Heart Rate Variability in Extremely Preterm Infants Receiving Non-Synchronized Nasal Intermittent Positive Pressure Ventilation and Continuous Positive Airway Pressure Immediately After Extubation

Thesis by

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August 2016

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

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Acknowledgments

There are many people who merit recognition for their support of this thesis. First and foremost, my sincerest thanks goes to my supervisor, Dr. Guilherme Sant'Anna, for providing me with the opportunity to explore the beautiful, complex, and continuously developing world of neonatology. By being involved in various research projects, Dr. Sant'Anna has provided me with a stimulating and challenging environment, which has enriched my academic experience and encouraged me to think critically in search of ways to improve the medical field. My training was financially supported through Dr. Sant'Anna's operating grant from the Canadian Institute of Health Research.

I am grateful to Ali Al-Jabri, Philippe Lamer, and Lara Kanbar for their work in acquiring the data used for this thesis.

I would also like to thank to Dr. Wissam Shalish for training me in clinical research and teaching me about neonatology, and Dr. Robert Kearney, Dr. Karen Brown, Dr. Doina Precup, Lara Kanbar, and Carlos Robles-Rubio for their input and suggestions throughout my Master's training.

I thank our research assistant, Smita Rao, and fellow Master's students, Cristina Sciortino and Monica Bhuller, for their ongoing support, and for creating a friendly and helpful environment to work in.

For the manufacturing of the RIP bands, I thank my father, Mike Latremouille, and Dr. Ross Wagner for their teaching and guidance in electronics and biomedical engineering, areas of expertise that I knew very little about. Additionally, I would like to thank Lara Kanbar and Dr. Shalish for the validation study on the RIP bands.

Finally, I thank my parents for their never-failing support. Their encouragement in my academic endeavours has instilled the confidence in me necessary to pursue my graduate work. To them, I dedicate this thesis.

Abstract

Background: After extubation, extremely preterm infants are supported with some type of non-invasive respiratory therapy to prevent extubation failure. The two most common modes are nasal intermittent positive pressure ventilation (NIPPV) and continuous positive airway pressure (CPAP). Both types have similar physiological effects on stable infants, but in infants who were recently extubated and are unstable, any physiological difference between those modes has never been investigated. Heart Rate Variability (HRV) has been shown to be a useful marker of physiological wellbeing in preterm infants during weaning from mechanical ventilation, with significant differences between infants that succeed or fail extubation. Therefore, evaluation of HRV may provide some insight into the physiological differences between NIPPV and CPAP in preterm infants recently extubated. There is no consensus on what is the best methodology to analyze HRV in neonates.

Objective: To investigate for physiological differences between NIPPV and CPAP applied during the immediate post-extubation phase using several different methodologies for HRV analysis. The secondary objective was to investigate for differences in HRV with relation to the extubation outcome.

Methods: Infants born with birth weight ≤1250g and undergoing their first extubation attempt were studied 30 min after extubation. Electrocardiogram (ECG) recordings were obtained while these infants were receiving NIPPV at a rate of 20 breaths per minute (NIPPV20) and 40 breaths per minute (NIPPV40), and nasal CPAP in a random order for 30-60 min each. Initial comparisons revealed no differences between NIPPV20 and 40, thus final comparisons were only performed between NIPPV20 and CPAP. Using time domain and frequency domain methods, HRV parameters were calculated from 5-minute segments of ECG obtained using the following four methodologies: 1) average of all acceptable (≥80% normal intervals) segments, 2) the last acceptable segment, 3) the last acceptable segment band-pass filtered, and finally 4) the best obtainable segment (100% normal intervals). Non-parametric comparisons were done between NIPPV20 and CPAP, for the absolute difference between NIPPV20 and CPAP (ΔHRV), and the relative difference (ΔHRV(%)) for all infants and between infants that failed and succeeded extubation. Extubation failure was defined as the need for reintubation within 72 hours.

Results: Twelve out of 15 infants were analyzed (7 success and 5 failures); 3 infants were excluded due to low quality of ECG signals. No differences were found between NIPPV20 and CPAP for the overall population. From the third segment analysis, utilizing a single band-pass filtered segment, a significantly higher ΔHRV and ΔHRV(%) were observed in infants that failed when compared to success for all time domain HRV parameters. All these parameters showed high accuracy in predicting extubation failure, with area under the ROC curves >0.9.

Conclusion: There were no differences in HRV between NIPPV20 and CPAP. However, infants who failed extubation had significantly greater HRV on NIPPV20 compared to CPAP. This difference was only observed in the third segment analysis, which uses the most systematic approach to segment selection. Analysis of HRV may be a useful tool to identify infants at high risk of failing extubation as early as 2 hours post-extubation.

Résumé

Contexte: Suite à l'extubation, les nourrissons extrêmement prématurés sont soutenus avec un certain type de thérapie respiratoire non-invasif afin de prévenir l'échec de l'extubation. Les deux modes les plus courants sont la ventilation nasale à pression positive intermittente (VPPIn) et la pression positive continue (PPC). Les deux types ont des effets physiologiques semblables sur les nourrissons stables. Par contre, aucune étude a été effectuée sur la différence physiologique entre ces deux modes chez les nourrissons qui ont été récemment extubés et sont instables. La variabilité du rythme cardiaque (VRC) est un marqueur utile pour démontrer le bien-être physiologique des nourrissons prématurés pendant le sevrage de la ventilation mécanique, avec des différences significatives entre les nourrissons qui ont été extubés avec ou sans succès. Par conséquent, l'évaluation de la VRC peut élucider les différences physiologiques entre la VPPIn et la PPC chez les nourrissons prématurés récemment extubés. Il n'y a pas de consensus de la meilleure méthode pour analyser la VRC chez les nourrissons.

Objectif: Pour étudier les différences physiologiques entre la VPPIn et la PPC appliquées au cours de la phase post-extubation immédiate en utilisant plusieurs méthodes différentes pour l'analyse de la VRC. L'objectif secondaire est d'étudier les différences de la VRC reliées au succès de l'extubation.

Méthodes: Les nourrissons nés avec un poids ≤1250g et subissant leur première essai d'extubation ont été étudiés 30 min après l'extubation. Les enregistrements d'électrocardiogramme (ECG) ont été obtenus alors que ces nourrissons recevaient la VPPIn à un taux de 20 respirations par minute (VPPIn20), et 40 respirations par minute (VPPIn40), et la PPC dans un ordre aléatoire pour 30-60 min chaque. Les comparaisons initiales n'ont révélé aucune différence entre les taux sur la VPPIn, donc les comparaisons finales ont été effectuées uniquement entre la VPPIn20 et la PPC. En utilisant des méthodes dans le domaine temporel et le domaine fréquentiel, des paramètres de la VRC ont été calculées en utilisant des segments de 5 minutes de l'ECG obtenus en utilisant les quatre méthodes suivantes: 1) moyenne de tous segments acceptables (≥80% des intervalles normaux), 2) le dernier segment acceptable, 3) le dernier segment acceptable filtré passe-bande, et enfin 4) le meilleur segment obtenu avec la plus faible quantité d'artéfacts. Des comparaisons non-paramétriques ont été effectuées entre la VPPIn20 et la PPC, la différence absolue entre la VPPIn20 et la PPC (ΔHRV), et la différence

relative (ΔHRV(%)) pour tous les nourrissons, et entre les nourrissons qui ont échoués et réussis l'extubation. L'échec de l'extubation a été défini comme la nécessité de réintubation dans les 72 heures subséquentes.

Résultats: Douze des 15 nourrissons ont été analysés (7 succès et 5 échecs); 3 enfants ont été exclus en raison de la faible qualité des signaux ECG. Aucune différence n'a été identifiée entre la VPPIn20 et la PPC pour la population globale. De la troisième analyse du segment, en utilisant un seul segment de 5 minutes filtré passe-bande, un ΔHRV et un ΔHRV(%) beaucoup plus élevé à été observée chez les nourrissons qui ont échoué par rapport à la réussite de tout des paramètres de la VRC dans le domaine temporel. Tous ces paramètres ont montré une grande précision dans la prédiction de l'échec de l'extubation.

Conclusions: Il n'y avait aucune différence dans la VRC entre la VPPIn20 et la PPC. Toutefois, les nourrissons qui ont échoué l'extubation avaient significativement plus de VRC sur la VPPIn20 en comparaison à la PPC. Cette différence n'a été observée que dans la troisième analyse du segment, qui utilise l'approche la plus systématique à la sélection de segment. L'analyse de la VRC peut être un outil utile pour identifier les enfants à risque élevé d'échec à l'extubation dès 2 heures post-extubation.

List of Abbreviations

NICU – Neonatal Intensive Care Unit

GA – Gestational Age

LPT – Late Preterm

MPT – Moderate Preterm

VPT – Very Preterm

EPT – Extremely Preterm

RDS – Respiratory Distress Syndrome

ANS – Autonomic Nervous System

MV – Mechanical Ventilation

BPD – Bronchopulmonary Dysplasia

PIP – Peak Inflation Pressure

PEEP – Positive End-Expiratory Pressure

MAP – Mean Airway Pressure

FiO_2 – Fraction of Inspired Oxygen

SBT – Spontaneous Breathing Trial

CPAP –Continuous Positive Airway Pressure

NIPPV – Nasal Intermittent Positive Pressure Ventilation

HFNC – High Flow Nasal Cannula

NIV-NAVA – Non-Invasive Neurally Adjusted Ventilatory Assist

$PaCO_2$ – Arterial Partial Pressure of Carbon Dioxide

RIP – Respiratory Inductive Plethysmography

HRV – Heart Rate Variability

ECG – Electrocardiogram

SDNN – Standard Deviation of NN intervals

SD delta NN – Standard Deviation of successive differences between NN intervals

RMSSD – Root Mean Square of Successive Differences between NN intervals

pNN50 – Percentage of NN intervals differing by >50ms

VLF – Very Low Frequency

LF – Low Frequency

HF – High Frequency

TP – Total Power

LF/HF – Low Frequency to High Frequency ratio

RSA – Respiratory Sinus Arrhythmia

CO_2 – Carbon Dioxide

ICU – Intensive Care Unit

STV – Short-Term Variability

LTV – Long-Term Variability

PPV – Positive Predictive Value

NPV – Negative Predictive Value

SpO_2 – Oxygen saturation

PCO_2 – Partial pressure of carbon dioxide

BPM – Beats Per Minute

TA – Thyroarytenoid muscle

AU – Arbitrary Units

Section 1 - Introduction

1.1 Preterm birth

Preterm birth occurs for a variety of reasons, including maternal stress and medical conditions, but often occurs spontaneously with the exact cause unknown. The rate of preterm births in Canada was approximately 8% in 2012, with a global rate of 10% [1]. This represents approximately 29,000 preterm births per year. Preterm infants make up 62% of neonatal intensive care unit (NICU) admissions, representing a large number of infants being treated for a variety of respiratory, cardiac and gastrointestinal problems due to their immature physiological systems [1, 2].

Preterm infants, defined as infants born at less than 37 weeks gestational age (GA), can be categorized into four major groups: late preterm (LPT) infants, born at 34^0-36^6 weeks GA; moderate preterm (MPT) infants, born at 32^0-33^6 weeks GA; very preterm (VPT) infants, born at 28^0-31^6 weeks GA; and extremely preterm (EPT) infants, born at <28 weeks GA [1].

1.2 Premature lungs and respiration

Normal lung development occurs in overlapping stages. For preterm infants, the most notable stages are the canalicular (16 to 26 weeks gestation), the saccular (24 to 38 weeks gestation), and the alveolar stages (36 weeks gestation to the 2^{nd} year of life) [3]. During the canalicular stage, respiratory epithelial cells begin differentiation into type I and type II cells; specifically, the type II epithelial cells begin differentiation at 24-26 weeks gestation in the transition between canalicular and saccular stages [4]. The type II epithelial cells are responsible for producing surfactant, a lipid- and protein-based fluid that coats the inner surface of alveoli, thereby reducing their surface tension and preventing atelectasis. Given the gradual development

of the type II cells, the majority EPT and VPT infants lack adequate surfactant production and suffer from respiratory distress syndrome (RDS) [4]. The differentiation of type I cells is vital, as they consist of the thin cells that line the air-blood barrier, allowing gas exchange to occur [5]. Within the canalicular phase, the lung tissue is rapidly becoming capillarized; in particular, those capillaries that contact the type I epithelial cells become eventual sites for gas exchange. As such, infants born before this capillary and epithelial development occurs will lack the ability for gas exchange and will, therefore, not be viable [2, 5].

The impaired cardiorespiratory regulation that is highly prevalent in the preterm population is due in part to the immature autonomic nervous system (ANS), with greater degree of severity linked to the smaller GAs. Neuronal differentiation, axon growth, dendritic connections, and myelination in the central nervous system occur by 6 months gestation and continue on into childhood [2]. Specifically, the myelination of vagus fibers is linearly increasing from 26 weeks GA to 10 weeks after birth; this immaturity of the vagus nerve has been implicated in the impaired Hering-Breuer reflex and, consequently, the unstable breathing patterns of these infants [6].

