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Article *in* Physiological Measurement · August 2009

DOI: 10.1088/0967-3334/30/8/009 · Source: PubMed

IOP PUBLISHING PHYSIOLOGICAL MEASUREMENT

Physiol. Meas. **30** (2009) 847–860 doi:10.1088/0967-3334/30/8/009

Heart rate variability during sleep in healthy term newborns in the early postnatal period

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Received 6 March 2009, accepted for publication 22 June 2009
Published 10 July 2009
Online at stacks.iop.org/PM/30/847

Abstract

Normative time- and frequency-domain heart rate variability (HRV) measures were extracted during quiet sleep (QS) and active sleep (AS) periods in 30 healthy babies. All newborn infants studied were less than 12 h old and the sleep state was classified using multi-channel video EEG. Three bands were extracted from the heart rate (HR) spectrum: very low frequency (VLF), 0.01–0.04 Hz; low frequency (LF), 0.04–0.2 Hz, and high frequency (HF), >0.2 Hz. All metrics were averaged across all patients and per sleep state to produce a table of normative values. A noticeable peak corresponding to activity in the RSA band was found in 80% patients during QS and 0% of patients during AS, although some broadband activity was observed. The majority of HRV metrics showed a statistically significant separation between QS and AS. It can be concluded that (i) activity in the RSA band is present during QS in the healthy newborn, in the first 12 h of life, (ii) HRV measures are affected by sleep state and (iii) the averaged HRV metrics reported here could assist the interpretation of HRV data from newborns with neonatal illnesses.

Keywords: newborn, sleep, EEG, ECG, heart rate variability, respiratory sinus arrhythmia

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Heart rate variability (HRV) is a useful, non-invasive method for analysing the function of the autonomic nervous system function. It reflects the interplay between the sympathetic and

parasympathetic autonomic nervous system, which serves to speed up and slow down the heart rate, respectively (Askelrod *et al* 1981). HRV analysis in the newborn infant has previously been used in outcome studies (Patzak 1999, Doussard-Roosevelt *et al* 2001), assessing the severity of neonatal illnesses (Griffin *et al* 1994, Longin *et al* 2006, Filtchev *et al* 1994), and seizure detection (Greene *et al* 2007, Malarvili *et al* 2007). However, it is still not widely used in the clinical domain and remains primarily a research methodology. There are a number of reasons for this; the main reason being the variety of methodology used.

There are two main approaches to the analysis of HRV: time-domain and frequency-domain analyses. Time-domain indices are derived from simple statistical calculations based on interbeat intervals. These indices are sensitive to transients and trends in the sample of heartbeats, and as such provide estimates of overall and beat-to-beat variability (Rosenstock *et al* 1999). Frequency-domain analysis, which is based on the power spectral density of the heart rate time series, highlights the issue of the underlying rhythms of the mechanisms controlling heart rate (HR).

The main physiological mechanisms influencing HRV are respiration, baroreceptor reflex and thermoregulation. As each of these contributes to HR oscillations at a particular frequency, the relative contribution of these mechanisms can be read from the HRV power spectrum (Rosenstock *et al* 1999). Askelrod *et al* (1981) identified three major spectral peaks in the adult HR spectrum. A high-frequency (HF) spectral peak, the result of respiratory sinus arrhythmia (RSA), is generally observed between 0.15 and 0.4 Hz. A low-frequency (LF) peak, synchronous to blood pressure Mayer waves occur between 0.04 and 0.15 Hz. Very low-frequency (VLF) HR oscillations that are attributed to peripheral vascular resistance fluctuations caused by thermoregulation, are observed below 0.04 Hz (Rosenstock *et al* 1999).

In the newborn, the same autonomic generators of HRV are present, however, these oscillatory frequencies are different and in some cases absent (Rosenstock *et al* 1999, Longin *et al* 2005). RSA is derived from the increase in the heart rate that occurs during inspiration followed by the decrease during expiration (Hathorn 1987). Some studies in the newborn have reported an absent RSA (Longin *et al* 2005); others have reported an RSA peak in 11 out of 20 newborns studied (Baldzer *et al* 1989). The time and frequency domain parameters of HRV are also affected by the maturation of the autonomic nervous system (ANS) in newborns (Longin *et al* 2005).

In 1996, a European and North American task force was formed in an attempt to set standards for future studies of HRV (Malik *et al* 1996). While important recommendations were made for the length of recordings and the required spectral indices, their conclusions were based on adult studies only. All spectral frequency bands specific to the newborn infant differ from those in adults. Therefore, these recommendations for division of the power spectrum should not be used in foetal, neonatal or infant studies. The task force also addressed the issue of parametric and non-parametric methods for the computation of frequency domain measures, however, no recommendation was made.

