0.0096	0.009	0.07
Maternal smoking	−0.0940	−0.1733, −0.0146	0.020	
CV_{post_slope}				
BPD	0.0188	0.0122, 0.0255	<0.001	0.15
CV_{end}				
Gestational age (w)	0.00102	−0.0010, 0.0031	0.025	0.16
Sex, male	−0.0323	−0.0528, −0.0118	0.003	
Birth weight *z*-score	0.0172	0.0078, 0.0265	<0.001	

Bronchopulmonary dysplasia (BPD) was expressed as the number of days on supplemental oxygen. Respiratory rate was averaged over the duration of the measurement. $V_{I,max}/V_{T,pre}$, maximal inspiratory volume during the sigh normalized to mean tidal volume at the beginning of the measurement; $V_{E,max}/V_{T,pre}$, maximal expiratory volume during the sigh normalized to mean tidal volume at the beginning of the measurement; $V_{E,max}/V_{I,max}$, ratio of maximal expiratory volume during the sigh/maximal inspiratory volume during the sigh; $V_{T,pre}$, mean tidal volume over the first 30 breaths of the measurement; $V_{T,post}$, mean tidal volume over the first five breaths after the sigh; $V_{T,diff}$, difference between $V_{T,pre}$ and $V_{T,post}$; $V_{T,high}$, number of breaths after a sigh that exceeded 2 SD of $V_{T,pre}$; $V_{T,end}$, mean tidal volume over the last 30 breaths of the measurement; CV_{pre}, coefficient of variation in $V_{T,pre}$; CV_{prior}, coefficient of variation in $V_{T,prior}$; CV_{post}, coefficient of variation in $V_{T,post}$; CV_{diff}, difference between CV_{pre} and CV_{post} as an estimate of change in variability in tidal breathing upon a sigh; CV_{post_slope}, difference between CV_{VT} of first window after the sigh and second window after the sigh normalized to individual baseline CV_{VT}; CV_{end}, coefficient of variation in V_T.

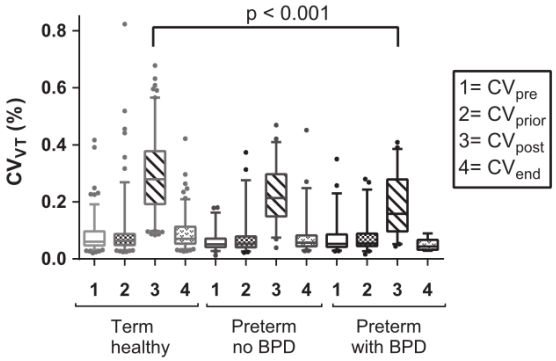

Figure 3. Short-term variation in tidal volume (CV_{VT}) for different subgroups (Term Healthy; Preterm infants without BPD at 36 weeks GA; Preterm infants with BPD at 36 weeks GA). CV_{VT} was measured using moving window technique at the start of the measurement (CV_{pre}), prior to the sigh (CV_{prior}), just after a sigh (CV_{post}), and at the end of a measurement (CV_{end}).

assessed in their study. It is somewhat surprising that Qureshi et al. did not find differences in sigh frequency between preterm infants at about 33–34 weeks PCA versus term infants at 41 weeks PCA given the maturational discrepancy of over 8 weeks during a critical period of development of respiratory control (Engoren et al. 2009); many investigators believe that sighs occur more frequently in preterm infants due to their pronounced need of restoring lung volume (Brockmann et al. 2011). However, postnatal age of preterm infants in Qureshi et al. was close to that of term infants (17 ± 3 vs. 11 ± 2 days), and the occurrence of spontaneous sighs during quiet sleep in infants is indeed related to their postnatal age: Within the first weeks of life, sigh frequency drops from about 0.9 sighs/min to 0.2 sighs/min as indicated by serial pneumography in infants studied from day one of life until 7 months of age (Fleming et al. 1984). Further, differences in the definition of a sigh (≥100% above baseline V_T in our study vs. ≥50% above baseline V_T in Qureshi et al.) and experimental conditions (supine position 30 min postfeed in our study versus supine or lateral position pre- or postfeed in Qureshi et al.) might explain the discrepancy to our results as breathing pattern of preterm infants measured in left lateral and prone position differs from that obtained in supine position (Gouna et al. 2013). Our findings of a decrease in V_T and increased short-term variability in V_T after a sigh are in agreement with our earlier findings reported by Baldwin et al. who studied variability as well as short- and long-range memory of V_T in term healthy infants at 4–6 weeks postnatal age (Baldwin et al. 2004). In this previous study, we found stability in long-range memory but improved variability and short-range memory of V_T after a sigh. This study further shows that changes in V_T and the temporary gain in short-term variability in V_T following a sigh are less pronounced in former preterm infants and that infants suffering from BPD return faster to their lower baseline variability in V_T after a sigh (CV_{post_slope}). This suggests that both immaturity at birth and residual lung disease accelerate an infant's return to baseline breathing pattern after a sigh. However, the effect size of both preterm birth and BPD is small indicating that unmeasured factors substantially influence those outcomes.

