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Features Based on Heart Rate Variability Capture Regulatory Changes during Kangaroo Care in Preterm Infants

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Running title: Kangaroo Care Changes HRV in Preterm Infants

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List of key words not in the title: Autonomic Regulation

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Abstract

**Objective:** We hypothesize that heart rate variability (HRV) can be used as a surrogate measure to track regulatory changes during kangaroo care (KC), a period of parental co-regulation believed to be superior to regulation within the incubator. **Study Design:** Nurses annotated the start and end times of KC for three months. The pre-KC, during-KC and post-KC period of monitor data were retrieved in infants that had at least 10 accurately annotated KC sessions. Eight HRV-features (five in time-domain and three in frequency-domain) were used to visually and statistically compare the pre-KC and during-KC periods. Of these features two were novel, capturing the percentage (pDec) and extent of heart rate decelerations (SDDec) respectively. **Results:** A total of 191 KC sessions were investigated in 11 preterm infants and despite clinically irrelevant changes in vital signs, six of the eight HRV-features showed a visible and statistically significant difference between stable periods of KC and pre-KC (SDNN, RMSSD, pNN50, SDDec, HF power and LF/HF ratio; \( p < 0.01 \)). HRV reduced during KC due to a decrease in the extent of transient heart rate decelerations. **Conclusion:** HRV-based features may be clinically useful to capture the dynamic changes in autonomic regulation in response to KC.

Key words: Heart rate variability, Kangaroo care, Preterm infants; Dynamic Regulation
Abbreviations

HRV – Heart rate variability
KC – Kangaroo care
HR – Heart rate
SpO₂ – Oxygen saturation
ANS – Autonomic nervous system
BR – Breathing rate
SNS – Sympathetic nervous system
PSNS – Parasympathetic nervous system
NN - Normal-to-normal
SDNN – Standard deviation of normal-to-normal
RMSSD – Root mean square of the standard deviation
pNN50 – Percentage of consecutive NN-intervals that differ by more than 50 ms
LF – Low frequency
HF – High frequency
SDDec – Standard deviation of deceleration
pDec – percentage of decelerations
Introduction

Kangaroo care (KC) refers to a period of direct skin-to-skin contact in which infants are placed in the prone position on the naked parental chest. It is proven to be safe and is known to reduce morbidity and mortality, even in extremely preterm infants (1,2). KC is associated with important physiological benefits such as promoting quiet sleep, enhancing thermoregulation and reducing crying/fussy behavior (3). It even mitigates physiological responses to procedural pain since painful stimuli during KC, as opposed to during routine care, are associated with smaller increases in heart rate (HR) and more stable values of oxygen saturation (SpO₂) (4,5). This indicates that parental co-regulation is superior to regulation within the incubator environment and that KC positively influences autonomic regulation (6). The ability to track and quantify any changes that occur due to KC can help in detecting and establishing patterns of improved regulation in preterm infants and thereby offer opportunities to enhance neurodevelopmental care and homeostatic regulation.

The role of the autonomic nervous system (ANS) in controlling homeostatic regulation can be evaluated by tracking cardiorespiratory parameters such as HR, breathing rate (BR), SpO₂ and temperature. In a number of previous studies, these parameters were found to be stable during KC (7), supporting the conclusion that KC is safe. However, this finding in itself does not provide insight into any underlying physiological changes that were triggered by KC.

While the average HR may not divulge information on any regulatory changes, the physiological phenomenon of heart rate variability (HRV), i.e., the variation in the time intervals between consecutive heartbeats can provide additional physiological insight. HRV reflects the dynamic, fast occurring changes in autonomic regulation caused by the primary systems controlling the HR. In addition to humoral factors, these are the sympathetic nervous system (SNS) and the parasympathetic nervous system (PSNS) and these can influence the heart rate instantly, i.e. from beat-to-beat (8,9). Multiple features analyzing these beat-to-beat changes have been constructed and studied in adults, with at least some consensus on the interpretations for the same. For instance, the standard deviation (SD) of the RR-peak
intervals reflects overall variability whereas differences in successive RR-intervals largely reflect the activity of the PSNS (8).

In neonates, however, HRV has been less thoroughly explored. Moreover, the behavior of a neonatal heart and in particular that of a premature neonate is significantly different from that of an adult heart, reflecting underlying differences in autonomic regulation. This suggests that interpretations of HRV-based features in neonates may differ from those of adults (9,10). For example, unlike adults, neonates display a significantly larger range of variation in their heart rate and respiratory rate and they are prone to both acute tachycardia and bradycardia (9,11), suggesting that HRV-features should account for this intrinsically different aspect of neonatal physiology.