HR data possess several unique properties not usually accounted for in traditional frequency-domain techniques. Essentially, RR intervals are not evenly sampled in time; yet it is this phenomenon that HRV analysis attempts to characterize. Standard methods for power spectral density (PSD) estimation, including Fourier transform and autoregressive methods, operate on time series with uniform intervals. Therefore, it is necessary to resample at uniform intervals when analysing the heart rate time series (Berger *et al* 1986). This resampling process can have a low pass filtering effect on the frequency content of the HR series (Moody 1993).

More recently, a method that addresses the issue of irregularly sampled signals, the Lomb Periodogram, has been studied. Laguna *et al* (1998), Moody (1993) and Chang *et al* (2001)

Table 1. Methods of HRV calculation.

Author	Subjects	Method	Window length	Bands (Hz)			Sleep sstate classification
				VLF	LF	HF	
(Giddens and Kitney 1985)	Newborns	FFT	200s	0–0.04	0.04–0.2	>0.2	N/A
(Aarimaa *et al* 1988)	Newborns	FFT	200 beats		0.02–0.2	0.2–1	Clinical
(Baldzer *et al* 1989)	Newborns	FFT	100s	0–0.04	0.04–0.2	>0.2	Clinical
(Schechtman *et al* 1989)	Infants	Time domain	60s	0.033–0.083	0.133–0.25	0.33–1.11	Anders
(Clairambault *et al* 1992)	Newborns	STFT		0.02–0.083	0.083–0.25	0.25–1	Clinical
(Finley and Nugent 1995)	Infants and children	FFT	150s		0.03–0.15	0.15–0.6	Clinical
(Patzak *et al* 1996)	Newborns	FFT			0.02–0.2	0.2–1.5	Clinical
(Massin *et al* 2001)	Infants	FFT	300s	0.004–0.04	0.04–0.15	0.15–0.4	Polysonogram
(Mehta *et al* 2002)	Newborns	FFT	300s	0.0033–0.04	0.04–0.15	0.15–0.4	N/A
(Longin *et al* 2005)	Newborns	FFT	64s	0.01–0.05	0.05–0.2	0.2–1	Clinical

compared the Lomb Periodogram to autoregressive, Fourier and interpolation followed by Fourier PSD estimates. All three studies concluded that the Lomb Periodogram was superior to all other methods assessed. The Lomb and resampled Fourier methods can introduce some high-frequency contamination when either the deviations from uniform sampling are dramatic or the frequency of variation is very high (Laguna *et al* 1998). However, neither of these are likely to occur over a stationary signal. Previous work by Garde *et al* found that neonatal HR data are stationary over periods of at least 1 min, the behaviour of longer segments of neonatal HR data is undocumented (Garde *et al* 2001).

From a cross section of the current literature on newborn and infant HRV (see table 1) a standardized method of HRV calculation is clearly required. The results from previous studies cannot be directly compared due to varying: (i) lengths of data used, (ii) algorithms used and (iii) frequency bands used to segment the power spectrum of the HR data. Little is known about the characteristics of HRV in newborn infants in the early postnatal period. This is an important period for newborns, particularly those that have suffered a hypoxic injury. Targeted therapies for hypoxic ischaemia are now available but must be ideally instigated within 6 h of birth. It is often difficult to identify those babies that may benefit most from therapy but features of HRV analysis may prove useful. The aim of this paper is to describe the current methods for HRV in newborns and to use an optimum methodology to characterize HRV in newborn infants. This will serve to provide a robust reference dataset for studies of sick newborn infants. The early postnatal period is a vulnerable time for sick newborns. In babies that have suffered perinatal asphyxia, cooling therapy, investigated in the first few hours after birth may improve long term outcome. Early identification of newborns at rick or that have suffered a significant asphyxial event, in the early postnatal period is crucial for interventional therapies.

2. Methods

2.1. Subjects

Healthy term newborns were enrolled in this study if they met the following criteria:

- gestation >37 weeks;
- no requirement for resuscitation following delivery;
- apgar scores of >8 at 5 min;
- normal cord pH (>7.1).

Exclusion criteria were

- maternal epilepsy or diabetes;
- birth weight <2.5 kg;
- congenital abnormalities;
- admission to the neonatal ward for special or intensive care.

Following parental consent, potential newborns were examined using the Amiel-Tison assessment, a standardized neurological examination (Amiel-Tison 1977, 2002). Only newborns with a normal neurological examination were then recruited for the study. The study had full approval from the Clinical Ethics Committee of the Cork Teaching Hospitals.

2.2. Data acquisition

Video electroencephalogram (EEG) and electrocardiogram (ECG) data were recorded synchronously for each patient using the Viasys NicoletOne EEG system. All data were sampled at 256 Hz. Each newborn was in the supine position at their mother's bedside during each recording. All recordings were taken as soon as possible after birth and were continued for 1–2 h to include active sleep and quiet sleep (QS) phases. EEG was recorded from seven scalp electrodes positioned using the 10–20 system modified for newborns. Two shoulder ECG electrodes were used to record a single channel heart rate signal.