Strengths and limitations

All measurements were conducted according to American Thoracic Society/European Respiratory Society standards for infant lung function testing. Infants were studied at a comparable PCA and were assessed in unsedated quiet sleep using modern, miniaturized lung function equipment. Limitations of our study include a recruitment period of over 7 years, potentially introducing observer bias as several investigators conducted the measurements. Nevertheless, all personnel followed standard operating instructions and we did not find any trends in outcomes over time, that is, observer-dependent effects as origin of our findings are unlikely. A general methodological difficulty lies in the precise quantification of residual lung disease in preterm infants as the clinical definition of BPD is entirely based on duration, and level of oxygen supplementation and respiratory support during neonatal intensive care stay (Jobe and Bancalari 2001). This simplistic approach might lead to misclassification, however, currently there is no superior alternative diagnostic tool and it remains a valid predictor of poor outcome including death and long-term respiratory and neurological sequelae (Kugelman et al. 2007; Schmidt et al. 2003).

Interpretation and mechanisms

We expected a higher sigh frequency in preterm versus term infants due to the particular need of preterm infants to restore lung volume, optimize compliance and resistance, and, presumably, to reset autonomic tone (Alvarez et al. 1993; Davis and Moscato 1994; Poets et al. 1997). The observation that sigh frequency normalized to respiratory rate did not differ between preterm and term infants underlines the importance of baseline breathing pattern as a marker of maturation in preterm infants: Both $V_{T,pre}$ and $V_{T,end}$ were significantly associated with GA at birth; further, respiratory rate in preterm infants was increased compared to term infants although all patients were measured at equivalent PCA. This is in agreement with Schmalisch et al. who found similar

associations in a lung function study of 386 very low-birth weight infants measured at 48–52 weeks of PCA (Schmalisch et al. 2013). Arguably, sigh frequency in former preterm infants during quiet sleep is a function of respiratory rate, which in turn reflects biological maturity of the respiratory system.

The specific mechanisms that trigger sighs in human infants are essentially unknown (Alvaro and Rigatto 2011). We found that contrary to baseline breathing pattern, V_T, variability in V_T immediately prior to a sigh, and morphology of the sigh itself are fairly similar between preterm and term infants. We can only speculate on the reasons for such "uniformity of sigh-breathing"; based on our findings, the presigh and sigh period during quiet sleep represent epochs of respiration that are genuinely independent of maturity at birth, degree of residual lung disease, and baseline demographics such as body weight or sex. Most remarkably, inspiratory sigh volume ($V_{I,max}$) of preterm infants was comparable to that of their term peers although these infants have restrictive lung disease (Thunqvist et al. 2014). However, the reaction to a sigh seems to be influenced by GA and BPD; depth and duration of change in breathing pattern after a sigh are associated with both prematurity and residual lung disease: After the sigh, preterm infants, and particularly those with BPD return faster to their lower baseline variability in V_T. These observations are consistent with the hypothesis that although sighs play an important role in restoring lung volume in preterm infants (Poets et al. 1997), these infants might not tolerate prolonged deviations from baseline breathing pattern due to underlying maturational deficits. The reason(s) for this phenomenon are unclear. We speculate that the potential threat of hypoventilation after a sigh requires preterm infants, and especially those with BPD, to quickly return to baseline breathing pattern in order to avoid an epoch of periodic breathing/hypoxia under conditions of an immature respiratory feedback loop (Thach and Taeusch 1976; Bradley 2002; Khan et al. 2005; Qureshi et al. 2009). This behavior may indicate an immature respiratory pattern generator which potentially could be important for cardio–respiratory coupling and survival of infants under stress (Ramirez 2014).

We conclude that breathing pattern following a sigh is moderately influenced by the degree of prematurity and residual lung disease in preterm and term infants measured at equivalent corrected age shortly after the expected date of delivery. The precise mechanisms triggering sighs, and the major determinants of breathing pattern prior to and after a sigh in preterm infants remain to be investigated in future studies. Whether or not sigh architecture is a predictive marker of later respiratory morbidity should ideally be investigated in future longitudinal studies. Such studies should potentially include measurement of long-range memory of control of breathing given that the latter provided novel insights into breathing dynamics in term healthy infants.

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Conflict of Interest

None declared.

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6. Discussion, Conclusion, and Outlook

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"Until we know what we are looking for,
the secrets of complex networks will remain elusive."

- Steven Strogatz –
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Immaturity, as other illnesses, can impair autonomic control. A preterm infant would naturally have weeks to months more time in utero to "learn" how to regulate body temperature, heartbeat and breathing, and how to coordinate those elements. Thus, it is not surprising that intensive care treatment and monitoring is needed to support them and keep them under surveillance during their first weeks of life. Even though treatment of preterm infants made large progress in the last years, the understanding of the immature regulatory processes still is limited. Complexity and dynamic properties of these immature regulatory systems can objectively be described using nonlinear analysis. The results from this PhD work add more information about autonomic dysregulation of vital signs in preterm infants.