While KC has been shown to enhance autonomic maturation (12), only a few studies have looked into the dynamic changes in HRV during KC, notably without consensus on the findings (13,14). No studies have visualized changes in HRV during KC and compared it with HRV while the baby was in the incubator.

We hypothesize that regulatory changes in preterm infants can be captured using HRV-based features. The aim of this study is to employ HRV-based features in order to track regulatory changes that occur as a result of KC, a period of improved regulation. Furthermore, the HR, BR, temperature and SpO₂ are also analyzed in order to provide a holistic perspective on regulation.
Methods

Clinical setting and patient monitoring

The Máxima Medical Center has an 18-bed, level III, tertiary NICU with private rooms where KC is practiced routinely. Parents are encouraged to perform KC for durations of sixty minutes or longer. Routine patient monitoring continues during KC, including measuring the ECG, HR, BR (using impedance pneumography), SpO\textsubscript{2} and temperature (measured in the diaper). Patient monitors from Philips (IntelliVue MX 800, Germany) are used for monitoring and have the provision to save the parameter data (HR, BR, SpO\textsubscript{2} and temperature; one value every minute) and the 3-lead ECG (sampled at 125 Hz) of the past 72 hours of measurement. Notably, ECG sampled at 125 Hz have proven to be sufficient for determining HRV-based features (15). For this study, nurses annotated all KC sessions by recording the start time (placement on parental chest) and the end time (placement into the incubator) of kangarooing based on the patient-monitor time for a period of three months, between August-October 2015.

Participants

This study was part of a comprehensive observational perinatal monitoring research program (IMPULS 1) conducted at the Máxima Medical Center, in collaboration with Eindhoven University of Technology and Philips Research, for which approval was provided by the local ethical committee (Core and peripheral temperature measurement in the NICU during interventions ID 2012-0120; 2\textsuperscript{nd} February, 2015). We selected neonates with more than ten annotated KC sessions within the study period to account for intra-patient variability and maturational differences. This yielded a total of 220 KC sessions from 11 neonates, six male and five female. We excluded KC sessions where infants were mechanically ventilated or diagnosed with an infection, a congenital anomaly or a severe brain pathology (periventricular leukomalacia or intraventricular hemorrhage grade III/IV). In addition, the KC sessions had to (i.) last one hour or longer, (ii.) have data for at least one hour in the pre-KC and post-KC periods and (iii.) the pre-KC/post-KC period did not overlap with the post-KC/pre-KC period of
another KC session within the same infant. Table 1 characterizes the patient metadata at birth (first two rows) and across the KC sessions that were used in the study.

**Study design**

In order to observe regulatory changes in infants as a result of KC, HRV-features from the pre-KC period were visually and statistically compared with HRV-features during KC. For the purpose of visualization, we chose to retrieve data for the 60 minutes before KC, the variable duration of KC and for the 60 minutes post-KC. Since the duration of KC was variable, but at least 60 minutes long, the first 30 minutes and the last 30 minutes of each KC session were retained for visualizations (Figure 1).

For statistical analyses of parameter data (vital signs) and HRV-features, stable epochs were determined in the pre-KC and during-KC periods (Figure 1). Based on expert opinions we determined that the first 30 minutes of the pre-KC epoch are stable, whereas in the last 30 minutes nursing care events such as diaper-change take place with or without parental assistance. The period of KC itself is assumed stable after 15 minutes, allowing any physiological changes arising because of the transition to decay. Therefore, the first 30 minutes from the pre-KC period were compared with the 30-minute epoch in between the 16th - 45th minute of KC (displayed as the 76th – 105th minute of continuous data in Figure 1). Note that this epoch is centered about the middle and visualized in its entirety only for those KC sessions that are exactly 60 minutes long. The median and interquartile ranges for the mean values of the HRV-features corresponding to these 30-minute epochs were calculated.