2.3. Data analysis

2.3.1. Sleep state classification. The electrographic sleep states were categorized as follows: (i) active sleep combined theta and delta frequency waveforms with intermixed low-amplitude irregular segments (alpha frequency and faster rhythms), (ii) quiet sleep is a combination of high amplitude slow waves, predominantly in the delta band, and tracé alternant segments (beta and theta activities alternating with delta activity) and (iii) indeterminate sleep (IS) according to Scher *et al* (2002). The EEG recordings were visually analysed by an expert in neonatal EEG (IK) and periods of AS, QS, IS and wakefulness were identified and annotated. In addition, the periods of QS were classified into trace alternant (TA) or high voltage slow waves (HVS). The scoring criteria for EEG sleep states, as defined by Scher *et al* (1992), are summarized in table 2. All instances of artefact or events that could influence the heart rate of the patient, such as deep breath, movement, etc, were manually labelled and excluded from the analysis.

2.3.2. HRV analysis.

QRS detection. A QRS detector was used to extract the HR from the ECG signal. The QRS detection algorithm was based on that developed by Hamilton and Tompkins (1986). This is a time-domain-based method which performs peak detection on the time-averaged first

Table 2. Scoring criteria for EEG sleep states as defined by Scher *et al* (1992).

Sleep state	Sleep state segment	Onset of sleep state
Active sleep	Period from onset of AS to onset of QS, excluding any intervals of IS at the transition	Beginning of segment of AS = 3 consecutive minutes of 3 out of 4 consecutive minutes scored as AS
Quiet sleep	Period from the onset of QS to onset of AS, excluding any periods of IS at the transition	Beginning of segment of QS = 3 consecutive minutes of 3 out of 4 consecutive minutes scored as QS.
Complete sleep cycle	Episode from onset of QS through a required period of AS (and IS if present) to onset of the next QS	

derivative of the ECG and then uses these markers to find the original R-wave maxima. The resulting R-points were visually inspected to ensure that the records contained no ectopic beats or artefact, i.e. that the RR tachogram contained only 'normal' beats, i.e. the NN intervals.

HRV-time domain. Five time domain measures were extracted from the NN intervals to characterize the interbeat variability:

- The mean HR in beats per minute, calculated as 60 divided by mean RR interval.
- The standard deviation of the NN interval (SDNN) which represents all the cyclic components of responsible for variability in the period of recording.
- The square root of the mean-squared differences of successive NN intervals (RMSSD) which is a measure of short-term variation.
- The percentage of successive RR intervals that differ by more than 25 ms, PNN25.
- The coefficient of variation (CV) is a normalized measure of the dispersion of a probability distribution.

HRV-frequency domain. PSD analysis quantifies how the power (i.e. the variance) of a signal is distributed as a function of frequency. Here the Lomb PSD estimation method is used, which can accommodate unevenly sampled data, hence the need for resampling is removed. The Lomb method for PSD estimation is based on the minimization of the squared differences between the projection of the signal onto the basis function and the signal under study. The Lomb Periodogram ($P_x(f)$) of a non-uniformly sampled real-valued data sequence $x(t_n)$ of length N as defined by Press *et al* (1992), is

$$P_x(f) = \frac{1}{2\sigma^2}\left\{\frac{\left[\sum_{n=1}^{N}(x(t_n)-\overline{x})\cos(2\pi f(t_n-\tau))\right]^2}{\sum_{n=1}^{N}\cos^2(2\pi f(t_n-\tau))} + \frac{\left[\sum_{n=1}^{N}(x(t_n)-\overline{x})\sin(2\pi f(t_n-\tau))\right]^2}{\sum_{n=1}^{N}\sin^2(2\pi f(t_n-\tau))}\right\} \tag{1}$$

where $\overline{x}$ and σ^2 are the mean and variance of the series and τ is an offset that makes the periodogram insensitive to time shift, where τ can be calculated (Press *et al* 1992), as follows:

$$\tau = \frac{1}{4\pi f}\arctan\left(\sum_{n=1}^{N}\sin(4\pi f t_n)\Big/\sum_{n=1}^{N}\cos(4\pi f t_n)\right). \tag{2}$$

The window length was chosen based on three factors: (i) the requirement of stationarity, (ii) minimum data length to ensure adequate resolution for the frequency range of interest and

(iii) maximum data length per patient, per sleep state that can be guaranteed to be free of any ectopy or artefact. The neonatal HR is stationary over 60s segments Garde *et al* (2001), however this requirement must be balanced with the minimum window length of two minutes, recommended by a task force on standards in HRV Malik *et al* (1996). Using a window length of 2 min, the minimum frequency that can be analysed is 1/120 (0.0083) Hz. The selection of a relatively short window length also ensured that few records had to be excluded due to ectopy or artefact.