6.1. Complexity in physiology

When we look at the human body as a conglomerate of physiological systems, we might get a fraction of the impression of its complexity when recalling the changes in scale from subcellular molecules, to the cell itself, to the organ, and finally to the organism. Thus, it is not surprising that the regulatory systems and their interactions in living organisms are not linear processes. On the other side they are not random in behavior. Nonlinear mathematical tools have been useful in the description of complex organizations of cells (pacemaker cells in the sinus node[99]), symptoms (cardiac arrhythmias[100]; tremor in patients with Parkinson's disease[101]), and the behavior of small organisms (fireflies[102, 103]). To truly understand, how billions of unconscious neurons lead to both, conscious and unconscious regulations and decisions in the human body, presents an even bigger challenge and until now seems impossible.

6.2. Comparison with the literature

Signal processing

To describe regulatory systems, small changes in amplitude or frequency of the signals, such as milliseconds of variation between consecutive heartbeats, have to be detected reliably. This illustrates the need of accurate measuring systems for the detection of alterations in fluctuations of those signals. We could show that inappropriate data cleaning influences outcomes of time series analysis (5.3. Diaphragmatic surface electromyography in preterm infants -A systematic way to clean data for analysis of heart rate variability-). The use of time series data that includes noisy signal parts might have a large effect in the description of nonlinear systems. This becomes evident when recalling the strong dependency of nonlinear systems on the initial conditions[76, 104], also known as the butterfly effect. On the other side, extensive cleaning is leading to fragmentation of the signal, which itself could influence results from time series analysis[105]. Once reliable analysis from cleaned study data is provided, complexity and dynamics of vital signs can lead to deeper understanding of (patho-) physiological mechanisms.

Temperature

Our study is the first to show that complexity of body temperature in incubator-nursed preterm infants can be described using DFA and SampEn. Stern et al found an increasing scaling factor alpha in temperature time series of healthy term born children, when they observed them over several weeks[30]. They interpreted this to be a sign of maturational effect towards a more deterministic system with increasing age post term. In contrast, our data showed a decrease in mean (SD) alpha from 1.67 (0.18) on the first day of life to 1.46 (0.22) on the fifths day of life. The differences in study setting (incubator vs home setting without active external heat input; difference in frequency, time range, duration and sampling frequency of temperature date) and cohort characteristics (very preterm infants vs healthy term infants) might explain the discrepancy in the direction of the association between scaling factor alpha and postnatal age.
We found lower complexity (higher alpha and lower SampEn) to be associated with the degree of prematurity and growth restriction at birth, and with several comorbidities of preterm infants. In fact, an increase in alpha was associated with a stepwise increase in the level of respiratory support required during the first days of life after adjusting for prematurity and intrauterine growth restriction. Varela at el found significantly higher DFA and lower SampEn values in hourly temperature data in adult patients who did not survive their intensive care unit stay after adjustment for age[84]. Hence, and similar to our findings, the authors interpreted the loss of complexity in temperature curves as a marker of severity of disease and, additionally, as indicator for poor prognosis.

Sleep

The amount of quiet sleep and of active states including active sleep and wakefulness, as well as sleep cycle duration of preterm infants did not change under light-deprivation. The ultradian rhythm of sleep in immature newborns seems more independent than the one in adults, as a lack of light changes (light-deprivation during phototherapy) was not affecting this rhythm. These results are in line with other sleep studies[33, 106]. Nevertheless, the earlier birth of premature infants is affecting sleep behavior, as the entrainment of circadian sleep-wake rhythm was shown to be earlier in former premature infants compared to term born controls[44, 107].

Heart rate

The association of altered fluctuations in heart rate and increased mortality in post-myocardial infarct patients has first been described by Wolf et al[108]. Later, mathematical description of IBI of patients with life-threatening cardiac pathologies, showed significant differences in short- and long-range correlation properties when compared with healthy controls[49, 82, 109].
In newborns, variability in heart rate is useful as an additional, early marker of a sepsis or sepsis like illnesses during their stay on a NICU[55-58]. It has been shown that stress and painful procedures during the vulnerable phase of infancy and potentially fetal life have long lasting effects. Rakow et al[61] showed that infants with intra-uterine growth restriction have altered HRV at the age of 9 years when compared to healthy controls. Morin et al[95] studied HRV of former preterm children and children that underwent surgery in the newborn period. At the age ranging from 7-25 years they were exposed to a painful procedure (they had to place their hand in ice water). Frequency domain analysis of study participant's heart rate showed changes reflecting a higher sympathetic activity. These findings were interpreted as persisting changes in cardiac autonomic control with potential impairment in responding to stress or pain. In summary, mathematical properties of HRV have been shown to be associated with acute and chronic morbidity and mortality. Our data, to our knowledge the first to measure HRV in very preterm infants during their first days of life, adds the fact that HRV also has a predictive value to determine resolution of autonomic dysregulation in terms of termination of pharmacological and respiratory support: SampEn of IBI during the first days of life significantly adds predictive value on top of well-known risk factors such as degree of prematurity, intra-uterine growth and relevant co-morbidities.