1.3 Respiratory support

Due to their immature respiratory regulation and underdeveloped lungs, most EPT infants are unable to maintain adequate spontaneous ventilation [2]. Indeed, up to 89% of extremely low birth weight infants (<1000g) require mechanical ventilation (MV) via an endotracheal tube during the first days of life [7]. While MV is a life-saving intervention, it can also lead to complications such as bronchopulmonary dysplasia (BPD), pneumothorax, ventilator-associated pneumonias, as well as long-term problems such as neurodevelopmental impairment [7 – 11]. In

order to avoid prolonged MV and its associated complications, physicians often advocate extubating as soon as possible.

General guidelines for basic extubation readiness have been proposed, utilizing the infants birth weight, age, ventilator mode and settings for peak inflation pressure (PIP), positive end-expiratory pressure (PEEP), rate, mean airway pressure (MAP), fraction of inspired oxygen (FiO_2), and tidal volume [12]. Similarly, guidelines have been proposed for successful extubations, using factors such as oxygen saturation, blood gas results, and the establishment of protocols for the weaning and extubation process [13]. However, most Canadian NICUs do not have established protocols for MV, which could lead to a wide variety in extubation practices and outcomes [14]. Infants that are extubated too soon may fail extubation and require reintubation. Risk factors associated with failure include low GA, low birth weight, prolonged MV, and previous extubation failure [12, 15].

Prediction of extubation failure in the preterm population is difficult, with failure rates that can be as high as 75% [16]. Extubation failure rates are higher amongst the more extremely preterm infants (i.e. lower birth weights or smaller GAs) [17]. The use of spontaneous breathing trials (SBTs) is being widely used amongst the adult and pediatric population. As a mean of assessing extubation readiness, the patients are challenged to spontaneously breathe through the endotracheal tube, usually with PEEP as support, for a given duration. The use of the SBT has been studied within the preterm population, however the large variability in the SBT practices and uncertain benefits prevent any recommendations from being established [18]. Other methods of predicting the extubation outcome have been investigated with success, including the use of pulmonary function tests [19 – 22], assessment of respiratory muscles [23], and physiological variability [24, 25]. Although many of these studies had accuracy in assessing extubation

readiness, still most extubations are performed under the decision of the attending neonatologist, based on their clinical experience and the general guidelines described above.

Non-invasive respiratory support is usually used as a part of the weaning process from MV to prevent extubation failure. In infants with less severe respiratory distress, the use of non-invasive respiratory support can also prevent the need for intubation altogether. The most common modes of non-invasive respiratory support include nasal continuous positive airway pressure (CPAP), non-synchronized nasal intermittent positive pressure ventilation (NIPPV), and heated humidified high flow nasal cannula (HFNC) [13]. A newer mode that is gradually being integrated into the NICU setting is the neurally adjusted ventilatory assist (NIV-NAVA), which uses the electric signal from the diaphragm to trigger ventilation proportional for each individual breath [26]. The most frequently used modes in EPT infants are non-synchronized NIPPV and CPAP [27].

1.4 NIPPV vs. CPAP

1.4.1 Clinical studies

Multiple studies have compared the clinical differences between preterm infants on non-synchronized NIPPV and CPAP. Some studies of which have compared the two modes within the context of preventing intubation. Bisceglia *et al.* compared NIPPV and CPAP in a total of 88 patients, matched for GA, birth weight, sex, APGAR scores, and oxygen needs. Their results showed that infants on NIPPV had less apneas, shorter respiratory support duration, and partial pressure of carbon dioxide ($PaCO_2$) that were closer to normal physiological values than infants receiving CPAP, but no differences in intubation rates [28]. Armanian *et al.* investigated 54 infants on CPAP and 44 on NIPPV with GAs less than 34 weeks and/or birth weights less than

1500g. Similarly, their results showed no differences in intubation rates, however infants within the NIPPV group had shorter duration of respiratory support, oxygen dependency, intraventricular hemorrhage, time to establish full enteral feeds, and shorter overall length of hospitalization than those on CPAP [29]. Meneses *et al.* also compared 100 infants in both modality groups of GA less than 34 weeks, and found no significant differences between the intubation rates within 72 hours of life [30]. In contrast, one study had demonstrated a lower intubation rate within the NIPPV group at 48 hours and 7 days from randomization, including 39 infants on CPAP and 37 infants on NIPPV of 28 – 34 weeks GA [31].

Few studies have compared these modes as post-extubation respiratory support to prevent extubation failure. Khorana *et al.* studied 24 infants on NIPPV and on CPAP of GA less than 34 weeks or birth weight less than 1500g, and demonstrated similar reintubation rates in both groups [32]. By contrast, Ramanathan *et al*. showed that infants in the NIPPV group had lower rates of reintubation as well as BPD, with 53 infants on NIPPV and 57 on CPAP with GA less than 30 weeks [33]. The largest randomized control trial comparing NIPPV and CPAP enrolled 1009 preterm infants with birth weight less than 1000g and less than 30 weeks GA. The results showed no differences in rates of intubation (either for initiation of mechanical ventilation or reintubation), BPD, death, as well as many other secondary outcomes [34]. However, this study must be interpreted with caution as the delivery of NIPPV in 43% of infants was through SiPAP. SiPAP cycles regularly between different levels of PEEP at a set rate, while conventional NIPPV applies shorter bursts of PIP with a baseline PEEP. Finally, a recent systematic review comparing post-extubation NIPPV and CPAP in preterm infants found that there were no significant effects on the rates of chronic lung diseases or mortality, but NIPPV was found to reduce reintubation rates within 48 hours to 1-week post-extubation when compared to CPAP.

However, these results must also be interpreted with caution, as 5 of the 8 studies included used synchronized NIPPV, which is not clinically available and may have provided greater benefit than the standard, non-synchronized NIPPV currently used [35]. Overall, the clinical studies have mixed results, with most indicating that neither mode (NIPPV or CPAP) is better than the other, while some promote NIPPV as having less intubation/reintubation rates. This lack of clarity is confirmed by a recent clinical report from the American Academy of Pediatrics on the use of non-invasive respiratory support [36].

1.4.2 Physiological studies

There is only one study that compared the physiological effects of NIPPV and CPAP in preterm infants following a period of mechanical ventilation. The study included 16 preterm infants with median post-conceptional age of 30 weeks that were extubated for > 7 days and stable. Infants were placed on different respiratory support modalities for one hour each. Through the measurement of esophageal pressures and respiratory induced plethysmography (RIP), the results showed no differences in ventilation (tidal volume, minute ventilation, and respiratory rate), gas exchange (transcutaneous partial pressure of CO_2, oxygen saturation, and FiO_2), and inspiratory effort. However, NIPPV had an increased expiratory effort when compared to CPAP, likely due to the 56% of breaths that were delivered during the expiratory phase of the infant's spontaneous breathing [37]. No studies have investigated physiological differences between these two modes in unstable preterm infants, such as during the post-extubation period.

1.5 Heart Rate Variability

Heart rate variability (HRV) refers to the fluctuation of beat-to-beat intervals over time. HRV is the result of the continuous counterbalancing input from the parasympathetic and sympathetic branches of the ANS, which generate different frequencies in the power spectrum. Consequently, HRV is used as a measure of ANS function. HRV is a common way to assess patient wellbeing and has been extensively investigated in adult medicine for different conditions with specific recommendations for analysis [38, 39].

1.5.1 Linear methods for HRV analysis

There are two common methods for linear HRV analysis: time domain and frequency domain [38]. Time domain methods utilize the intervals between each QRS complex from the electrocardiogram (ECG), called the RR interval, due to the detection of R waves, or the NN interval, for "normal-to-normal" referring to the beats initiated by sinus node depolarization. Common time domain parameters include the standard deviation of NN intervals (SDNN), standard deviation of successive differences between NN intervals (SD delta NN), root mean square of successive differences (RMSSD), and percentage of NN intervals differing by >50ms (pNN50). Frequency domain methods use power spectrum analyses to break down the time series of NN intervals (tachogram) into spectral components: very low frequency (VLF, ≤0.04 Hz), low frequency (LF, 0.04-0.15 Hz), and high frequency (HF, 0.15-0.4 Hz). The total power (TP) of the spectrum as well as the LF/HF ratio are also commonly used HRV parameters. The vagal and respiratory sinus arrhythmia (RSA) activity are reflected in the HF component, while some controversy remains regarding the ANS contribution to the LF component: some consider

it under sympathetic control while others consider it to be under both sympathetic and parasympathetic control [39].

1.5.2 HRV and lung function

1.5.2.1 Adult clinical studies

In adults, some studies have demonstrated links between ventilation and changes in HRV. Pöyhönen *et al.* found during mechanical ventilation as well as spontaneous breathing that alteration in carbon dioxide (CO_2) concentrations as well as respiration rates affects the frequency domain components of HRV. Specifically, increased CO_2 increased the power in the LF and HF components, while reduced respiratory rate results in increased power in LF, reduced power HF, and increased the LF/HF ratio [40].

Few studies have investigated HRV during an SBT prior to extubation. Shen *et al.* investigated the changes in HRV when weaning 24 patients from mechanical ventilation. The results showed reduced frequency domain components (LF, HF, and TP) during the SBT from pressure support ventilation and assist control mechanical ventilation in the patients that failed the SBT or subsequently failed extubation. There was no such change in HRV observed in the patients that were successfully extubated, indicating that changes in HRV could be a prediction of extubation failure [41]. Further studies support this finding, with one studying 101 patients undergoing an SBT prior to extubation that also used frequency domain analyses. They found a significant reduction in HRV in patients that failed the SBT; furthermore, the patients that were extubated and subsequently failed within 72 hours had lowered HRV after being extubated [42]. A large multicenter study agrees with the results; 434 patients extubated in the intensive care unit (ICU) had HRV measured during their SBTs prior to extubation and their results showed a

similar decrease in HRV for those that failed the SBT, as well as in those that failed after extubation [43].

1.5.2.2 Neonatal clinical studies

In neonates, some of the early literature using HRV investigates infants suffering from RDS. Cabal *et al.* studied 92 preterm infants from 28-36 weeks GA over their first week of life. They compared 4 groups (healthy infants, infants with mild or moderate RDS, infants with severe RDS who survived, and infants with severe RDS who died) using short-term variability (STV) and long-term variability (LTV). STV and LTV use NN interval differences similar to time domain methodologies. Amongst the first three groups, HRV increased as the post-natal age increased while heart rates decreased. This effect was not observed in the group of severe RDS infants who subsequently died during the first week of life, where HRV decreased with post-natal age. Furthermore, HRV was reduced in infants with RDS and the reduction was proportional to the severity of the RDS; indeed, HRV within the first few hours of life was able to predict 84% of infants who would suffer from RDS. Essentially, HRV was found to be a useful predictor of RDS as well as mortality in preterm infants [44].

Similar studies confirm Cabal's findings; Jenkins *et al.* studied 66 ill newborn infants within the first 72 hours of life divided into three groups: those not suffering from RDS (Group 1), those suffering from RDS requiring CPAP support (Group 2), and those suffering from RDS requiring intermittent positive pressure ventilation (Group 3). All infants with RDS had lower HRV than those without, with the lowest values found in Group 3. This study confirms that the reduction in HRV is linked to the degree of severity of RDS, finding similar predictive abilities for mortality in these infants [45]. A similar study used a variety of methods to calculate STV

and LTV in 60 preterm infants <33 weeks GA for the first 72 hours of life. The overall results confirm those by Cabal and Jenkins, with reduced HRV in infants with RDS and linked to the degree of severity. Furthermore, they demonstrated a similar reduction trend in infants with symptomatic patent ductus arteriosus and periventricular hemorrhage [46]. Prietsch *et al.* studied 105 preterm infants <33 weeks GA during first week of life divided into four groups: control infants without RDS <30 weeks GA, infants with RDS <30 weeks GA, infants without RDS ≥30 weeks GA, and infants with RDS ≥30 weeks GA. In accordance with the previously described studies, the results showed an increase in HRV over the first 7 post-natal days, and significantly reduced HRV in patients suffering from RDS, with worsening effect in those who subsequently died. Moreover, they demonstrated that the older healthy controls (>30 weeks GA) have higher HRV than the younger healthy controls (<30), confirming the maturational effect observed with HRV [47].

One study investigated changes in HRV through frequency domain analysis in 11 preterm infants with RDS, 21 healthy preterm infants, and 22 healthy term infants in the first 5 days of life. The results indicate that the normal term development of HRV, beginning with only an LF peak and then developing an RSA peak in the HF, was not found in the healthy preterm infants, where the same LF peak was present but they did not develop the change in HF during the first 5 days. Furthermore, in preterm infants with RDS, the overall density in the LF and HF frequency bands were reduced compared to the other groups, and similar to the healthy preterm infants, no RSA peak within the HF developed. This study points to the maturational process that can be observed through HRV, as well as the difference in those preterm infants that are ill, with reduced capabilities for adaptation [48].

A recent study by Kaczmarek *et al.* used frequency domain HRV analysis to predict

extubation failure in EPT infants. They studied 47 preterm infants with birth weight ≤1250g immediately prior to extubation, of which 11 went on to fail their subsequent extubation, requiring reintubation within 72 hours. All parameters of the frequency domain analysis (TP, VLF, LF, HF, and LF/HF ratio) were significantly reduced in the failure group compared to the success group. To predict extubation failure, these HRV parameters had perfect specificities and positive predictive values (PPVs), but poor sensitivities and negative predictive values (NPVs) [25]. These results further confirm the potential usefulness of HRV to predict respiratory illness.