The bands applied to the HR spectra were selected by considering the placement of peaks in the HR spectra observed in this study and previous studies. The VLF band applied here was 0.01–0.04 Hz. Giddens and Kitney (1985) and Baldzer *et al* (1989) both reported a notable peak between within this band. However, the physiological explanation of this component of HRV is much less defined than the activity in the LF and HF bands (Malik *et al* 1996).

The cutoff frequency between the LF and HF bands was set at 0.2 Hz, suggested initially by Dykes *et al* (1986) and has since been employed in the majority of studies of neonatal HRV, see table 1. This corresponds to the highest component of breath amplitude, which in some newborns modulates the LF component of the HR spectrum (Dykes *et al* 1986). The cutoff frequency used in adult studies of 0.15 Hz is based on the respiration rate of resting adults which ranges from 0.15 to 0.3 Hz (Patzak 1999). The respiration rate per patient was not available for this study, so the ranges used in other studies were investigated:

(i) 0.35–0.9 Hz (Longin *et al* 2005);
(ii) 0.5–1.9 Hz (Giddens and Kitney 1985);
(iii) 0.5–1.5 Hz (de Beer *et al* 2004);
(iv) 0.3–1.5 Hz (Patzak 1999).

These ranges illustrate that a cutoff frequency of 0.2 Hz ensures that even the lowest (0.3 Hz) respiratory rates will be captured in the HF band.

The upper frequency limit of the PSD estimate can be found by considering the Nyquist limit of the power spectrum. The Nyquist frequency is defined as half the average sampling frequency which, for heart rate analysis is the mean heart rate. The neonatal HR has been reported to vary over a wide range, 100–200 BPM (de Beer *et al* 2004). Therefore, the upper limit was calculated dynamically for each patient as half the mean HR (in Hz), fmax. From the neonatal respiration rates listed above it is clear that this dynamic upper limit may not always capture the potential RSA component of the HR spectrum. However, it does ensure that the maximum meaningful frequency for this method is not surpassed.

The following frequency bands, specific to the newborn, were applied:

(i) VLF: 0.01–0.04 Hz;
(ii) LF: 0.04–0.2 Hz;
(iii) HF: 0.2–fmax.

The power in these three bands was extracted, PVLF, PLF and PHF and the ratio PLF/PHF, a reflection of both sympathetic and parasympathetic response. Higher values of the ratio correspond to greater sympathetic and/or less parasympathetic response and lower values are indicative of less sympathetic and/or greater parasympathetic response.

Poincaré plot. The Poincaré plot can be used to graphically describe the beat-to-beat variability of RR intervals. It is constructed by plotting each RR time interval as a function of the immediately preceding one. Figure 1 shows an example of a Poincaré plot from a subject in this dataset. The dispersion points on the *X*- and *Y*-axes illustrate the overall range of the RR intervals. This can be quantified by extracting two measures from the plot, SD1 and SD2. SD1 is defined as the length of the transverse axis which is perpendicular to the line of identity and

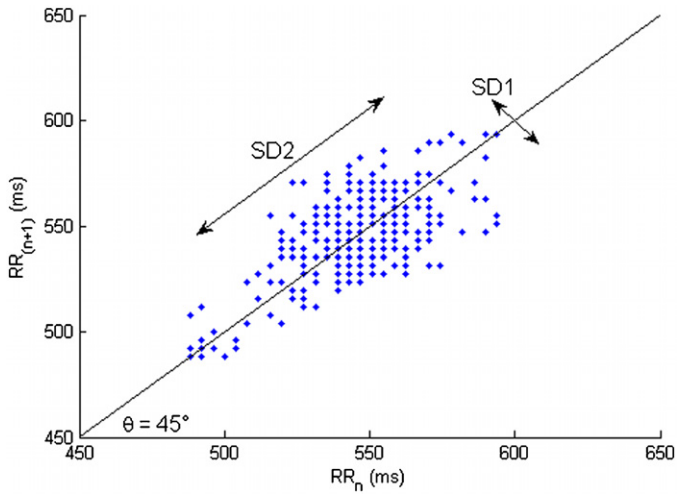

Figure 1. Example of Poincaré plot using RR data from *s* subject in this dataset during quiet sleep.

SD2 as the length of the longitudinal axis, parallel to the line of identity. When two adjacent RR intervals differ greatly (larger beat-to-beat variation) the resulting point will deviate from the line of identity, resulting in a larger SD1. Conversely, when the fluctuation in RR intervals is larger but continuous, the plotted points are distributed widely but along the line of identity, resulting in large SD2 but small SD1. Therefore, the two parameters measure different aspects of RR fluctuations. These are calculated by rotating the x- and y-axes by 45° and projecting the original points onto the transformed axes using the equations:

$$x' = x\cos\theta + y\sin\theta \tag{3}$$

$$y' = x\sin\theta - y\cos\theta \tag{4}$$

where x' and y' are the transformed points, x and y are the original co-ordinates and $\theta = 45°$. The standard deviation times four (in order to approximate the visual image on the Poincaré plot) of the differences along the rotated x- and y-axes were then calculated to give SD2 and SD1, respectively. From SD2 and SD1 a further two measures were computed, cardiac vagal index (CVI) and cardiac sympathetic index (CSI), where CVI = log(SD1 × SD2) and CSI = SD2/SD1.