Breathing

As shown by Latzin et al, the functional residual capacity of former preterm infants is only marginally different from healthy term controls when measured at a comparable PCA[110]. It seems as if the capacity of former preterm infants to maintain their lung volume is very high, as long as there is no perturbation. Sigh morphology was astonishingly uniform and independent of degree of prematurity, body weight or gender. Breathing pattern, however, is persistently different in former preterm infants when compared to healthy controls shortly

after due date. This is even more evident after a sigh. The breathing pattern upon a sigh in healthy term born infants has been previously described by Baldwin et al[11]. They observed an increase in variability of tidal volume after a sigh. When we compared the effect of a sigh between healthy term born and preterm infants at a comparable PCA, we found that the reaction in breathing pattern upon a sigh is associated with degree of prematurity and residual lung disease. Hence, the event of a sigh seems to unmask the underlying limits of the respiratory system in preterm infants.

6.3. Physiological mechanisms

Alterations in physiological systems

The goal of a living organism is to find a balance between stability to maintain its functions and sensitivity to be able to respond to unpredictable stimuli or stress. This state is maintained by various feedback loops and interactions between several regulatory systems and thus is not static. The resulting fluctuations exhibit complex nonlinear properties, suggesting that under healthy stable conditions, the regulatory systems are operating far from equilibrium, which enables plasticity[111]. This complexity of physiological systems has been shown to be altered with increasing age and in case of disease[45, 112, 113].
In summary, there are the following two ways how alterations in human regulatory systems can be described:

- Increase in order: The transition to a strongly periodic behavior as for example in Cheyne Stokes breathing[111], tremor in Parkinson's disease[101], or heart rate variability in infants with sepsis[55] or in elderly people[45]. The emergence of a dominant mode results in loss of functional responsiveness and reduced plasticity of the system. This possibly leads to less adaptability in stressful situations.

- Decrease in order: A breakdown of the memory in a physiological system is leading to uncorrelated randomness[111]. The physiological organization following deterministic chaos is turned into outputs similar to "white noise". A well-described example of this deterioration is the ventricular response to atrial fibrillation[111].

Both, the undue increase or decrease in a regulatory system's order, are expressions of degradation of the correlated, multiscale dynamics of the system. This results in reduced ability to react upon external or internal changes, on one side as pathologic periodicity, on the other as possible loss of function due to uncorrelated randomness.

Temperature

The higher complexity in time series of body temperature in extremely preterm and/or very ill infants shown in our study could be explained as pathological alteration with an increased order. Or, taking into account our study setting of an external controlling system, trying to keep the infant's body temperature at a certain value, it could be a sign of a more passive temperature control of the infant. In this case, the incubator algorithm would thus be less influenced by temperature regulation from the infant's side and hence, the predictability of the signal would be rising.

Sleep

Light-deprivation usually results in significant melatonin increase and consequently one is falling asleep. Studies of melatonin production in term and preterm newborns have shown that in preterm infants, the necessary mechanisms for melatonin production are developed at 33-36 weeks GA[114], but that melatonin levels do not increase in response to light-deprivation[115]. The absence of melatonin production shown by Mantagos et al[115] could be one of the reasons for the resistance of the ultradian rhythm of preterm infants against external stimuli such as light-deprivation during phototherapy, shown in our study.

Heart rate

Variability of the heart rate shows increase of irregularity (SampEn) and decrease of memory (DFA) with increasing maturity in our study population of very preterm infants. The underlying mechanisms, making HRV, assessed during the first days of life, a predictive value for cessation of pharmacological and respiratory support, are not yet understood. Infants with a lower SampEn of IBI are longer treated with caffeine and respiratory support, even after correction for GA. Speculative, one could interpret the negative association of SampEn and postconceptional age at termination of those therapies as consequence of the increase in order of cardiac autonomic control. The observed increase in complexity of heart rate fluctuations in infants needing longer support could be a sign of pathological periodicity. This would lead to a decrease in the functional responsiveness to unpredictable stimuli.

Breathing

The principal triggers of sighs are still unknown. We can thus only speculate about the mechanisms that result in differences of breathing pattern upon a sigh in preterm infants when compared to healthy term infants. The novel finding of our study, that sigh frequency is a function of respiratory rate shortly after due date, makes it likely to be a results of the maturity of the respiratory system. We showed that degree of prematurity and residual lung disease is associated with magnitude and duration of changes in breathing pattern upon a sigh. This diminished reaction of the respiratory system upon an internal perturbation in preterm infants could be seen as a result of the higher periodicity in general (lower variability of tidal volume (CV_{VT}) also in the baseline breathing pattern). In other words, it could be described as an alteration in stable-limit cycle oscillations, resulting in a faster return to the restricted baseline breathing pattern in preterm infants. This behavior might indicate an immature respiratory pattern generator, which potentially could be important for cardio–respiratory coupling and survival of infants under stress[116].