HRV has been widely investigated in neonates but with significant variations in methods used, making it difficult for any comparisons and clear understanding of its clinical application. The numerous studies of HRV in neonates span a wide variety of topics other than respiration, including studies assessing brain injuries [49, 50], pain response [51, 52], prenatal drug exposure [53, 54], and maturation of preterm infants [55, 56]. A new monitoring device uses advanced methodology to calculate an HRV score and has been shown to be an accurate predictor of sepsis in neonates [57]. The array of HRV studies in this population demonstrates a great potential use for HRV as a clinical prediction and monitoring tool; however, none have investigated its use in EPT infants post-extubation. Assessment of heart rate variability while on non-invasive respiratory support has the potential to elucidate differences in physiological responses between the modes.

1.6 Thesis objectives

The objectives of this thesis are:

1. To investigate for differences between NIPPV and CPAP during the immediate post-extubation phase using different methodologies for HRV analysis.

2. To investigate for differences in immediate post-extubation HRV in relation with extubation outcome.

Section 2 – Methods

2.1 Population

All infants with birth weight ≤ 1250g, receiving mechanical ventilation and undergoing their first extubation attempt were eligible for the study. Patients were recruited from any of the 3 participating hospitals (Royal Victoria Hospital, Montreal Children's Hospital and Jewish General Hospital, Montreal, Canada). The research ethics board of each institution approved the study. Informed consent was obtained from parents or legal guardians as soon as any eligible infant was initiated on mechanical ventilation to avoid delays on extubation decisions and processes. Patients were excluded if they had congenital anomalies or were receiving narcotics, sedatives or inotropes at the time of extubation.

2.2 Peri-extubation management

This was a prospective, observational study to evaluate for differences in heart rate variability in infants receiving two common modes of post-extubation respiratory support during the specific period immediately after extubation. Thus, except for the study period, there was no interference in any aspect of the standard of care. Therefore, decisions concerning intubation, mechanical ventilation, weaning, timing of extubation, and type of post-extubation respiratory support to be provided outside of the study period were exclusively made by the treating physician. While the clinical team decided the respiratory management, the following guidelines for extubation were proposed: for birth weight <1000g, MAP ≤7 cmH_2O and FiO_2 ≤0.3, and for birth weight ≥1000g, MAP ≤8 cmH_2O and FiO_2 ≤0.3. Based on evidence from the literature

(Section 1.4.1), which has demonstrated no differences in the outcome of extubation failure between those two modes, current practice in the three units is to provide either non-synchronized NIPPV or CPAP after extubation as per physician choice. Extubation failure was defined as the need for reintubation within 72 hours, with the following criteria for reintubation proposed: a) FiO_2 >0.5 to maintain oxygen saturation (SpO_2) >88% or partial pressure of oxygen >45 mmHg; (b) partial pressure of carbon dioxide (PCO_2) >55–60 mmHg with pH <7.25; (c) apnea requiring positive pressure ventilation with bag and mask; (d) increased number of apneas (>6 in a 6 hour period); or (e) significantly increased work of breathing.

2.3 Study design and data acquisition

The research team was contacted once the treating physician made the decision to extubate. Each patient was instrumented approximately 1h prior to disconnection from the ventilator. This instrumentation consisted of placement of 3 ECG leads on the infants' limbs, located at least 1 cm apart from the existing leads to prevent interference. After extubation, a period of 30 min was allowed for stabilization prior to initiation of the recordings. During this transition period infants received the type of non-invasive respiratory support determined by the treating team, which was either non-synchronized NIPPV or CPAP. For the study, infants were kept in supine position, and the non-invasive respiratory support was provided in a random order between non-synchronized NIPPV at a rate of 20 breaths per minute (NIPPV20), non-synchronized NIPPV at a rate of 40 breaths per minute (NIPPV40) and CPAP. A time line of the study design is provided on Figure 1. During the study period, CPAP pressure levels were set at 5 or 6 cmH_2O (similar to pre-study PEEP levels) and peak inflation pressures during NIPPV were set 10 cmH_2O above the PEEP level. For each mode, the ECG was recorded using a

bioamplifier (FE132, ADInstruments, Colorado, U.S.A.) connected to the PowerLab acquisition system (ADInstruments, Colorado, U.S.A.) by using a six-pin DIN/MS socket to fit a 3-lead Bio Amp cable (Tronomed D-1340). Recordings were done during a period of 30 to 60 minutes for each mode and signals were stored in a research computer for later analysis using the LabChart software (Version 7.3.7, ADInstruments).

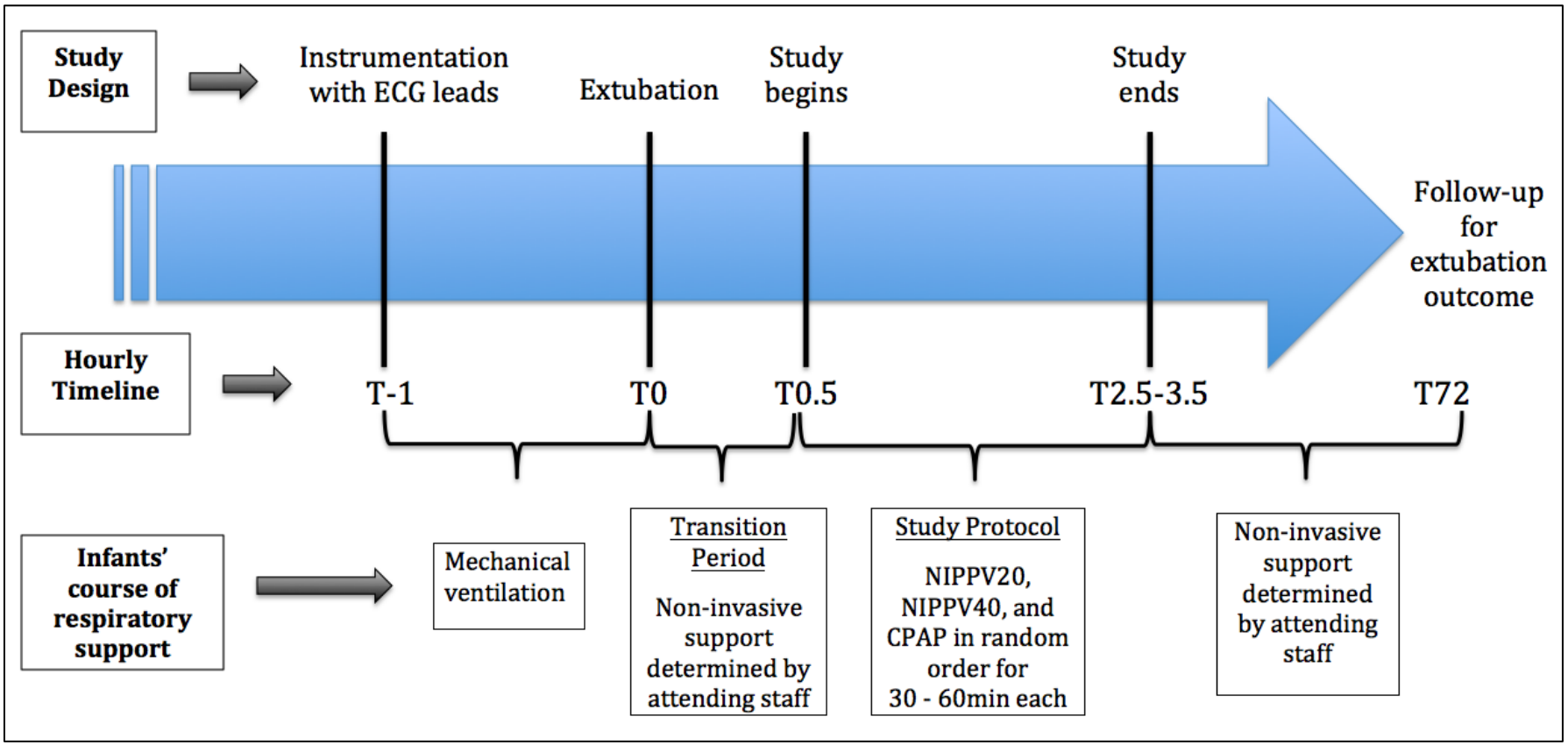

Figure 1. Schematic of the study design.
Legend: T-1 = 1 hour pre-extubation (mechanically ventilated) - instrumentation with ECG leads; T0 = time at extubation - mode of non-invasive support determined by attending staff; T0.5 = 30 min post-extubation - started on the study mode of non-invasive support in random order and recordings begins; T2.5-3.5 = study ends - approximately 2-3 hours of duration; and T72 = 72 hours after extubation – determine extubation outcome (success or failure).

2.4 Heart rate variability analysis

2.4.1 HRV analysis tools

HRV analysis was performed offline using the HRV module of the LabChart (version 1.4.2, ADInstruments). This module automatically conducts time domain and frequency domain analyses on the entire ECG recordings or selected segments. For each patient, the following sequence was used:

a. Open the LabChart software and select patient file

b. Open the HRV module and set-up cut-off ranges for the detection of the NN intervals

In the HRV module, the default settings are for adults. Therefore, they were modified for neonates: normal NN intervals = 300-600 ms (normal neonatal heart rate between 100 and 200 beats per minute [bpm]), ectopics = 200-300 ms (200 to 300 bpm) and 600-1000ms (60 to 100 bpm), and artifacts = <200 ms (>300 bpm) and >1000ms (<60 bpm)(Figure 2).

c. Select the ECG segment

We identified the beginning and end of each non-invasive support mode used. The first 10 minutes of ECG recording on CPAP, NIPPV20 or NIPPV40 was considered a transition period and not included in the analysis. For each mode, five-minute-long ECG segments were then identified. Details of the segment selection processes are provided in the following section.

d. Detect normal-to-normal (NN) intervals

Each selected ECG segment required manual adjustments of the voltage thresholds in order to maximize beat detection (Figure 3). From these detected beats, LabChart automatically calculates the NN intervals, which were then used for the time domain and frequency domain analyses of HRV.

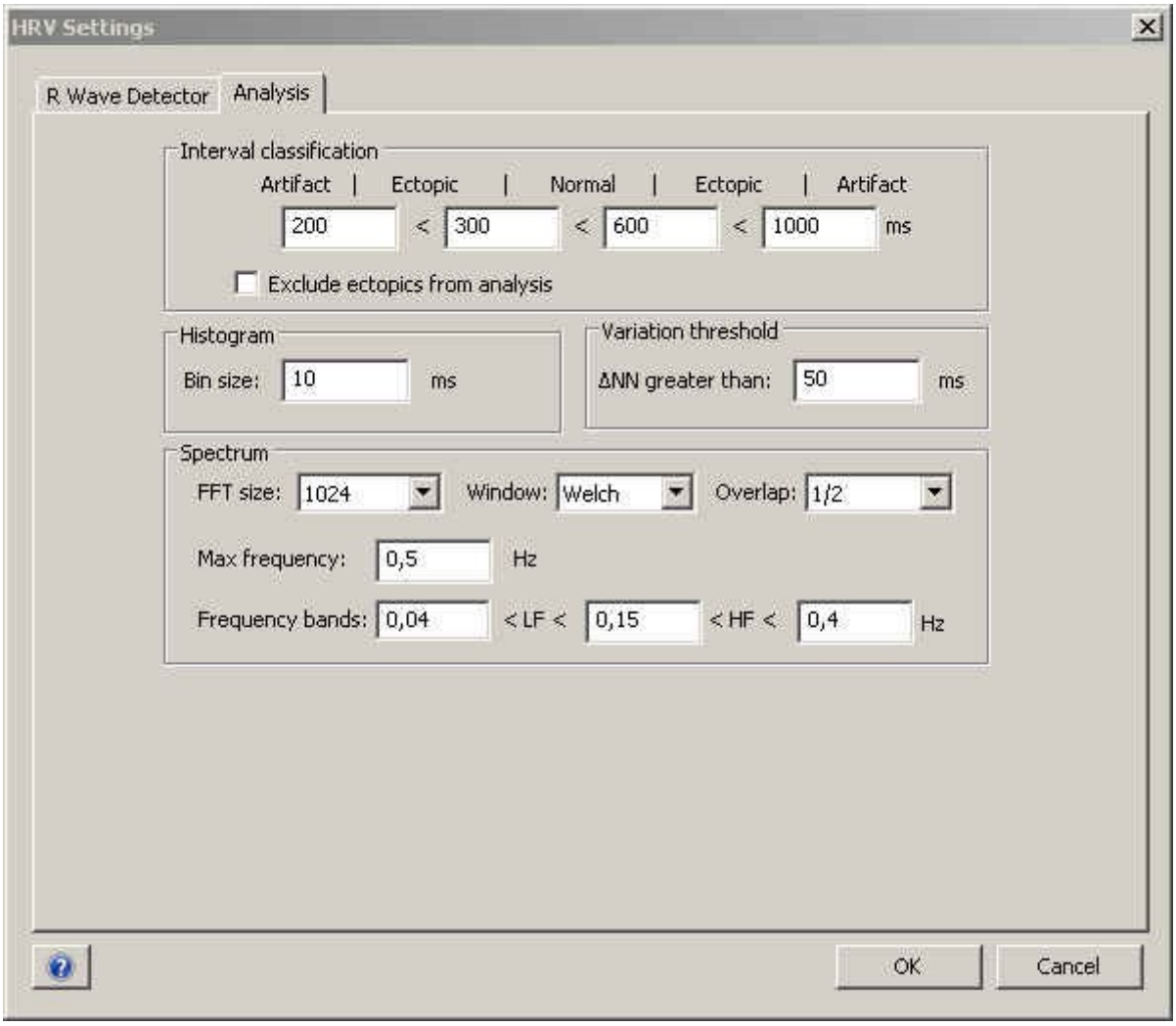

Figure 2. HRV settings window of the HRV module. From default values, cut-offs for interval classification and frequency bands can be modified.