Statistical analysis. To examine the separation between the measures in QS and AS a Mann–Whitney U test was used. A non-parametric test was applied as it cannot be assumed that all measures examined have a normal distribution. The strength of the relationships between some theoretically similar measures was evaluated by measuring their correlation using Spearman's coefficient, during each sleep state.

3. Results

EEG and ECG data were acquired from 30 newborn infants in the first 12 h of life. The recording length per baby ranged from 20.2 min to 80.5 min with an average length of 63.3 min. This dataset included 13 female and 17 male newborns with an average birth weight of 3.48 kg and gestational age of 39.8 weeks. All babies showed well-developed

Table 3. Mean results ± standard deviation across all patients and per sleep state, the *p*-values measure the separation between the means of the QS and AS groups.

	QS				AS				
Variables	Mean	SD	Median	IQR	Mean	SD	Median	IQR	*p*-Values
Time domain									
HR (BPM)	109.7	7.0	110.6	8.4	114	6.9	112.9	9.6	$p < 0.05$
SDNN (ms)	14.83	6.1	13.84	8.4	23.77	8.1	23.44	8.6	$p < 0.001$
RMSSD (ms)	11.91	5.4	11.20	9.7	11.43	5.1	11.85	5.84	NS
PNN25 (%)	5.7	6.15	1.4	12.9	4.7	7.58	4.0	6.3	NS
CV (%)	2.66	1.1	2.52	1.35	4.45	1.4	4.41	1.58	$p < 0.001$
SD2 (ms)	76.13	31.8	71.9	41.3	129.93	44.2	126.7	46.2	$p < 0.001$
SD1 (ms)	33.74	33.7	31.75	27.59	32.37	14.5	33.58	16.6	NS
CVI	7.66	0.96	7.73	1.3	8.19	0.78	8.31	0.7	$p < 0.05$
CSI	2.43	0.85	2.18	0.88	4.53	1.6	4.41	1.6	$p < 0.001$
Frequency domain									
PVLF (%)	32.9	15	29.9	23.5	41.3	17	39.8	17.7	$p < 0.05$
PLF (%)	36.4	13	32.78	11.9	46.36	16	49.3	19.2	$p < 0.01$
PHF (%)	32.2	15.3	30.8	23.7	13.0	5.7	12.9	8.5	$p < 0.001$
PLF/PHF	1.65	1.42	1.34	1.02	4.38	2.36	3.76	3.25	$p < 0.001$

sleep-wake cycling with the average percentage of AS-54.40%, QS-36.93%, IS-4.86% and wakefulness-4.1%. All recordings were made between 10:30 am and 17:00 pm.

The mean values across all patients and per sleep state were calculated and are shown in table 3. The separation between the mean of each measure in QS and AS was computed and the resulting *p*-values are also reported in table 3. The majority of measures were significantly different in QS than in AS, excluding RMSSD, PNN25 and SD1.

Three spectral regions of the HRV spectrum were analysed: (i) a VLF region from 0.01 to 0.04 Hz, (ii) a LF region from 0.04 to 0.2 Hz and (iii) a HF region >0.2 Hz. Figures 2(c), 2(d) and 3 are examples of HR spectra in QS and AS. From visual inspection, a dominant oscillation is observed in the VLF band at 0.01–0.02 Hz with at least one-third of the total power contained in this narrow band, across both sleep states. Several notable peaks are spread across the LF band with some appearing more locally dominant than others. The power in the HF band is variable across both sleep state and individual newborns. Figure 2(d) exhibits a well-pronounced peak in the HF band, this activity was observed in 24/30 (80%) of newborns during QS. On average 32% of the total power was contained in the HF band during QS. This was significantly reduced to 13% in AS, during which a similar prominent peak could not be identified in any patient. In the minority of patients (7/30) some occasional broadband activity which corresponded to approximately 20% of the total power was observed, see figure 3.

Figure 4 displays the trend of the power in the HF band, across all patients and both sleep states. This figure suggests that the power in the HF band is, on average, higher during QS. This increase was found to be statistically significant, see table 3.