**Parameter data**

The parameter values of HR, BR, SpO₂ and temperature were sampled every minute and required no further processing. The median and interdecile ranges of the mean values of the stable 30-min epochs from the pre-KC and during KC periods were calculated.
**HRV-features**

A peak detection algorithm was employed to detect the R-wave peaks in the ECG recordings and calculate the RR-intervals, also called normal-to-normal intervals (NN-intervals), corresponding to the time intervals between successive normal heart beats (e.g. not ectopic) (16). As a first step, normalized histograms were built for the concatenated pre-KC, during KC and post-KC periods for the purpose of visualizing the variations in NN-intervals. This was done using a bin size of 20ms (NN-intervals less than 200ms and greater than 600 ms were rejected on an empirical basis). Consecutively, time domain features were obtained by calculating the standard deviation of the NN-intervals (SDNN), the root mean square of the successive differences in NN-intervals (RMSSD) and the percentage of consecutive NN-intervals that differed by more than 50 ms (pNN50) every minute using data of the past five minutes. Furthermore, since transient repetitive heart rate decelerations are potentially a sign of distress/illness (17,18), yet still a component that increases HRV, a feature termed pDec (percentage of decelerations) was developed and defined as the percentage of NN-intervals larger than the mean NN-interval of the past five minutes. This feature aims at explicitly extracting variations in HRV arising due to decelerations. While the total number of transient decelerations yields additional information, the magnitude of decelerations (since a HR decelerating to 120 bpm differs from a deceleration to 60 bpm) is captured in the SDDec. The SDDec measures the standard deviation of all NN-intervals that contribute to pDec.

Frequency domain analysis was performed after resampling the NN-intervals at a frequency of 20 Hz along a uniform time axis using linear interpolation and calculating the discrete Fourier transform every minute using a moving average window of five minutes on detrended data. In adults, low frequency (LF) variation is reported to reflect SNS-driven baroreceptor activity while high frequency (HF) variation captures the vagally mediated respiratory sinus arrhythmia, resulting in an LF/HF ratio reflective of the sympatho-vagal balance. We calculated the LF power, HF power and the LF/HF ratio after defining the LF and HF bands as 0.04-0.15
Hz and 0.4-1.5 Hz respectively. Table 2 (online) gives an overview of the eight HRV-features that were used and their corresponding physiological interpretations in adults.

The mean and the standard error of the mean were calculated every minute for all HRV-features using a moving average window of five minutes. Since there can be differences in baseline values between different infants or between different KC sessions within the same infant (e.g., as a result of maturation), baseline removal was carried out by subtracting the mean value of the feature in the first 30 minutes of pre-KC from its corresponding time series.

**Statistical analysis**

We compared the differences in the mean values of the parameter data and the HRV-features corresponding to the stable epochs of the pre-KC and during-KC periods using the two-sided paired Wilcoxon signed rank test. A left tailed Wilcoxon rank-sum test was used for the concatenated pre-KC and during-KC NN-intervals to test the hypothesis that the median NN-intervals were higher during KC (i.e., lower HR). A p-value of 0.01 was considered significant.

**Results**

Data from the 30-minute long stable epochs during KC were analyzed for changes in vital signs and HRV-features with respect to the 30-minute long stable epochs from the pre-KC incubator period for a total of 191 KC sessions obtained from 11 preterm infants. For the vital signs, only HR and BR showed a statistically significant, albeit small change (p<0.01). For the pre-KC and KC epochs, the median (interdecile) values for HR are 159 (146-170) and 156 (145-167) respectively while for BR they are 49 (42-62) and 47(38-61) respectively.

The normalized histograms of the NN-intervals corresponding to the periods of the concatenated pre-KC, during-KC and post-KC data are shown in Figure 2 (online). The purpose of this figure was to visualize the distribution of NN-intervals. Overall, a greater
percentage of NN-intervals were shorter during the pre-KC period, when compared with the during-KC period (p-value <0.001).

An in depth analysis of the dynamic changes in NN-intervals in response to KC is visualized using the eight HRV-features in Figure 3. As described in the methodology (Figure 1), they represent the data of the pre-KC period, the first 30 minutes of KC, the last 30 minutes of KC and the post-KC period. Figure 3 shows that all features respond notably to the transition from incubator to the parental chest and vice-versa. Six of the eight features, with the exception of pDec and LF power, show a significant change in the baseline value between the pre-KC and KC periods (p<0.01, Table 3).

**Discussion**

In this study, 191 KC sessions were investigated in 11 preterm infants to analyze changes in HRV-features as a result of KC. Six features, the SDNN, RMSSD, pNN50, SDDec, HF power and the LF/HF ratio showed statistically significant and clearly visible differences during KC in comparison with the pre-KC period while changes in vital signs were small and clinically irrelevant. Although stable vital signs during KC have been reported previously (1,2,7), the novelty of this study is using interpretable features based on HRV to show changes in autonomic regulation as a result of KC.