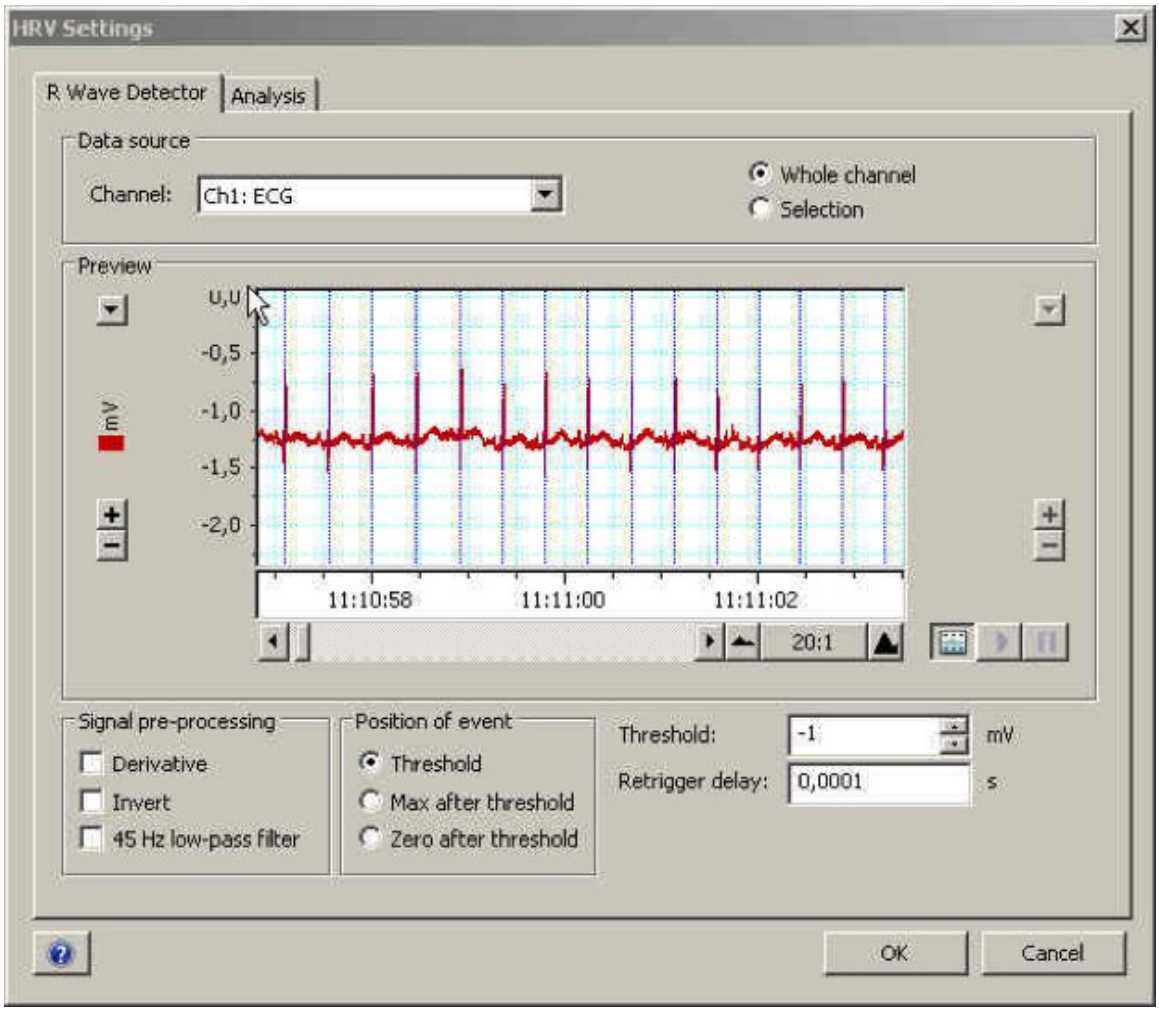

Figure 3. R-wave detection window in the HRV module of the LabChart software showing an example of perfect beat detection (dotted blue lines over the R waves). Note that the threshold must be adjusted for each ECG recording.

2.4.2 ECG segments selection

The Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology standardized guidelines were published in 1996 and never updated. These guidelines suggest recording lengths of 5 minutes (short) or 24 hours (very long). However, there is no information provided on the selection process of these 5-minute segments within longer recordings, and while some editing is generally expected, the extent to which editing should be acceptable is unclear [39]. We hypothesized that the segment selection process, as well as any cleanup manipulations of the signal itself would have an important effect on HRV results. Selecting a segment for HRV can either be done systematically (pre-determined selection criteria) or subjectively through visual inspection of the ECG signals recorded, with a wide variety of possible manipulations to remove artifacts and cleanup the signals. To explore these possibilities we devised 4 analytical methods using a variety of segment selection processes and signal manipulations:

***Analysis #1*:** Multiple 5-min segments *systematically* obtained and *no ECG signal manipulations*

Analysis #2: Single 5-min segment *systematically* obtained and *no ECG signal manipulations*

Analysis #3: Single 5-min segment *systematically* obtained with *ECG signal filtering*

Analysis #4: Single 5-min segment obtained *from visual inspection* (cleanest) of the recordings *with any ECG signal manipulation* necessary to obtain 100% normal NN intervals

2.4.2.1 Analysis #1

For each patient and non-invasive respiratory support mode, *all possible* 5-minute-long segments were identified starting from the end of the recording towards the beginning. The HRV

analysis was done on all those segments obtained, with the first 10 min excluded (transition period). After initial analysis, only 5 min segments with ≥ 80% normal NN intervals were deemed acceptable, and the average HRV values of these segments were used for comparisons. The preliminary analysis comparing NIPPV20 and NIPPV40 also used this methodology. A diagram of this analysis is provided as Figure 4.

2.4.2.2 Analysis #2

The same approach used on Analysis #1 was applied here except that *only the first acceptable* 5 min segment (≥ 80% normal NN intervals) starting from the end of each mode (or the 'last segment') was used for comparison. A diagram of this analysis is provided as Figure 5.

2.4.2.3 Analysis #3

Poor ECG signal quality is often due to high frequency noise that can occur from other electronic equipment and low frequency signal drift that can occur during movement. In order to reduce these effects, a *band-pass filter of 5 – 80 Hz was applied to each segment from Analysis #2* prior to HRV analysis. The LabChart band-pass filter uses finite impulse response filters to remove frequencies outside the range specified. The low frequency cut-off set at 5 Hz indicates that this frequency's amplitude has been reduced to 50% of its original amplitude, and all frequencies below 5 Hz will have gradually greater reductions until the amplitude is reduced to less than 1%; a similar function applies to frequencies above 80 Hz. The transition width, over which amplitudes for given frequencies are at 1-99% of their original amplitudes, occurs over a range that is set to 20% of the cut-off frequency, i.e. cut-off of 80 Hz means that between 72 and 88 Hz is the transition width where the frequencies' amplitudes are gradually attenuated. The attenuation of amplitudes per frequency is illustrated in Figure 6. The band-pass filter range was

chosen empirically based on the degree of noise contaminating the signals. A diagram of this analysis demonstrating the effect of a band-pass filter on the ECG signal is provided as Figure 7.

2.4.2.4 Analysis #4

A single segment was obtained for comparison by visually selecting *the best possible* 5-min long segment closest to the end of each mode. The best segment was defined in terms of overall signal quality, i.e. lack of noise or movement artifact as much as possible. If necessary, a variety of signal manipulations available from the LabChart software were applied to each segment in order to obtain 100% normal NN intervals. Such manipulations include the 5 – 80 Hz band-pass filter, inverting the signal, using the derivative of the signal, and manual deletion of artifacts identified through visual inspection. A diagram of this analysis and examples of the four manipulations are provided as Figure 8.

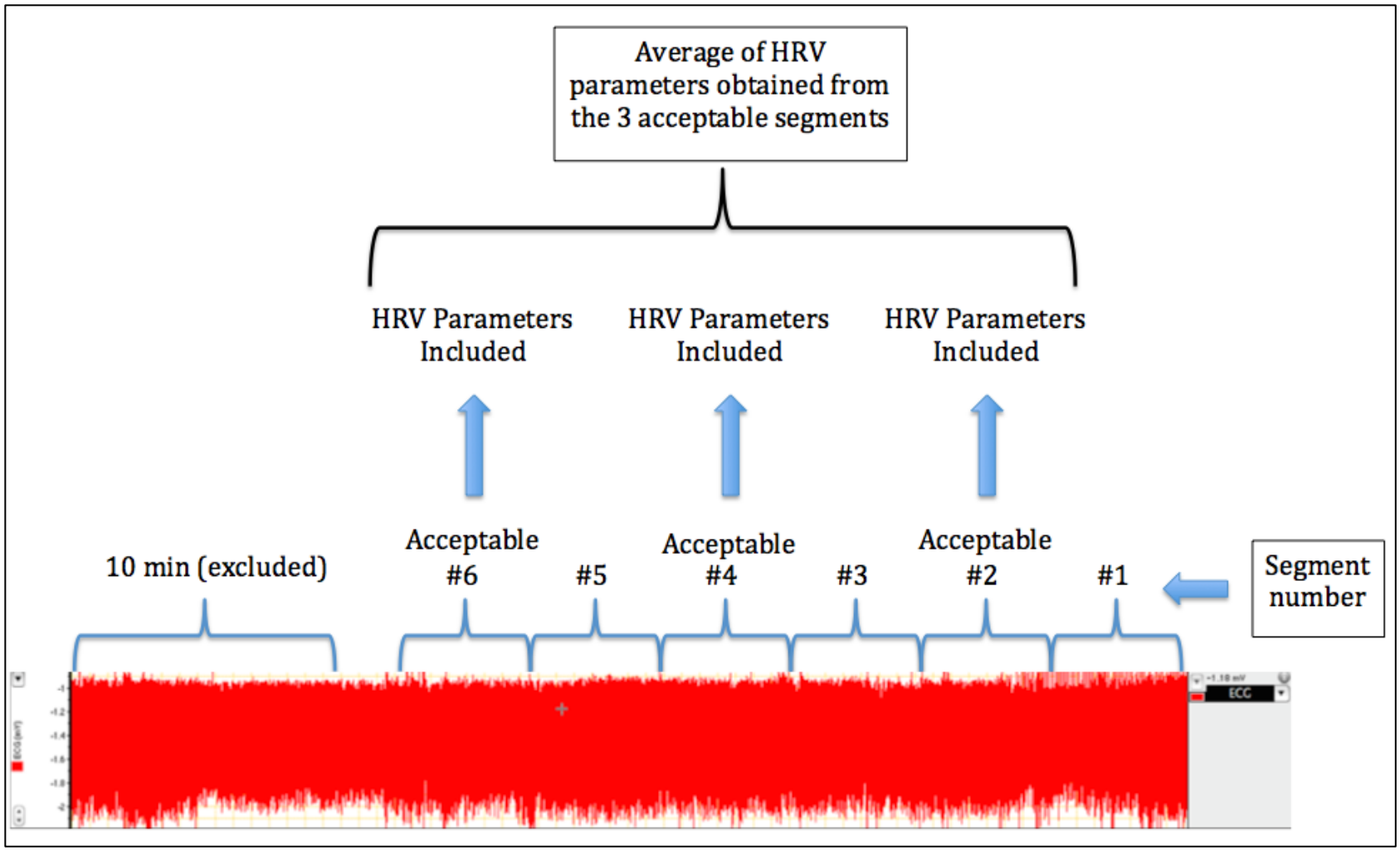

Figure 4. Diagram of Analysis #1. Example of a condensed ECG signal (43 min) obtained using the LabChart software. Working in reverse time, 6 segments of 5 min were identified. The first 10 min were always excluded (transition period). Only segments #2, #4, and #6 were deemed acceptable (having ≥80% normal NN intervals) and selected. HRV values were calculated and averaged for comparison. The other segments (#1, #3, and #5) were unacceptable and therefore excluded from the analysis.