The correlations between all measures were computed, see table 4. Three pairs of measures quantified slow and fast changes using three different approaches: time-domain (SDNN and RMSSD), frequency-domain (PLF and PHF) and Poincaré plot (SD1 and SD2) analyses. The time-domain and Poincaré analysis measures were highly correlated ($\rho > 0.9$) across both sleep states. However, the frequency-domain measures were not highly correlated ($\rho < 0.6$) with either pair. CSI was correlated ($\rho = 0.83$) with PLF/PHF only in QS, during

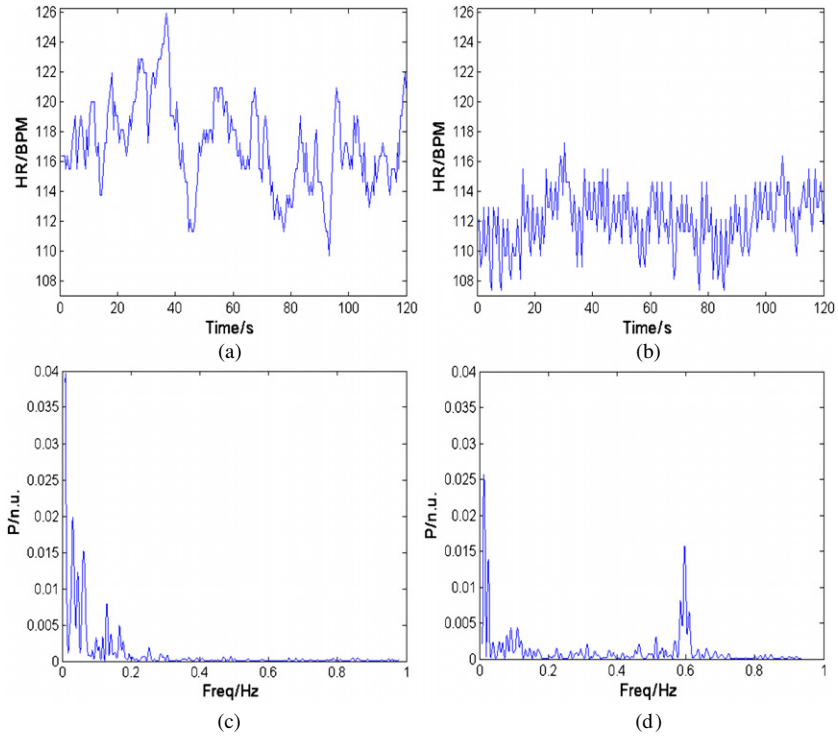

Figure 2. HR tachograms and Lomb PSDs of HR series during QS and AS (patient 10).

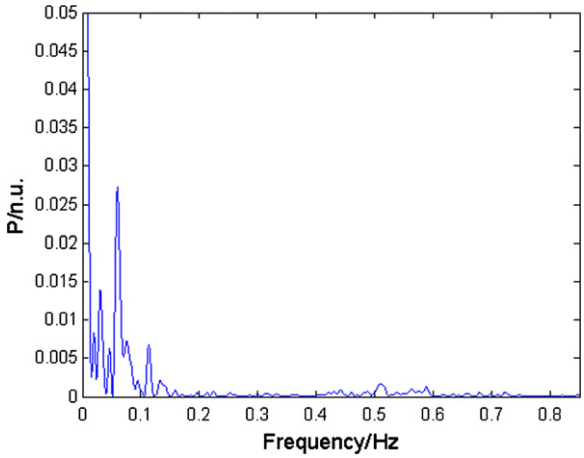

Figure 3. Lomb PSD of HR series during AS for patient 18.

which both measures decreased significantly ($p < 0.001$). No strong negative correlation ($\rho = -0.372$) was observed between CVI and PLF/PHF during QS nor was a strong positive correlation ($\rho = 0.234$) seen between PHF and CVI. In general, the time-domain (RMSSD, SDNN, PNN25, CV) and the Poincaré (SD1, SD2, CVI, CSI) measures all reported quite a high level of correlation ($0.7 < \rho < 0.99$) across both sleep states. The frequency-domain

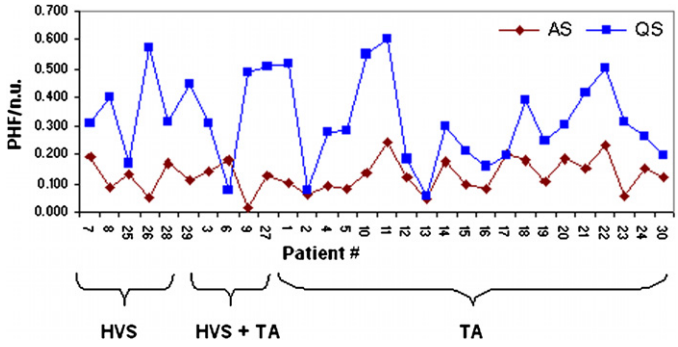

Figure 4. Power in the HF band (0.2 Hz and upwards), per patient and per sleep state. The data are ordered according to QS state: high voltage slow wave (HVS), tracè alternant (TA) and a combination of the two (HVS + TA).

Table 4. Spearman coefficient (ρ) of the correlations among measures.