It is undisputed that KC is a highly comfortable period for both the baby and the parent, and it is therefore assumed to represent a state of improved regulation in an infant (1,2,4–7). Remarkably the time domain measures of HRV – the SDNN, the RMSSD and the pNN50 – decrease during KC. In adults, a decrease in overall HRV is associated with cardiovascular morbidity and mortality (9). However, in premature infants the cardiorespiratory physiology is very different from adults. For example, preterm infants are prone to rapid and transient heart rate decelerations, which can flaw the interpretation of overall HRV.
Figure 2 (online) indirectly supports this, showing that despite HR being higher in the pre-KC period (larger number of short NN-intervals), there are similar number of NN-intervals that are longer, suggesting the presence of extensive decelerations.

Additionally, previous studies in the context of detecting neonatal sepsis show that abnormal heart rate characteristics are composed of transient heart rate decelerations in addition to the normal variability (19). Analogue to these observations, we constructed two new features, the pDec and the SDDec to independently study decelerations and the extent of decelerations, in addition to employing existing features to study overall HRV.

While the percentage of decelerations (pDec) remains remarkably similar in the periods of KC and pre-KC, the magnitude of deceleration (SDDec) decreases during KC. This is an interesting finding suggesting that fewer instances of extreme, transient bradycardia are in fact resulting in a decrease in overall HRV (SDNN, RMSSD, pNN50) seen during KC. Notably, more extreme decelerations can be seen during the stressful period corresponding to patient handling just preceding the start of KC and both periods of transfer.

We speculate that this instability leads to higher HRV as a result of an immature ANS and a dominant SNS that tends to overshoot during autoregulation. The SNS is a slower acting system, normally prevailed by the quicker, cholinergic, myelinated PSNS innervation (8). The dominance of the SNS at preterm birth has been established in previous research and is most likely a result of delayed maturation of certain PSNS branches (peaking at 31-38 weeks GA and maturing beyond term) (9,20–22). In addition, chronic exposure to stress in the NICU might also lead to a dominant SNS (23). In neonates, it has been proven that chronic stress disturbs the stress axis functioning, likely causing an overactive and unstable sympathetic system (6,24,25). The assumption that higher levels of HRV reflect instability is supported by the fact that the SDNN, RMSSD, pNN50 and SDDec appeared highest during the second half of the pre-KC period, a period in which patient handling frequently occurs.
Along the line of Porges’ Polyvagal Theory, we hypothesize that the immediate decrease in these HRV-features during KC may be caused by a switch in the neural mechanisms responsible for regulating the neurobehavioral state in order to deal with environmental challenges (26,27). Three such mechanisms have been described of which, evolutionarily speaking, the two most primitive mechanisms appear active in the pre-KC period. These involve the dorsal branch of the vagus, responding to challenges with immobilization and freezing of regulatory systems, and the SNS, which is necessary for the ‘fight or flight’ mode but sub-optimal for subtle regulation. The third mechanism, uniquely mammalian, involves a myelinated branch of the vagus and thus enables rapid regulation of cardiac output. We theorize that the decrease in the SDNN, RMSSD, pNN50 and SDDec features seen in this study demonstrates the rapid transition from the dominant unmyelinated PSNS and the SNS to the more stable regulation offered by the myelinated vagus in response to parental co-regulation. The myelinated vagus also happens to be neuroanatomically linked to other cranial nerves that are responsible for regulating social engagement and for responding to challenges by engaging in calming/soothing behavior through social communication (27). The existence of a rapid parasympathetic vagal reflex has been reported even in extremely preterm infants (28). The higher time-domain HRV values that are found with increasing gestational age in multiple longitudinal studies could also be the result of a developmental shift in the balance between these mechanisms (29,30).

This challenges the interpretation of HRV-features in the frequency domain for preterm infants. For instance, in adults the HF power is believed to reflect vagal activity, originating almost exclusively from the myelinated branches, whereas in preterm infants the balance between myelinated versus unmyelinated vagal activity might be altered. In a study in which atropine, a PSNS blocker, was administered to 12 preterm infants aged 26-32 weeks the LF power decreased more than HF power (28). This suggests that in preterm infants the PSNS has a relatively large contribution in the LF, leading to a non-interpretable LF/HF ratio. In our study the LF/HF ratio increased during KC, in contrast to what would have been expected in adults.
The HF power decreased significantly, likely due to the fact that decelerations during this period were less extreme and therefore the total signal (tachograms) has fewer HF-components. These findings and previous multiple studies suggest that in preterm infants, relaxation or the sympatho-vagal balance is not captured by the LF/HF ratio (13,28,31,32). Theoretically speaking an increased sympathetic drive in response to KC could also account for this. However, an entire body of literature that shows increased parasympathetic behavior in response to KC suggests otherwise (1,2,4–7). Other research investigating HRV in preterm infants (with or without additional stimuli like a heel prick) supports our findings in the frequency domain for routine KC (13,14,32–34).