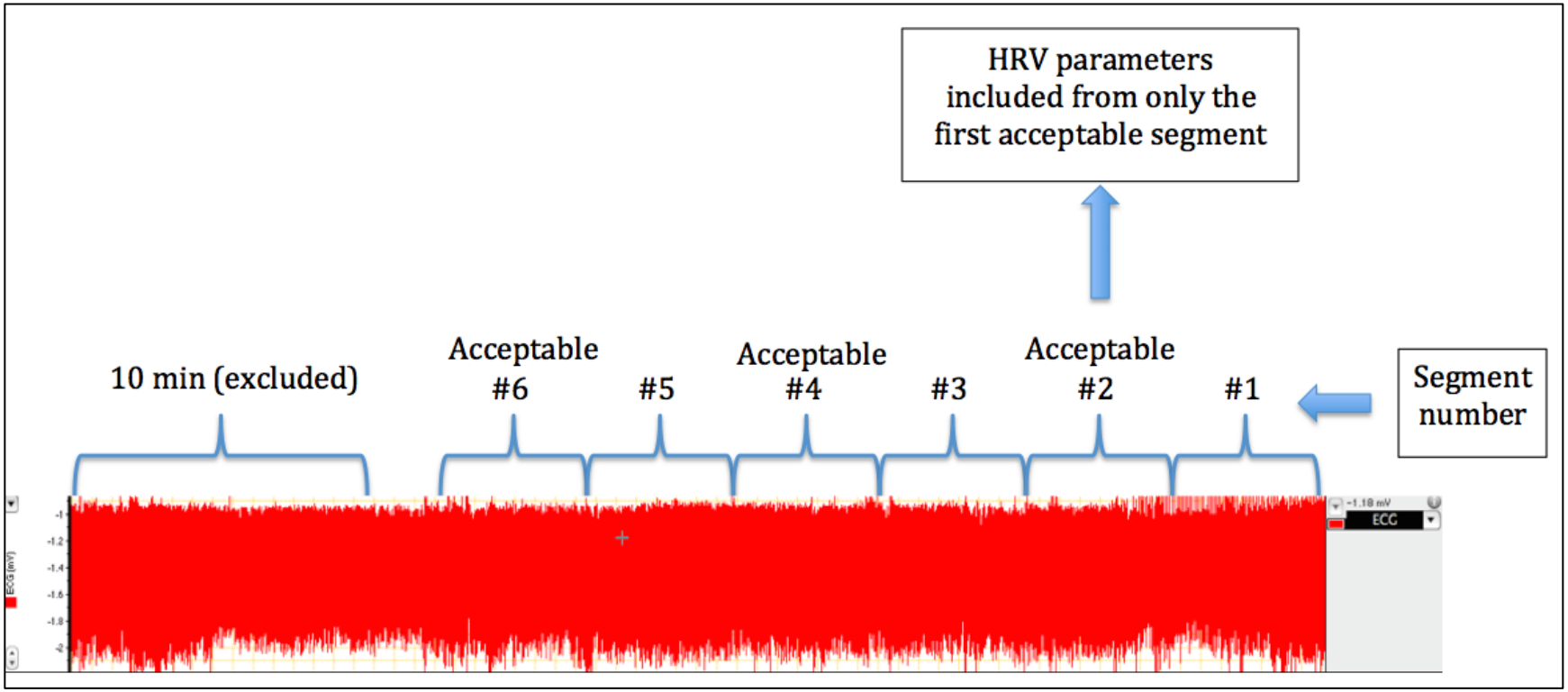

Figure 5. Diagram of Analysis #2. Example of a condensed ECG signal (43 min) obtained using the LabChart software. Working in reverse time, 6 segments of 5 min were identified. The first 10 min were always excluded (transition period). Only the first acceptable segment (having ≥80% normal NN intervals) starting from the end of the mode was selected. HRV values were calculated and used for comparisons.

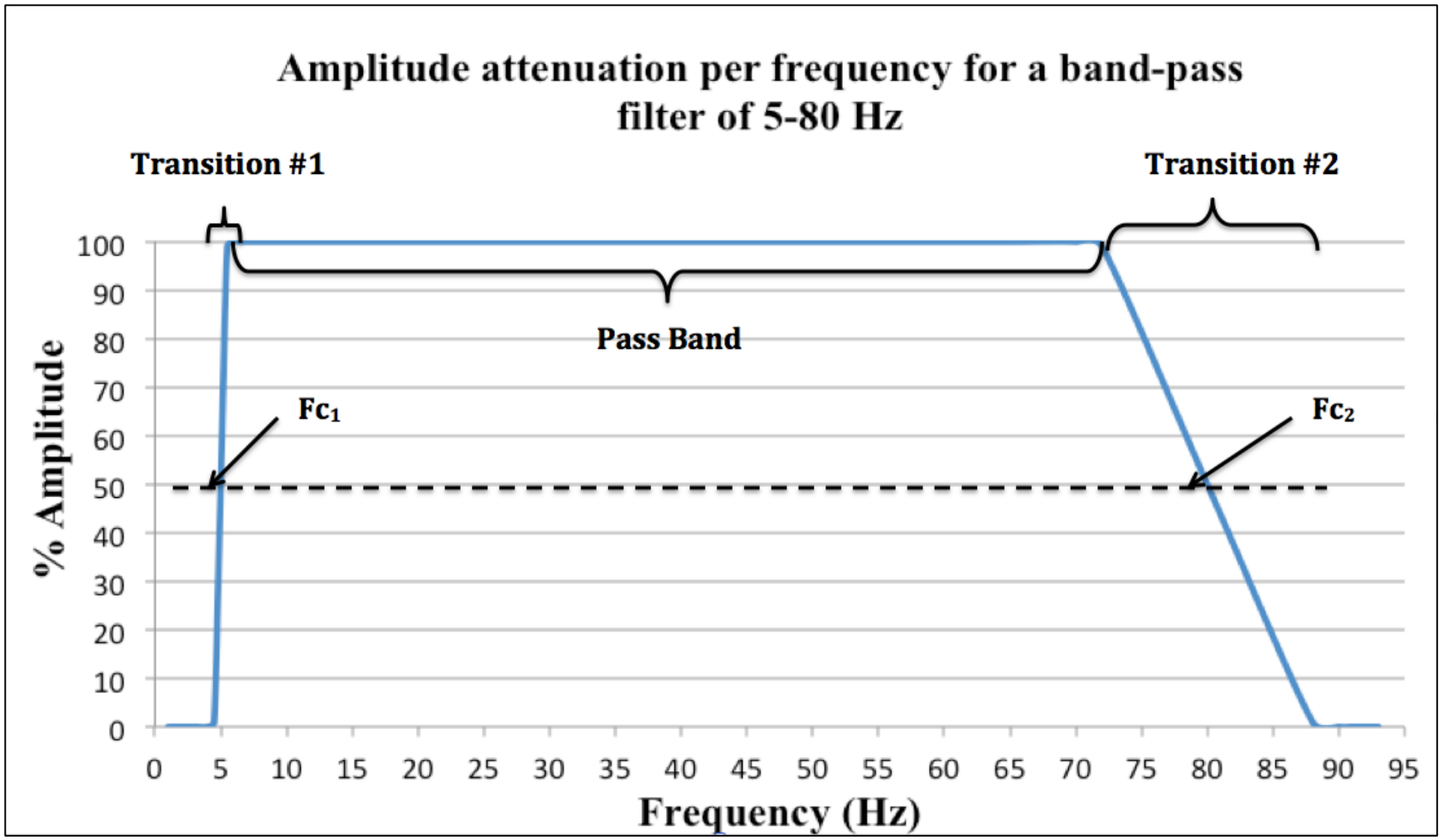

Figure 6. Example how a band-pass filter of 5-80 Hz attenuates the ECG signal amplitude.
Legend: Transition #1 = first transition width (between 4.5 to 5.5 Hz); Fc_1 = first set frequency cut-off at 5 Hz (50% of original amplitude); Pass Band = region with greater than 99% of original amplitude (between 5.5 to 72 Hz); Transition #2 = second transition width (between 72 to 88 Hz) and Fc_2 = second set frequency cut-off at 80 Hz (50% of original amplitude).

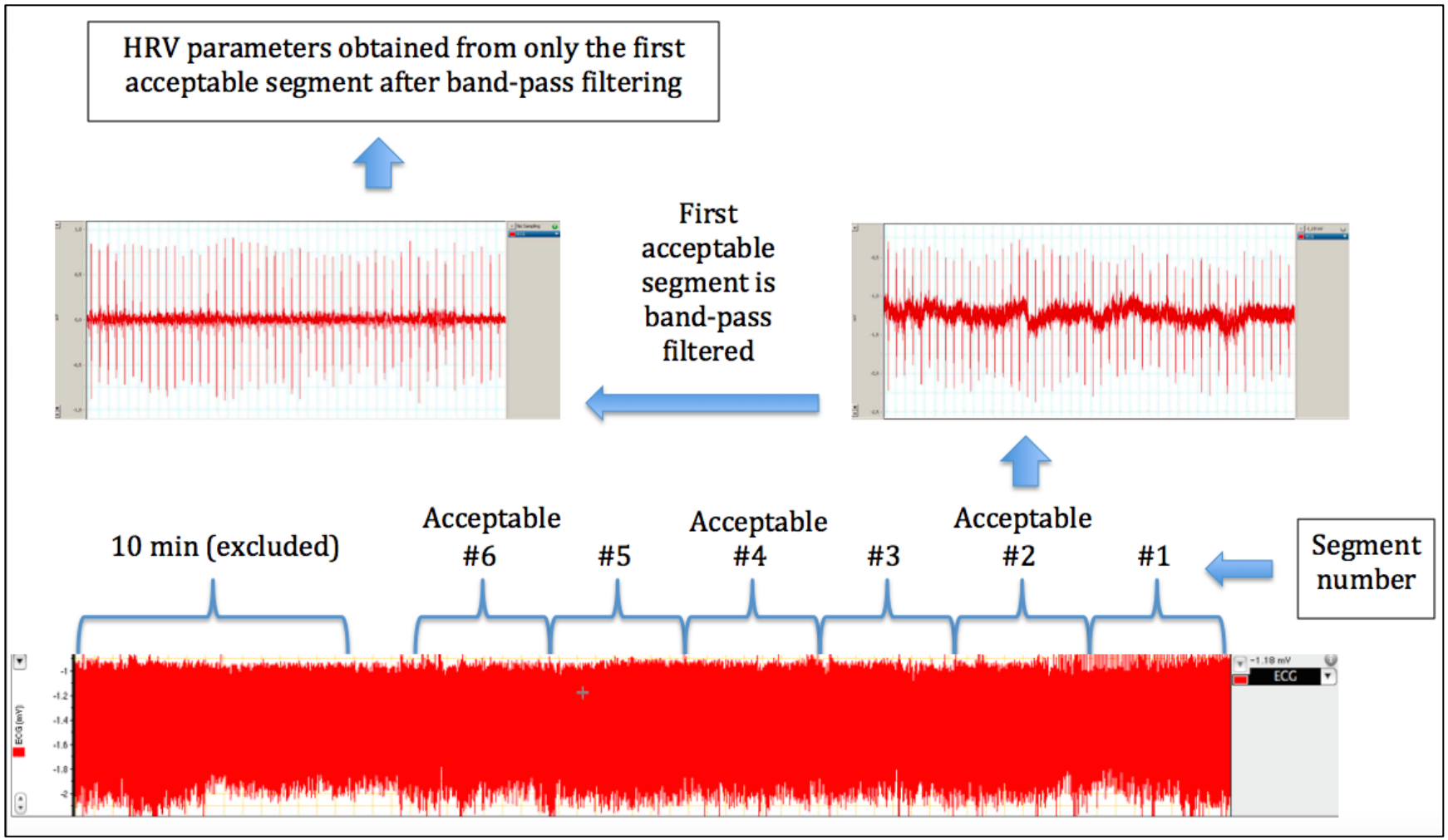

Figure 7. Diagram of Analysis #3. Example of a condensed ECG signal (43 min) obtained using the LabChart software. Working in reverse time, 6 segments of 5 min were identified. The first 10 min were always excluded (transition period). Only the first acceptable segment (having ≥80% normal NN intervals) from the end of the mode was selected and band-pass filtered (5 – 80 Hz) prior to analysis. HRV values were calculated and used for comparisons.