	SDNN	CV	pNN25	SD1	SD2	CVI	CSI	PLF	PHF	PLF/PHF
					AS					
RMSSD	0.708**	0.701**	0.945**	0.999**	0.666**	0.899**	−0.586**	−0.113	0.539**	−0.447**
SDNN		0.988**	0.638**	0.708**	0.994**	0.894**	0.006	−0.348	0.030	−0.126
CV			0.630**	0.701**	0.981**	0.882**	0.033	−0.381*	0.020	−0.130
PNN25				0.945**	0.589**	0.859**	−0.607**	−0.026	0.567**	−0.446*
SD1					0.666**	0.927**	−0.586**	−0.113	0.539**	−0.477**
SD2						0.860**	0.037	−0.355	−0.018	−0.094
CVI							−0.345	−0.228	0.348	−0.353
CSI								−0.236	−0.641**	0.447*
PLF									−0.056	0.506**
PHF										−0.865**
					QS					
RMSSD	0.830**	0.840**	0.922**	0.999**	0.736**	0.898**	−0.503*	−0.179	0.478**	−0.520**
SDNN		0.992**	0.822**	0.830**	0.988**	0.929**	0	−0.109	0	−0.176
CV			0.844**	0.875**	0.982**	0.882**	−0.037	−0.108	0.022	−0.205
PNN25				0.922**	0.814**	0.764**	−0.282	−0.139	0.187	−0.308
SD1					0.763**	0.909**	−0.461*	−0.211	0.539**	−0.520**
SD2						0.899**	0.088	−0.089	−0.087	−0.109
CVI							−0.151	−0.125	0.234	−0.372*
CSI								0.291	−0.811**	0.830**
PLF									−0.356	0.634**
PHF										−0.804**

* significant at the 0.05 level,
** significant at 0.01 level.

measures did not display a high level of correlation to the other two sets, with the exception of CSI and PLF/PHF during QS.

4. Discussion

In this study, both time and frequency-domain analyses were used to describe HRV measures in healthy newborn infants in the first 12 h after birth. Similar studies have also examined

these measures but comparisons of the numerical results cannot be made due to variations in methodology and in the age of the newborns. Significant differences in HRV measures were found during quiet and active sleep in healthy newborn infants in the early postnatal period.

Time and frequency-domain measures have previously been used to describe HRV in full-term, healthy newborn infants (Baldzer *et al* 1989, Aarimaa *et al* 1988, Longin *et al* 2005). All infants in these studies were generally older (over 24 h) and clinical sleep state classification systems were used. Our study is different to most of these previous studies for a number of reasons; all newborn infants were less then 12 h old and simultaneous EEG monitoring was used to assess sleep state. The methods used here to estimate the HRV measures are different to most in table 1 and include alternative frequency bands that are specific to newborns and a different method of HR spectrum calculation.

The frequency bands used in this study are relative to the physiological rates of the newborn, which differs greatly from the adult. Oscillation in the LF band, which generally occurs at 0.1 Hz in adults, is attributed to fluctuations in the baroreceptor loop. This oscillation is generally seen at 0.07 Hz in newborn infants reflecting the longer baroreceptor latency time (Giddens and Kitney 1985). The HF band in adults, 0.15–0.4 Hz, which captures the RSA component is based on the respiration rate of resting adults which ranges from 0.15 to 0.3 Hz (Patzak 1999). Neonatal respiration is much faster and this must be considered in HRV frequency-domain analysis. We did not measure respiration rate simultaneously during this study, though respiration was observed using video monitoring. In order to calculate the band which captures RSA we used neonatal respiration ranges from previous studies as a guide (Patzak 1999, Longin *et al* 2005, Giddens and Kitney 1985, de Beer *et al* 2004). We also used a lower frequency limit of 0.2 Hz and assigned the upper limit dynamically to each record (calculated at half the mean HR, in Hz). This ensured that the Nyquist criterion was always met and eliminated aliasing errors. It also ensured that no information was unnecessarily discarded.

We used the Lomb periodogram to extract the HRV spectrum from the unevenly sampled HR series. This technique avoids the low pass effect of resampling and prevents the introduction of artefactual components due to inadequate consideration of the highest frequency up to which the baseband extends. Several studies have concluded that the Lomb method does provide better estimates of the HR spectrum than classical methods (Moody 1993, Laguna *et al* 1998, Chang *et al* 2001).

Thirteen time-domain, frequency-domain and Poincaré plot geometry measures were extracted from the heart rate time series. Toichi *et al* developed the indices CSI and CVI. CVI was found to decrease significantly in the presence of a parasympathetic blockade (induced by atropine) and similarly, CSI was found to decrease significantly during a sympathetic blockade (induced by propranolol) (Toichi *et al* 1997). The PLF/PHF ratio is a reflection of both sympathetic and vagal response. Higher values of the ratio correspond to greater sympathetic and/or less vagal response (Baldzer *et al* 1989). RSA amplitude can also reflect the vagal innervation of the autonomic system. In this study, RSA has been quantified by measuring the power in the HF band and normalizing this by the total power. CV has been reported to display a high correlation with vagal tone (Hayano *et al* 1991).