This study has several limitations that need to be addressed, for example in a case study, McCain et al. attribute similar findings to sleep. Although changes in sleep stages could have contributed to HRV changes (22,35), the rapid manifestation of HRV changes following transfers suggests otherwise. Given this rapidity, we assume that changes in blood pressure due to transfer and the change in position from the incubator to KC are more relevant covariates. Additionally, mothers often start preparing for ending KC before the transfer by waking up, changing position, calling the nurse, etc. We believe this is the reason why the last period of KC (leading up to Ty, Figure 3) shows a dampened response. Despite these limitations, KC might be one of the best possible controlled settings for observational studies investigating HRV in a NICU. Due to the protocolized nature of KC, it offers greater consistency in positioning, handling, drug administration, etc. than daily life in an incubator.

Another limitation is the possibility of one-two minute errors in the nurse-annotations of KC. In addition to that, responses to KC might depend on the gender of the infant, whether KC was performed by the mother or the father, and on the infant’s age. It is well-known that HRV-values vary with age (both gestational and postnatal) and therefore it is difficult to interpret HRV-values in a heterogeneous population without correction for maturation (8). Nonetheless, in this study each infant was its own control and changes in HRV and not the absolute values were studied. Finally, infants did not contribute equal number of KC sessions to the study,
potentially skewing the findings as a result of habituation to KC. However, even after removing the contributions of infants one at a time, HRV-features responded to KC in a similar fashion. Also, the baseline removal carried out for all features inherently adjusts for inter and intra patient differences in baseline.

Therefore, taking the limitations into account, this study offers the possibility to track changes in autonomic regulation across periods of KC and thus helps in quantitatively establishing states of increased comfort. In the future, this technique holds potential to be used as a comfort-tracker. Moreover, the visualization of a physiological response to KC allows nurses to promote KC and increase the involvement of family. Furthermore, as can be seen from the HRV-features in Figure 3, there appears to be a lasting impact of KC even after the infant is moved from the parental chest to the incubator which we will investigate in future studies.

In conclusion, we used features based on HRV to quantify and visualize changes in regulation as a result of KC. Two new features were developed to decompose HRV in preterm infants in order to independently study the contribution of transient decelerations to HRV. HRV changes significantly during KC with respect to the pre-KC period, despite clinically insignificant changes in vitals.

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Figure legends

Figure 1. An illustration of the methodology

The pre-KC and post-KC sessions are 60 minutes long. The duration of KC can vary (first 30 minutes + $\Delta T$ + last 30 minutes) and therefore the first 30 minutes and the last 30 minutes of KC are used for visualization. The time required to transfer the baby from the incubator to parental chest (Tx) and vice-versa (Ty) are short but variable. A and B refer to the first minute on the parental chest and the first minute back into the incubator respectively. The first 30 minutes of the pre-KC period are free from nursing care and are thus considered to be a stable period within the incubator (pre-KC epoch). KC is considered to be stable 15 minutes after its onset since the initial transition from incubator to parental chest can effect physiology. The next 30 minutes, corresponding to the 76th-105th minute of data, are therefore used as stable epochs for statistical comparisons with the stable pre-KC epoch.

Figure 2. The normalized histograms of the pre-KC (blue), during KC (red) and post-KC (green) NN-intervals for the concatenated KC sessions.

The x-axis displays the NN-intervals in seconds while the y-axis represents the percentage of total NN-intervals. There are significantly more NN-intervals of smaller values in the pre-KC period (p-value<0.001, Wilcoxon rank-sum test), suggesting a higher heart rate during this period.

Figure 3. Composite figure for the time-series of eight HRV-features

The mean and standard error of the mean are shown for the pre-KC period ($T_1$), the first 30 minutes of KC ($T_2$), the last 30 minutes of KC ($T$) and the post-KC period ($T_4$). The vertical line between $T_2$ and $T_3$ is a gap of variable length since KC durations can be longer than 60 minutes. $T_x$ and $T_y$ represent the periods of transfer from the incubator to the parental chest and vice versa. The x-axes represent time in minutes while the y-axes represent the
normalized values of HRV-features. **A.** represents SDNN; **B.** RMSSD; **C.** pNN50; **D.** pDec; **E.** SDDec; **F.** LF power; **G.** HF power; **H.** LF/HF ratio.