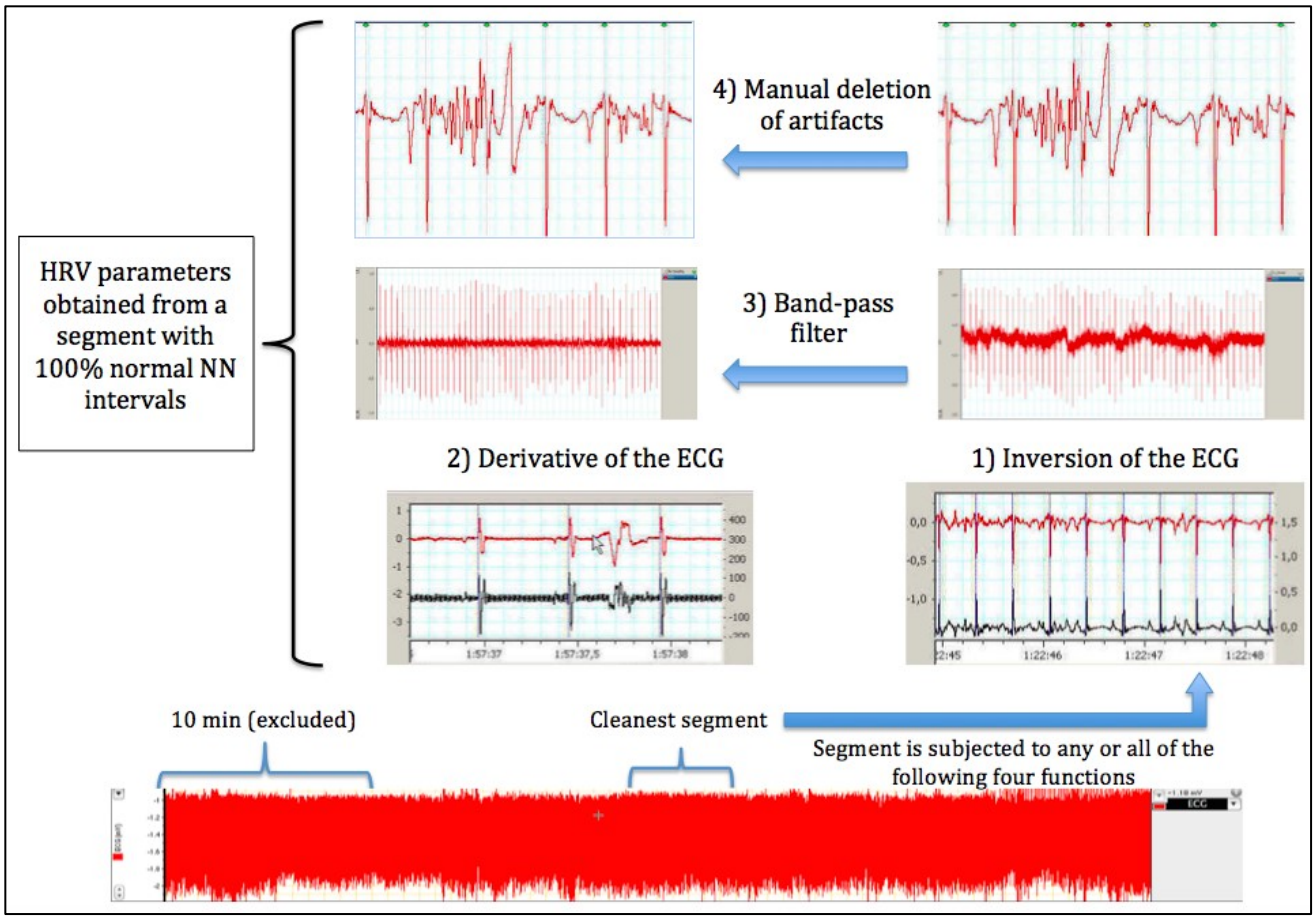

Figure 8. Diagram of Analysis #4. Example of a condensed ECG signal (43 min) obtained using the LabChart software. The ECG signal was visually inspected to obtain the cleanest, most artifact-free segment closest to the end of the mode, with the first 10 min of the recording always excluded (transition period). The selected segment was then subjected to any or all of the four functions to obtain 100% normal NN intervals: 1) Inversion of the ECG (red signal is the original signal, black is the inverted signal); 2) Derivative of the ECG (red signal is the original signal, black is the derivative of the signal); 3) Band-pass filter (5 – 80 Hz) and 4) Manual deletion of artifacts (green (normal), yellow (ectopic) and red (artifact) dots are beat indicators). HRV values were calculated and used for comparisons.

2.4.3 Time domain analysis

Time domain HRV measures are mainly markers of overall HRV. The common estimate used is the standard deviation of the NN intervals (SDNN), i.e. the variation of intervals measured between consecutive sinus beats. Some other commonly used HRV measures are based on the differences between NN intervals and variability is translated by using simple mathematical calculations: standard deviation of the successive differences between NN intervals (SD delta NN), root mean square of successive differences of NN intervals (RMSSD), percentage of adjacent NN intervals that differ by greater than 50ms (pNN50).

Using LabChart, for each selected ECG segment, the HRV module automatically calculated all of these time domain parameters and values were recorded in a separate data sheet. Given that the selected segments are short (5 minutes) and that most of these measures use NN interval differences, these time domain parameters represent rapid, high frequency changes in heart rate and are highly correlated with each other [38].

2.4.4 Frequency domain analysis

The frequency domain analysis utilizes the tachogram, which plots the NN intervals sequentially over time. Then, a power spectrum density analysis is applied to the tachogram in order to describe the relative contribution of different frequency components. As such, the frequency domain parameters are described by power within a given frequency range [38]. An example of a tachogram and its power spectrum density analysis are shown in Figure 9. The following frequency domain parameters were obtained, with frequency ranges set according to the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology standardized guidelines for HRV analysis [39]:

1. TP: total power (<0.4 Hz)
2. VLF: power within the very low frequency range (<0.04 Hz)
3. LF: power within the low frequency range (0.04-0.15 Hz)
4. HF: power within the high frequency range (>0.15 to <0.4Hz)
5. LF/HF ratio: ratio of the low frequency power to high frequency power

2.4.5 HRV interpretation

For the time domain analysis, a decreased HRV means reduced values of the parameters analyzed (SDNN, SD delta NN, RMSSD and pNN50). The same principle applies to the frequency domain analysis. However, when using frequency analysis, additional information can be obtained since the spectral measure of HF power is considered to be a measure of parasympathetic activity and the LF power as a measure of sympathetic activity. As a result, the ratio between low and high frequencies (LF/HF) represents the balance between both activities. In other words, reductions in HRV are reflected by decreases in the power of the frequency domain parameters, with certain frequency ranges providing specific information regarding the ANS branch involved [38]. Moreover, the medical literature has demonstrated that changes in HRV can be used as an indicator of altered physiological states, stress, response to treatment or intervention, and morbidity. Interpretation of HRV values are limited to comparisons within a group, individuals in longitudinal studies, or crossover study designs.

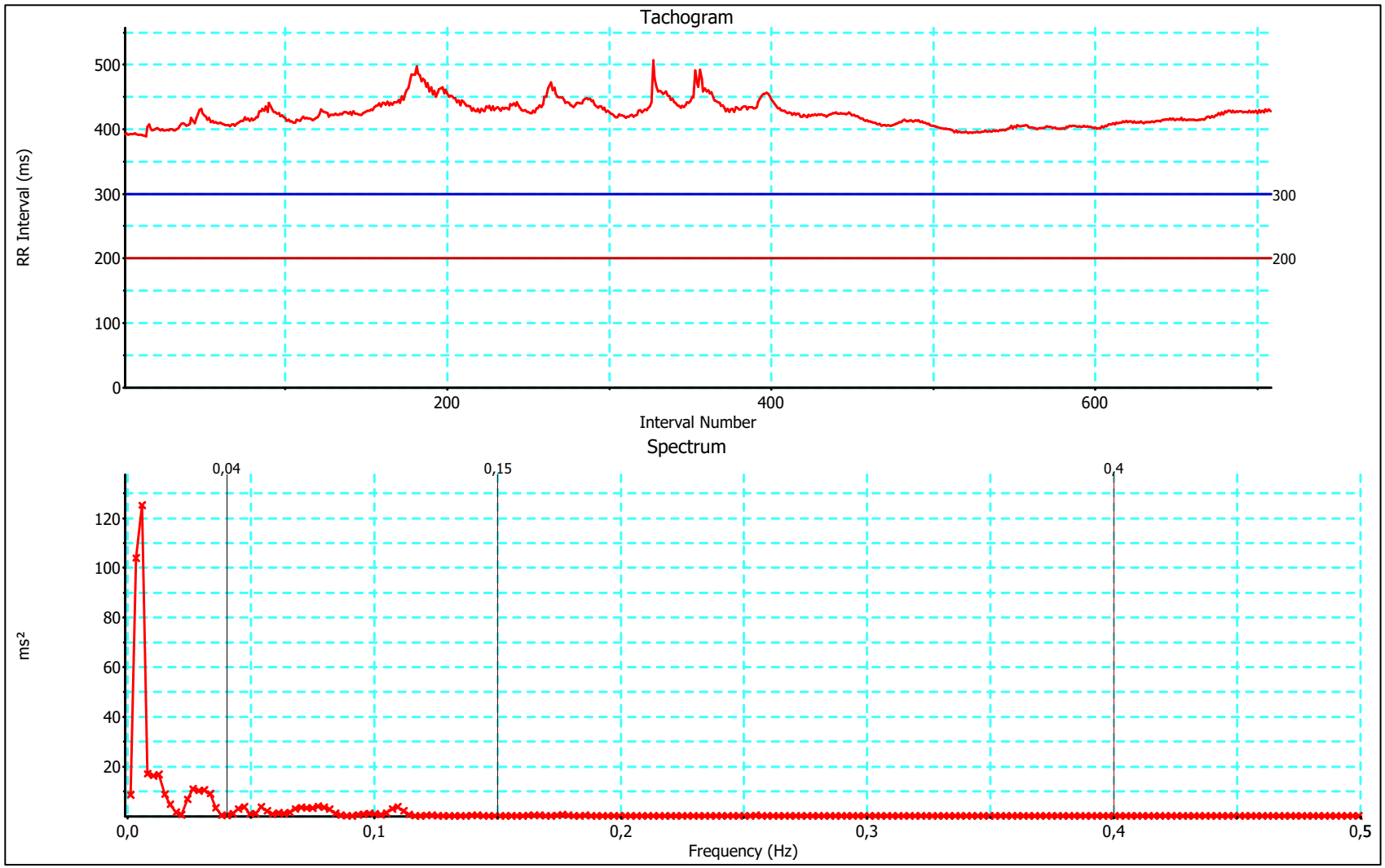

Figure 9. Example of a tachogram (upper panel) and power spectrum density (lower panel) generated by the HRV module and used for the frequency domain analyses.

2.5 Modes of non-invasive respiratory support analysis

HRV parameters of each patient (time and frequency domains) were compared between the modes of non-invasive respiratory support used. Since the most common rate used for non-synchronized NIPPV is 20 breaths per minute and previous studies were unable to demonstrate any differences between ventilatory rates while on non-synchronized NIPPV [37], we planned a first analysis comparing HRV values between NIPPV20 and NIPPV40. Given that this preliminary analysis revealed no differences between NIPPV20 and NIPPV40 (see Results 3.2), all subsequent analyses were restricted to compare NIPPV20 with CPAP.

For the secondary analysis, patients were grouped based on subsequent extubation outcome (success or failure) and for each patient the following comparisons of HRV parameters were made:

1) NIPPV20 vs. CPAP - Success group
2) NIPPV20 vs. CPAP - Failure group
3) NIPPV20 - Success vs. Failure groups
4) CPAP - Success vs. Failure groups
5) Absolute change in HRV values (ΔHRV) - Success vs. Failure groups
6) Relative change in HRV values (ΔHRV(%)) - Success vs. Failure groups

There is a lack of reference values for HRV in this population, given that values can be highly variable both between individuals as well as for a single individual at different times or circumstances. As such, comparisons of changes in HRV values allows the infants to act as their own control when exposed to the 2 different modes of non-invasive support. Thus, HRV changes will be calculated as the absolute and percent differences for each infant and compared between

groups (success and failure). For each patient, the absolute difference (ΔHRV) is calculated as the HRV values while on NIPPV20 minus the CPAP values, and the relative difference (ΔHRV(%)) is calculated as the ΔHRV divided by the CPAP values and multiplied by 100.

2.6 Clinical data

Baseline patient demographics (birth weight, gestational age, sex, APGAR scores, weight at extubation, day of life at extubation, and post-conceptional age at extubation), pre-extubation blood gas (pH, partial pressure of CO_2, bicarbonate, and base excess) and ventilation settings (ventilation mode, PIP, PEEP, inflation rates, FiO_2, and oxygen saturation), non-invasive support used during the transition period (ventilation mode, PIP, PEEP, and inflation rates) and extubation outcomes were prospectively collected using a data collection form. The primary reason for reintubation (apneas and bradycardias, increased work of breathing, and respiratory acidosis) was also collected. Any missing information from the data collection form and additional information regarding the infant's non-invasive respiratory support used following the end of the recordings was extracted from the medical records.

2.7 Sample size and statistical analysis

Due to the lack of data on HRV in preterm infants during the immediate post-extubation period, calculation of sample size was not possible. Therefore, a convenience sample size of 12 infants was chosen. Based on our previous experience, due to low quality of the ECG signals detected during the offline analysis, a loss between 10-20% of the patients was expected [25]. Thus, we planned to enroll 15 patients. Continuous variables are expressed as median (minimum, maximum) or number (percentage) and compared using the non-parametric two-sided *Wilcoxon rank sum* test and *Fisher's Exact* test using Matlab R2015a software (MathWorks Inc., Natick,

MA, USA). Similarly, two-sided non-parametric tests (*Wilcoxon signed-rank* and *Wilcoxon rank sum*) were used for all HRV parameter comparisons. The ability of each HRV parameter to accurately discriminate between the outcome of extubation success and failure was assessed using receiver operating characteristic (ROC) curves for the parameters with the most significant results using SigmaPlot 10.0 (Systat Software Inc., San Jose, CA, USA). The cut-off values were chosen to maximize the sensitivities and specificities. Standard formulae were used to calculate positive predictive value (PPV), negative predictive value (NPV), sensitivity and specificity.