6.4. Possible clinical implications in the future

Temperature

Temperature regulation in incubator-nursed very preterm infants during their first days of life is dependent on degree of prematurity and relevant comorbidities. The description of complexity and dynamics in temperature control in that population could be useful in the development of more individualized thermal care. It could be used to objectively determine the best timing for an infant to transit from the incubator to an open cot. Until now this transition is not based on scientific evidence but rather on an arbitrary body weight threshold[117] and not routinely adjusted for an individual patient's readiness from a systems control perspective. Hence, such transfers are typically a matter of trial and error despite the fact that thermo-neutrality is important for adequate weight gain and has beneficial effects on other autonomic control systems such as breathing and heart rate[14, 15, 26].
Timely transfer of infants from the incubator to an open cot might also have additional benefits in terms of environmental noise exposure, as in incubators noise levels have been shown to be approximately 10 times louder than recommended[31]. Furthermore, a recent study suggests that exposure of preterm infants to coherent language might have positive effects on language and motor development[32].

Sleep

Although sleep behavior has been shown not to be influenced by phototherapy, one of the routine therapies during a preterm infant's stay in the NICU, we consider it essential to limit potentially disturbing procedures and respecting the concept of minimal handling. This is of particular importance as sleep is known to play a key role in neuronal development and to influence other regulatory systems.

Heart rate

The gained knowledge about HRV during a preterm infants' first days of life showed to add predictive value to estimations aiming at prediction of the time of cessation of pharmacological treatment of autonomic dysregulation with caffeine and of termination of respiratory support. This information is not only interesting in terms of how early postnatal monitoring of HRV can have predictive value over weeks. It is also a major factor in resource allocation, as both, stop of caffeine treatment and respiratory support, are key features to transfer infants from a NICU or an intermediate care unit to a more general ward. Improved prediction of that time point would improve planning in daily clinical routine and optimize use of health care costs.
If the best time point for successful extubation could be estimated with analysis of HRV in preterm infants needs to be tested. In adult patients, suffering from respiratory failure, a successful extubation was associated with increased values in frequency domain analysis of HRV[118]. Al Ghonaimi et al found an association between preterm infants that failed to be extubated and reduction in frequency domain heart rate analysis, performed one hour prior to extubation[119]. In our study only a limited number of preterm infants were measured during mechanical ventilation. There was a negative association between scaling factor alpha of IBI and hours of respiratory support until successful extubation. Other well-known factors, such as degree of prematurity, intra-uterine growth, and sepsis were significantly associated with duration of ventilation. Thus, a higher number of ventilated infants with HRV analysis would be needed to estimate the usefulness of the scaling exponent alpha, derived by DFA, for prediction of extubation- readiness in that study population.

Breathing

Breathing pattern following a sigh is influenced by the degree of prematurity and residual lung disease in preterm and term infants measured at equivalent corrected age shortly after due date. Whether or not sigh characteristics are useful as predictive marker of later respiratory morbidity should ideally be investigated in future longitudinal studies. In case these markers could be used to measure resolution of autonomic respiratory control in former preterm infants, this would be an elegant, non-invasive way for risk-stratification on an individual level.

In general, deeper knowledge about changes in breathing pattern according to disease could be a helpful input in various fields. Polysomnographic records of different patient groups could be used to assess changes in breathing pattern upon internal and external stimuli. This knowledge could ultimately be used in clinical management of patients with need for respiratory support, e.g. for individualized ventilation strategies. Mechanical ventilation using variable tidal volumes is already discussed to be more physiological and thus more effective[120, 121]. Once this modified ventilation strategy has been proven to be safe and more effective in humans, variability of ventilation could be adapted to an individuals need based on the underlying pathology.

6.5. Conclusion

Autonomic dysregulation in preterm infants is a relevant burden of disease, affecting several organ systems, and leading to possible risk situations in the short- and long-term. Our data supports the importance of mathematical description of alterations in these physiological regulatory systems in terms of associations of indices derived from time series analyses with the degree maturity at birth, presence and severity of disease, and prediction of resolution of autonomic dysregulation. In this context, we conclude that our results about different aspects of autonomic control in preterm infants provide a good basis for further investigations in this interesting research field. This could lead to better support of preterm infants during their stay on the NICU and identification of patients at high risk for future deterioration.