In QS vagal tone predominates (Baldzer *et al* 1989). PLF/PHF and CSI are significantly reduced in the transition from AS to QS, as expected, as they both quantify sympathetic activity. PHF increased significantly, as expected as it quantifies vagal innervation. However, CVI and CV are both lower in QS than in AS. Toichi *et al* also reported that CV only reflected a mild cardiac autonomic change which was subject to conditions such as the subject being in the supine position. From table 4 it can also be seen that CVI and CV are highly correlated with SDNN, RMSSD and PNN25. This implies that for this population, CVI and CV capture

the overall variability of the heart rate more so than its vagal component. However, LF/HF and CSI are more sensitive to changes in the sympathetic-vagal balance.

The presence of RSA in newborn infants has been reported in previous studies but the results have been inconsistent. Longin *et al* (2005) did not find a peak corresponding to RSA in any of the 88 newborns examined, despite analysing periods of QS and AS. Baldzer *et al* (1989) reported the presence of RSA in 11/30 patients. None of the newborns in a study by Giddens and Kitney (1985) exhibited notable RSA. In a study by Aarimaa *et al* (1988) a peak corresponding to RSA emerged in the normal newborn in the first 5 days of life. Our results are not in agreement with the results of these studies. We observed a peak in the RSA range in 80% of newborn infants. On average, this peak contributed to 22.1% of total power during QS; this value was significantly lower in AS (6%) and a prominent peak was not observed.

We did not record a possible RSA peak in 20% of babies during QS. This cannot be explained by the influence of circadium rhythms as all recordings were made during daylight hours, ranging from late morning to early evening. The PLF/PHF ratio is significantly lower in QS, implying that the parasympathetic system is dominant and the respiratory drive is under autonomic control resulting in slower and more regular respiration. This is the most probable physiological explanation for prominent RSA in QS. The absence of RSA could not be attributed to a certain type of QS, either TA or HVS or TA/HVS. From figure 2, it can be seen that newborns with PHF <0.2 are distributed evenly across each of the three QS states with some of the highest and lowest values observed during TA.

Several hypotheses were proposed by previous studies to explain the reported absence of RSA:

(i) the respiratory peak is difficult to detect because of the high respiration rate and thereby small breathing amplitude, termed as breathing amplitude sinus arrhythmia (BASA) (Longin *et al* 2005).
(ii) cardiac aliasing, whereby the respiration rate exceeds half the mean HR;
(iii) immature cardiorespiratory control.

A direct measure of respiration rate was not available for this study. However, we did observe clear respiratory movements on video recordings and were able to estimate the respiratory rate for each infant. We believe that the presence of a probable RSA peak in 80% of babies reflects well-developed cardiorespiratory control. The upper frequency limit in the HF and RSA band was dynamically assigned to ensure only meaningful frequencies were included in the HR spectrum. However, if the respiration rate of the newborn exceeded half the mean heart rate then a potential occurrence of RSA would be missed by our method of quantification. This could explain why RSA was not observed in all newborns during QS.

Three measures RMSSD, PNN25 and SD1 did not exhibit a statistically significant difference between QS and AS. RMSSD can be thought of as a measure of fast changes in the RR series or short-term variability. SD1, as calculated from the Poincaré plot, is also a measure of short-term variability on a beat-to-beat basis, i.e. high frequency change. PNN25, a measure suggested by Longin *et al* (2006), is based on the adult measure PNN50, i.e. the percentage of consecutive intervals that differ by greater than 50 ms. The threshold is calculated as 6.25% of the average RR interval length, therefore a markedly lower value of 25 ms is used in this study as newborn infants have a higher mean heart rate than adults. All three measures were found to exhibit a significant mutual correlation, as expected. Bernston *et al* (2005) reported a high correlation between RMSSD and HF variability in the respiratory range. A strong correlation was not observed ($\rho < 0.6$) between PHF and RMSSD, PNN25 or SD1. This suggests that while RMSSD, PNN25 and SD1 can be thought of as high frequency measures, neither measure accurately captures the high frequency activity in QS, namely RSA.

To our knowledge, this is the first study to examine normative time- and frequency-domain HRV data at such an early age in the normal newborn. These normative set of HRV indices based on both time- and frequency-domain values may provide useful reference data in this population particularly, when the same measures are examined in sick newborns. We have studied term babies in the first 12 h to avoid maturational effects that have been reported to occur over the first 5 postnatal days (Aarimaa *et al* 1988). These normative data may provide useful references for the assessment of babies that have suffered significant hypoxic ischaemic injury at birth.

Acknowledgments

This work was supported by a Science Foundation Ireland (SFI), Principal investigator career advancement award (SFI/05/PICA/1836)

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