Section 3 – Results

3.1 Population characteristics

A total of 15 patients were studied in the immediate post-extubation period. Due to poor quality ECG signals, 3 patients had to be excluded (2 successfully extubated and 1 failure). Of the 12 patients with sufficient quality ECGs, 7 subsequently succeeded extubation and 5 failed.

Baseline patient demographics, blood gas results, respiratory support settings and post-extubation management are described and compared between success and failure groups in Table 1. Included patients were born with a median birth weight of 855 grams and GA 27.0 weeks. Extubation occurred at a median age of 3.25 days, which corresponded to a post-conceptional age of 27.6 weeks. Infants were extubated from either AC (5/12) or SIMV (7/12) and the majority of them (75%) were placed on NIPPV after extubation (6/7 in the success and 3/5 in the failure group). Pressure levels (PIP and PEEP) during the pre-extubation period (n=12), transition phase (n=9; 3 patients received CPAP), study recordings (n=12), and after the end of the study (n=10; 2 patients received CPAP) are presented on Figure 10. For each patient, all respiratory support settings during the study period for CPAP, NIPPV20 and NIPPV40 are

described in Table 2. Only one infant (patient 9) received a PIP of 9 cmH_2O above PEEP level (Table 2).

All characteristics were similar between success and failure groups (Table 1), except for an increase in the pre-extubation PEEP and MAP levels in the failure group (p = 0.038 and 0.043, respectively). After the study protocol, one infant in the success group that received NIPPV during the transition period was put on CPAP, while 2 infants in the failure group that received CPAP during the transition period were started on NIPPV. Most patients (4 out of 5) within the failure group were reintubated between 12 and 24 hours post-extubation. The most common reason to reintubate patients was because of apneas and bradycardias (3 out of 5).

Table 1. Patient characteristics and peri-extubation management.

	All patients (n=12)	Success (n=7)	Failure (n=5)	p-value
Characteristics				
Birth weight (g)	855 (645, 1190)	890 (660, 1190)	760 (645, 1130)	0.639
Gestational age (wks)	27.0 (24.2, 29.4)	28.0 (25.3, 29.4)	25.3 (24.2, 27.4)	0.068
Male sex	6 (50)	3 (42.9)	3 (60)	1.000
5-minute APGAR score	7 (5, 8)	7 (5, 8)	7 (5, 8)	0.780
Weight at extubation (g)	895 (620,1190)	970 (710, 1190)	820 (620, 1040)	0.268
Age at extubation (days)	3.25 (1, 21)	2 (1, 21)	3.3 (1, 14)	0.672
Post-conceptional age at extubation (wks)	27.6 (25.3, 31.0)	28.1 (25.4, 31.0)	26.2 (25.3, 27.6)	0.096
Pre-extubation - ventilation				
Mode: AC	5 (42)	1 (14)	4 (80)	0.072
SIMV	7 (58)	6 (86)	1 (20)	
Peak inflation pressure (cmH_2O)	13 (10 ,15)	12 (10, 15)	13 (12, 15)	0.174
Peak end-expiratory pressure (cmH_2O)	5 (4, 6)	4 (4, 5)	5 (5, 6)	0.038*
Mean airway pressure (cmH_2O)	6.75 (4.8, 9.8)	6.0 (4.8, 9.8)	8.0 (6.9 – 9.0)	0.043*
Fraction of inspired oxygen	0.23 (0.21, 0.25)	0.21 (0.21, 0.28)	0.25 (0.21, 0.30)	0.470
Rate (inflations per minute)	28 (10, 40)	25 (20, 40)	30 (10, 40)	0.758
Oxygen saturation (%)	94 (90, 100) [n=11]	94.5 (90, 100) [n=6]	94 (93, 96)	1.000
Pre-extubation - blood gas				
pH	7.29 (7.24, 7.39)	7.29 (7.25, 7.36)	7.26 (7.24, 7.39)	0.242
PCO_2	53 (32, 69)	42.0 (32, 69)	54.0 (48, 59)	0.250
Bicarbonate (HCO_3)	23 (19, 29)	23 (19, 27)	25 (22, 29)	0.215
Base excess	-2.35 (-7.2, 4.4)	-2.4 (-7.2, -2.35)	-0.7 (-3.5, 3.4)	0.343
Post-extubation (pre-study) - respiratory support				
Mode: NIPPV	9 (75)	6 (86)	3 (60)	0.523
CPAP	3 (25)	1 (14)	2 (40)	
Peak inflation pressure (cmH_2O)	15 (12, 16) [n=9]	14 (12, 16) [n=6]	15 (15, 16) [n=3]	0.262
Peak end-expiratory pressure (cmH_2O)	5 (4, 6)	5 (4, 6)	5 (5, 6)	0.515
Rate (inflations per minute)	20 (15, 50) [n=9]	25 (20, 50) [n=6]	20 (15, 30) [n=3]	0.429
Post-extubation (post-study) - respiratory support				
Mode: NIPPV	10 (83)	5 (71)	5 (100)	0.470
CPAP	2 (17)	2 (29)	0 (0)	
Peak inflation pressure (cmH_2O)	15 (10, 20) [n=10]	14 (10, 16) [n=5]	15 (15, 20)	0.087
Peak end-expiratory pressure (cmH_2O)	5 (4, 6)	5 (4, 6)	5 (5, 6)	0.636
Rate (inflations per minute)	20 (15, 50) [n=10]	20 (20, 50) [n=5]	20 (15, 30)	0.524
Time of reintubation				
12 – 24hrs post-extubation	-	-	4 (80)	-
24 – 48hrs post-extubation	-	-	1 (20)	-
Reasons for reintubation				
Respiratory acidosis	-	-	1 (20)	-
Increased work of breathing	-	-	1 (20)	-
Apneas & bradycardias	-	-	3 (60)	-

*Legend: values presented as median (min, max), or n (%). * = p <0.05*

Table 2. Respiratory support settings during study period.

Patient number	CPAP	NIPPV20		NIPPV40	
	PEEP (cmH_2O)	PIP (cmH_2O)	PEEP (cmH_2O)	PIP (cmH_2O)	PEEP (cmH_2O)
1	5	15	5	15	5
2	6	16	6	16	6
3	5	15	5	15	5
4	5	15	5	15	5
5	5	15	5	15	5
6	5	15	5	15	5
7	5	15	5	15	5
8	5	16	6	16	6
9	5	14	5	14	5
10	5	15	5	15	5
11	5	15	5	15	5
12	5	15	5	15	5

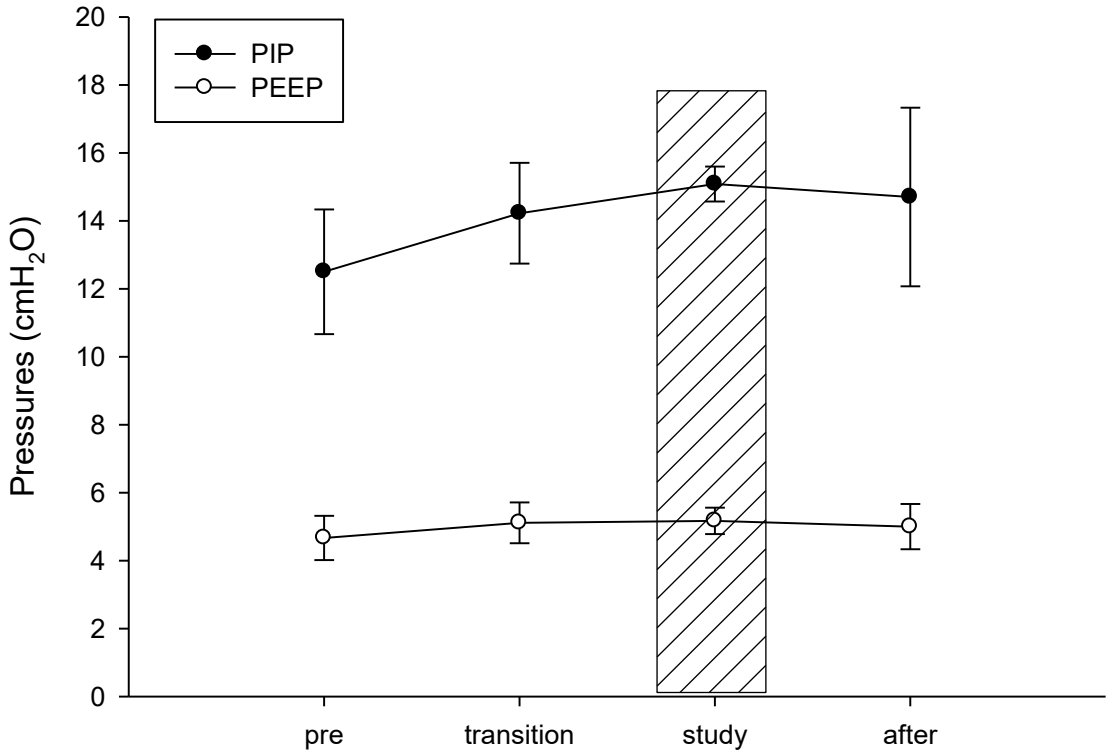

Figure 10. Means and standard deviations of pressures (PIP and PEEP) during the peri-extubation period. Shaded area highlights the study period where most infants received PIP of 15 and PEEP of 5 with small variability. Legend: pre = pre-extubation period [n=12]; transition = transition phase (30 min between extubation and study period) [n=9]; 3 infants received CPAP]; study = study period [n=12]; after = immediately after the study period [n=10; 2 infants received CPAP].

3.2 Preliminary results (NIPPV20 vs. NIPPV40)

The total number of segments included and excluded per patient for CPAP, NIPPV20, and NIPPV40 were not statistically different from each other (Table 3). Comparisons between NIPPV20 and NIPPV40 for all patients yielded no significant differences in any of the time domain or frequency domain HRV parameters (Table 4; Analysis #1).

3.3 Results of Analysis #1

No significant differences in any HRV parameters were observed between NIPPV20 and CPAP (Table 5). Similarly, no significant differences in any time domain (Table 6) or frequency domain (Table 8) HRV parameters were noted between NIPPV20 and CPAP for infants that were successfully extubated or failed. For the changes in HRV between NIPPV20 and CPAP, two parameters were significantly different between success and failure groups (Tables 7 and 9): ΔHRV(%) for pNN50 (time domain) -9.8 vs. 108.8%, $p = 0.030$, and ΔHRV(%) for HF (frequency domain) -17.1 vs. 224.7%, $p = 0.048$.

Table 3. Number of included and excluded segments per patient for CPAP, NIPPV20, and NIPPV40.

Patient number	CPAP Segments		NIPPV20 Segments		NIPPV40 Segments	
	Included	Excluded	Included	Excluded	Included	Excluded
1	7	2	8	2	10	0
2	2	6	5	4	8	2
3	10	0	8	0	6	0
4	3	1	5	0	4	0
5	3	0	4	1	3	0
6	3	0	4	0	3	0
7	3	1	2	0	3	0
8	4	0	4	0	2	1
9	5	2	2	4	5	1
10	6	0	6	0	6	0
11	6	0	6	0	6	0
12	5	2	2	5	6	1

Table 4. NIPPV20 and NIPPV40 for all patients (Analysis #1).

HRV Parameter	NIPPV20	NIPPV40	P-value
SDNN (ms)	26.884 (9.528, 67.723)	32.732 (6.908, 50.224)	0.380
SD delta NN (ms)	29.497 (6.973, 88.936)	26.474 (1.544, 60.541)	0.233
RMSSD (ms)	29.477 (6.968, 88.863)	26.458 (1.543, 60.492)	0.233
pNN50 (%)	2.337 (0.694, 5.896)	1.948 (0, 3.875)	0.266
TP (ms^2)	1113.104 (135.762, 7570.290)	1258.242 (32.630, 3192.717)	0.176
VLF (ms^2)	421.000 (104.456, 2508.903)	426.875(26.260, 1650.966)	0.129
LF (ms^2)	282.318 (8.974, 1425.075)	215.939 (2.435, 980.456)	0.176
HF (ms^2)	150.247(3.810, 1837.674)	173.067 (0.125, 764.315)	0.092
LF/HF Ratio	2.029 (1.001, 14.073)	2.149 (0.811, 31.101)	0.266

Legend: values presented as median (min, max)

Table 5. NIPPV20 and CPAP for all patients (Analysis #1).

HRV Parameter	NIPPV20	CPAP	P-value
SDNN (ms)	30.365 (9.015, 54.598)	24.080 (7.969, 39.704)	0.077
SD delta NN (ms)	29.631 (4.646, 67.640)	21.238 (7.743, 52.171	0.092
RMSSD (ms)	29.612 (4.643, 67.585)	21.222 (7.738, 52.132)	0.092
pNN50 (%)	2.044 (0.397, 4.197)	1.455 (0.472, 3.777)	0.129
TP (ms^2)	1068.071 (91.563, 4287.110)	853.319 (62.232, 1675.834)	0.110
VLF (ms^2)	404.882 (70.944, 1674.499)	306.505 (12.370, 531.850)	0.092
LF (ms^2)	216.421 (6.172, 1173.784)	236.964 (3.435, 584.843)	0.077
HF (ms^2)	174.398 (2.445, 1032.654)	108.990 (5.273, 344.244)	0.129
LF/HF Ratio	2.109 (1.039, 21.371)	2.024 (0.878, 9.572)	0.791

Legend: values presented as median (min, max)

Table 6. Group comparisons of NIPPV20 and CPAP for time domain HRV parameters (Analysis #1).