6.6. Outlook

Interactions

A human being is never isolated from the environment and all sorts of external stimuli and internal interactions are influencing the behavior of our regulatory systems[122]. There are not only well described immediate effects, such as acceleration of heart rate and respiratory rate as a response to pain or excitement, but also more complex situations were vital signs are influencing each other over longer time periods. It has been known since 1980 that sleep duration is dependent on body temperature at the time point of falling asleep[123]. The group around Czeisler built an impressive setting, were adult study participants lived over weeks in absence of any knowledge about daytime. This way they aimed to exclude all external stimuli potentially influencing their circadian rhythm. The study participants sometimes had very long sleeping periods (averaging 15 hours). This was always the case whenever their body temperature was high at the time point of falling asleep. In contrast, they were sleeping on average 8 hours whenever their body temperature was at a minimum level at the time they went to bed.

Another amazing fact of different systems, in this case even different subjects, influencing each other, is the synchronization of ovulation among women within one peer group. McClintock, at that time an undergraduate student in psychology, was the first to show that fellow students synchronize their menstrual cycle over the time of a semester. A randomly selected control group did not show this effect[124]. The chemical communication between women, leading to this phenomenon, is believed to be driven by pheromones[125, 126].

After describing output signals of single systems, a more complicated task, the interactions of systems between each other should regain more attention in the future. Stéphan-Blanchard et al recently showed that changes in ambient air temperature lead to reduction in short- and long-term variability of heart rate in sleeping preterm infants, measured near term[14]. Persistence of impaired temperature regulation has been discussed as a risk factor for SIDS for a long time[127]. The observed changes in HRV as reaction to different ambient air temperature in the high-risk population of preterm infants provides new insights in potential mechanisms behind SIDS[14]. Pikkujämsä et al showed that HRV, assessed with 24 hours ECG measurements in children and adults, changed systematically during the night among all age groups[45]. During sleep, heart rate showed increased variance and complexity, thus a lower regularity and predictability. When we assessed associations between HRV and sleep stage in 186 measurements of our study population of very preterm infants, we found significant differences between awake and quiet sleep. Some of these outcomes, as for example SampEn of IBI, showed a degree of change between behavioral states that dependent on GA. If the degree of variation of HRV parameters could be a measure of maturity in cardiac autonomic control would have to be tested in a more systematic way.

The interdependency of the different regulatory systems, as outlined in Figure 3, is not only a challenge but also a chance. If we are able to describe how stabilization in one system is improving the other immature processes as well, we could target our care more efficiently. We therefor think that a better understanding of immaturity in the physiological systems and of their interactions could improve clinical care and risk stratification in the population of preterm infants

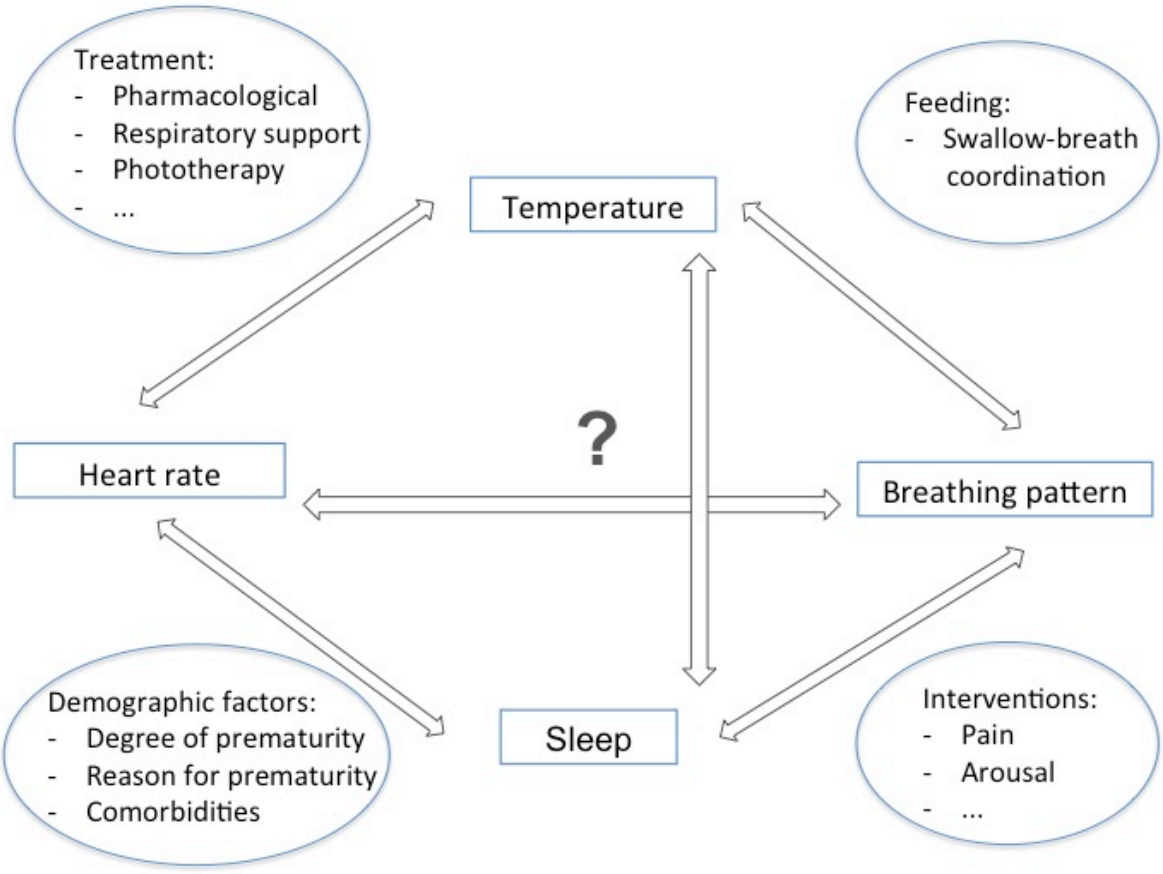

Figure 3 Schematic overview of interactions between the immature regulatory systems in preterm infants

Coordination

Besides the described parts of the immature regulatory system in preterm infants, the inability to coordinate swallowing and breathing is a further expression of autonomic dysregulation. Most commonly, preterm infants are fed using gastric tubes for weeks to months until they are deemed mature enough to introduce suck feeds. Thus, similar to the other components of autonomic dysregulation, the resolution of this type of immaturity in NICU patients is a key factor to be fulfilled before they can be discharged home. Whether coordination abilities depend only on gestational age and how they can be diagnosed objectively remains controversial[128-131]. In addition, the ideal time point to introduce suck feeds and to challenge coordination of swallowing and breathing is unknown and largely remains a matter of trial and error with the risk of causing iatrogenic aspirations of milk (potentially causing life-threatening pneumonia or sepsis) if the infants are not ready for suck feeds. Successful swallow-breath coordination is generally considered a higher neurological function and, consequently, represents a milestone in overall child development. Therefore, measurement of its level of function might serve as predictor for the neurological development and future outcome of preterm infants. However, the utility and predictive power of such indicators remains unkown[97, 132, 133]. One of the main reasons for the questions above lies in the lack of diagnostic tools that objectively measure suck-swallow-breath coordination with sufficient accuracy[134-136].

Therefore, we established a research-collaboration between the Department of Neonatology at the University of Basel Children's Hospital (UKBB), the Institute for Human Centred Engineering (HuCE), Bern University of Applied Science, and the ARTORG Center-Cardiovascular Engineering, University of Bern. This collaboration, combining clinical expertise in neonatology and clinical research with electrophysiology and technical expertise in (esophageal) signal recording and processing, aims to investigate the swallow-breath coordination in preterm infants and to develop tools to quantify such coordination.

In a recently started observational study, we aim to assess the swallow-breath coordination in preterm infants as possible early markers of autonomic development. Since July 2015 we successfully measured 9 preterm infants during their stay in the NICU. All of them were in need of a gastric feeding tube, which we replaced by the CE-labeled Edi tube (Maquet, Solna, Sweden), which is normally used to derive signals for neuronally adjusted ventilation assist (NAVA). Currently, we are processing the signals to identify periods with swallowing events to then determine the coordination of swallowing, breathing, and heart rate. A preliminary look at the data is very promising as can be seen in Figure 4. An advantage of this new method is not only the possibility to measure one additional, immature function. The esophageal location, close to the organs of interest (heart, diaphragm) could result in a better signal to noise ratio of examining HRV and/or diaphragmatic EMG signals. To determine whether the use of this esophageal technique is associated with less motion artifacts, synchronized surface EMG measurements and heart rate traces from the standard monitoring are captured.

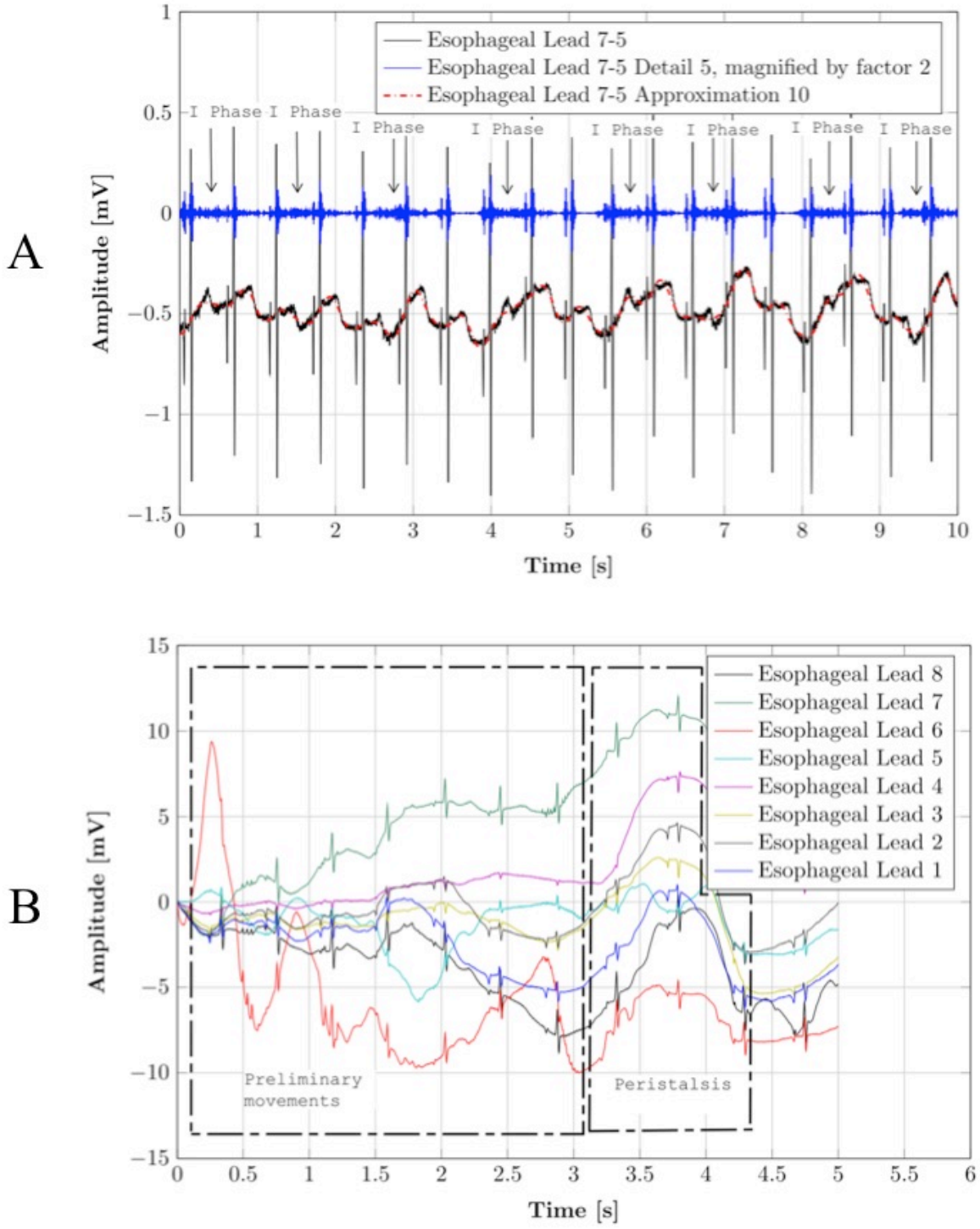

Figure 4
A) High-pass filtered, bipolar esophageal lead 7-5 (detail 5) shows diaphragm EMG during the inspiration phase that triggers the mechanical answer seen as baseline wander (approximation 10).
B) Peristaltic activity is recorded from the most proximal (8) to the most distal esophageal lead (1) during oral feeding.

Tracking

Even after discharge from the NICU, former preterm infants remain a high-risk population. They are, among others, known to be at risk for re-hospitalization and SIDS. Poor respiratory control[16, 63] and simplified lung structure due to interrupted maturation of the lung periphery[62] are two of the discussed possible reasons for persistence of the elevated risk of respiratory complications in former preterm infants. Risk stratification on an individual level, based on objective parameters, would be very desirable.

Although the description of vital signs, using distinct mathematical tools, has become more prominent in the last years, there is not enough knowledge about biological maturation and development of an individual patient. Tracking is well known for variables as growth, and weight gain in children, but has also been described in physiological parameters such as individual breathing pattern[137]. In this review of Benchetrit the persistence of a respiratory personality, the way each subject has its own, specific way of breathing, is described under diverse conditions[137].

When a child leaves its percentile range in a standardized growth chart, this is an immediate indication for pediatricians to systematically assess variables that could influence growth. If changes in mathematically described vital functions, comparable to crossing of percentiles in a growth chart, could be a marker of improvement or deterioration of a physiological system in an individual patient, is unknown. Therefore, a better understanding of tracking in physiological systems is very important to improve our understanding and support patients with impaired autonomic control. In a first step, one would have to build a set of values representing a target population. This could be done by using the open databases, as for example Physionet, containing large datasets[70]. With this normative data one could assess if single markers of physiological systems can be tracked over time. In a seconds step, one could describe alterations from physiological tracking in case of resolution of autonomic dysregulation or newly acquired problems in autonomic control for example upon an acute disease.

If characteristics of temperature regulation, HRV or swallow-breath coordination during their first days of life or maybe sigh-induced changes in breathing pattern shortly after due date could be useful for the identification of high-risk patients within the group of very preterm infants, is unknown. This would have to be tested in a large prospective, longitudinal study including long-term follow-up of patients, ideally with a normative dataset as comparison. A possible study design could include repetitive measurements of physiological parameters during the infants first days of life and in the context of later hospital visits due to routine assessments (e.g. immunization or neurodevelopmental follow up).

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“The important thing is not to stop questioning.
Curiosity has its own reason for existing.“

- Albert Einstein –

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