HRV Time Domain Parameter	Group & Mode	Values	P-values			
			NIPPV20 vs. CPAP Success	NIPPV20 vs. CPAP Failure	Success vs. Failure CPAP	Success vs. Failure NIPPV20
SDNN (ms)	Success NIPPV20	29.090 (9.015, 36.797)	0.219	0.313	0.530	0.202
	Success CPAP	24.077 (7.969, 36.102)				
	Failure NIPPV20	34.273 (18.746, 54.598)				
	Failure CPAP	24.083 (17.285, 39.704)				
SD delta NN (ms)	Success NIPPV20	21.492 (4.646, 50.550)	0.813	0.125	0.639	0.343
	Success CPAP	21.913 (9.942, 46.442)				
	Failure NIPPV20	32.160 (20.904, 67.640)				
	Failure CPAP	19.707 (7.743, 52.171)				
RMSSD (ms)	Success NIPPV20	21.478 (4.643, 50.512)	0.813	0.125	0.639	0.343
	Success CPAP	21.896 (9.934, 46.407)				
	Failure NIPPV20	32.139 (20.888, 67.585)				
	Failure CPAP	19.694 (7.738, 52.132)				
pNN50 (%)	Success NIPPV20	1.628 (0.397, 3.765)	0.938	0.063	0.755	0.343
	Success CPAP	1.806 (0.551, 3.777)				
	Failure NIPPV20	2.617 (1.683, 4.197)				
	Failure CPAP	1.104 (0.472, 3.465)				

Legend: values presented as median (min, max)

Table 7. Group comparisons of ΔHRV and ΔHRV(%) for time domain HRV parameters (Analysis #1).

HRV Time Domain Parameter	Group & Variable	Values	P-values	
			Success vs. Failure ΔHRV	Success vs. Failure ΔHRV(%)
SDNN (ms)	Success ΔHRV	2.558 (-8.148, 11.876)	0.432	0.268
	Success ΔHRV(%)	21.1 (-40.0, 69.0)		
	Failure ΔHRV	10.189 (0.810, 33.333)		
	Failure ΔHRV(%)	37.5 (2.7, 162.4)		
SD delta NN (ms)	Success ΔHRV	0.930 (-20.382, 12.483)	0.073	0.073
	Success ΔHRV(%)	4.5 (-71.2, 57.0)		
	Failure ΔHRV	15.469 (-1.126, 23.945)		
	Failure ΔHRV(%)	63.2 (-5.1, 250.0)		
RMSSD (ms)	Success ΔHRV	0.930 (-20.367, 12.475)	0.073	0.073
	Success ΔHRV(%)	4.5 (-71.2, 57.0)		
	Failure ΔHRV	15.453 (-1.126, 23.926)		
	Failure ΔHRV(%)	63.2 (-5.1, 250.0)		
pNN50 (%)	Success ΔHRV	-0.177 (-1.316, 0.855)	0.073	0.030*
	Success ΔHRV(%)	-9.8 (-62.7, 155.2)		
	Failure ΔHRV	1.513 (0.434, 2.187)		
	Failure ΔHRV(%)	108.8 (12.5, 409.2)		

*Legend: values presented as median (min, max), * = p <0.05*

Table 8. Group comparisons of NIPPV20 and CPAP for time domain HRV parameters (Analysis #1).

HRV Frequency Domain Parameter	Group & Mode	Values	P-values			
			NIPPV20 vs. CPAP Success	NIPPV20 vs. CPAP Failure	Success vs. Failure CPAP	Success vs. Failure NIPPV20
TP (ms^2)	Success NIPPV20	854.444 (91.563, 2428.663)	0.578	0.313	0.639	0.343
	Success CPAP	1050.072 (62.232, 1675.834)				
	Failure NIPPV20	2497.287 (378.472, 4287.110)				
	Failure CPAP	616.332 (332.722, 1521.309)				
VLF (ms^2)	Success NIPPV20	394.076 (70.944, 812.465)	0.375	0.188	0.639	0.268
	Success CPAP	363.152 (12.370, 531.850)				
	Failure NIPPV20	988.554 (131.511, 1674.499)				
	Failure CPAP	262.076 (53.863, 441.962)				
LF (ms^2)	Success NIPPV20	201.358 (6.172, 483.260)	0.297	0.188	1.000	0.343
	Success CPAP	266.514 (3.435, 584.843)				
	Failure NIPPV20	468.832 (71.513, 1173.784)				
	Failure CPAP	207.413 (59.929, 462.269)				
HF (ms^2)	Success NIPPV20	122.811 (2.445, 393.725)	0.813	0.125	0.639	0.343
	Success CPAP	148.127 (5.273, 344.244)				
	Failure NIPPV20	267.516 (58.805, 1032.654)				
	Failure CPAP	68.069 (40.688, 318.009)				
LF/HF Ratio	Success NIPPV20	2.178 (1.442, 21.371)	0.375	0.438	0.530	0.755
	Success CPAP	2.020 (0.878, 2.999)				
	Failure NIPPV20	2.040 (1.039, 4.566)				
	Failure CPAP	2.603 (1.072, 9.572)				

Legend: values presented as median (min, max)

Table 9. Group comparisons of ΔHRV and ΔHRV(%) for frequency domain HRV parameters (Analysis #1).

HRV Frequency Domain Parameter	Group & Variable	Values	P-values	
			Success vs. Failure ΔHRV	Success vs. Failure ΔHRV(%)
TP (ms^2)	Success ΔHRV	210.613 (-1015.732, 752.829)	0.268	0.432
	Success ΔHRV(%)	44.9 (-82.7, 320.0)		
	Failure ΔHRV	1880.956 (45.749, 2965.574)		
	Failure ΔHRV(%)	181.8 (13.7, 862.3)		
VLF (ms^2)	Success ΔHRV	58.573 (-410.670, 521.937)	0.268	0.432
	Success ΔHRV(%)	54.8 (-77.2, 473.5)		
	Failure ΔHRV	666.072 (-192.272, 1495.703)		
	Failure ΔHRV(%)	206.5 (-43.5, 836.5)		
LF (ms^2)	Success ΔHRV	2.736 (-372.175, 189.705)	0.432	0.639
	Success ΔHRV(%)	48.9 (-85.6, 527.0)		
	Failure ΔHRV	261.419 (-146.741, 1060.348)		
	Failure ΔHRV(%)	59.6 (-47.4, 934.8)		
HF (ms^2)	Success ΔHRV	-2.828 (-152.520, 144.539)	0.268	0.048*
	Success ΔHRV(%)	-17.1 (-90.7, 295.2)		
	Failure ΔHRV	223.086 (-9.264, 714.645)		
	Failure ΔHRV(%)	224.7 (-13.6, 557.5)		
LF/HF Ratio	Success ΔHRV	0.939 (-0.587, 19.351)	0.432	0.530
	Success ΔHRV(%)	53.1 (-28.9, 958.1)		
	Failure ΔHRV	0.585 (-8.534, 1.268)		
	Failure ΔHRV(%)	14.7 (-89.2, 118.3)		

*Legend: values presented as median (min, max), * = p <0.05*

3.4 Results of Analysis #2

Given that averaging multiple ECG segments (as done in Analysis #1) may not be appropriate because biological signals are not stationary, a single segment analysis was used in Analysis #2. Comparisons between NIPPV20 and CPAP for all patients yielded no significant differences for most HRV parameters, except for VLF within the frequency domain parameters where NIPPV20 values were higher than the CPAP values (491.601 vs. 223.033 ms^2, $p = 0.001$) (Table 10). Similar to Analysis #1, the results for NIPPV20 tended to be higher than those for CPAP. Comparisons between extubation success and failure groups yielded no significant differences in any time domain parameter, although results for the failure group for NIPPV20 and had a tendency to be greater than in the success group (Table 11). Most frequency domain HRV parameters yielded no significant differences for any of the group comparisons, except for VLF when comparing NIPPV20 vs. CPAP within the success group, with NIPPV20 values again higher than the CPAP values (356.554 vs. 241.521 ms^2, $p = 0.031$) (Table 13).

When comparing the absolute and relative differences between groups, many time domain and frequency domain parameters had significant differences: SDNN for ΔHRV (3.793 vs. 21.737 ms, $p = 0.048$), SD delta NN for ΔHRV(%) (19.4 vs. 357.0% ms^2, $p = 0.048$), RMSSD for ΔHRV(%) (19.4 vs. 357.0%, $p = 0.048$), TP for ΔHRV(%) (26.3 vs. 670.4%, $p = 0.030$), VLF (158.7 vs. 1070.4%, $p = 0.030$), and HF (-44.0 vs. 996.8%, $p = 0.030$)(Tables 12 and 14).

Since this analysis used only a single 5-min ECG segment and no signal manipulation, it became important to assess whether or not the presence of ectopic beats would significantly affect the final results. Thus, we compared all the HRV parameters with the percentage of

ectopic NN intervals per segment for all 24 segments (all patients on both modes). This comparison indicated a strong linear association between percent of ectopic NN intervals and all time domain parameters (R^2 values > 0.7; Figure 11), which was not the case for the frequency domain parameters (R^2 values between 0.019 and 0.534; Figure 12). Therefore, these results demonstrate that this type of methodology (Analysis #2) can be altered by the presence of ectopics and in Analysis #3 a filter was applied to overcome this problem, i.e. to remove any false ectopic beats that could have occurred due to noise.

Table 10. NIPPV20 and CPAP for all patients (Analysis #2).

HRV Parameter	NIPPV20	CPAP	P-value
SDNN (ms)	28.857 (15.468, 62.477)	21.715 (5.638, 56.595)	0.077
SD delta NN (ms)	31.493 (13.015, 75.093)	18.071 (1.698, 77.127)	0.129
RMSSD (ms)	31.471 (13.006, 75.044)	18.059 (1.697, 77.069)	0.129
pNN50 (%)	2.581 (0.586, 5.808)	0.794 (0, 6.557)	0.301
TP (ms^2)	1450.390 (102.086, 11515.800)	817.903 (24.105, 4501.860)	0.064
VLF (ms^2)	491.601 (54.274, 7171.930)	223.033 (12.611, 517.825)	0.001*
LF (ms^2)	354.951 (2.012, 1671.200)	201.721 (1.501, 1387.120)	0.064
HF (ms^2)	163.075 (3.804, 907.166)	89.213 (0.245, 1023.430)	0.424
LF/HF Ratio	2.050 (0.529, 3.849)	1.260 (0.265, 13.052)	0.569

*Legend: values presented as median (min, max), and * = p <0.05*

Table 11. Group comparisons of NIPPV20 and CPAP for time domain HRV parameters (Analysis #2).

HRV Time Domain Parameter	Group & Mode	Values	P-values			
			NIPPV20 vs. CPAP Success	NIPPV20 vs. CPAP Failure	Success vs. Failure CPAP	Success vs. Failure NIPPV20
SDNN (ms)	Success NIPPV20	20.783 (15.468, 36.726)	0.688	0.063	0.755	0.149
	Success CPAP	21.229 (8.439, 56.595)				
	Failure NIPPV20	47.893 (18.758, 62.477)				
	Failure CPAP	22.202 (5.638, 45.255)				
SD delta NN (ms)	Success NIPPV20	23.483 (13.015, 52.429)	0.813	0.125	0.202	0.202
	Success CPAP	24.660 (8.806, 77.127)				
	Failure NIPPV20	31.904 (25.634, 75.093)				
	Failure CPAP	6.981 (1.698, 64.452)				
RMSSD (ms)	Success NIPPV20	23.466 (13.006, 52.396)	0.813	0.125	0.202	0.202
	Success CPAP	24.642 (8.800, 77.069)				
	Failure NIPPV20	31.883 (25.614, 75.044)				
	Failure CPAP	6.976 (1.697, 64.403)				
pNN50 (%)	Success NIPPV20	1.961 (0.586, 5.808)	0.938	0.063	0.136	0.432
	Success CPAP	2.256 (0.295, 6.557)				
	Failure NIPPV20	3.134 (1.558, 4.427)				
	Failure CPAP	0.661 (0, 2.417)				

*Legend: values presented as median (min, max), and * = $p < 0.05$*

Table 12. Group comparisons of ΔHRV and ΔHRV(%) for time domain HRV parameters (Analysis #2).

HRV Time Domain Parameter	Group & Variable	Values	P-values	
			Success vs. Failure ΔHRV	Success vs. Failure ΔHRV(%)
SDNN (ms)	Success ΔHRV	3.793 (-37.310, 20.158)	0.048*	0.106
	Success ΔHRV(%)	22.3 (-65.9, 238.9)		
	Failure ΔHRV	21.737 (2.638, 40.276)		
	Failure ΔHRV(%)	181.4 (5.8, 294.6)		
SD delta NN (ms)	Success ΔHRV	6.527 (-53.644, 26.208)	0.073	0.048*
	Success ΔHRV(%)	19.4 (-69.6, 297.6)		
	Failure ΔHRV	24.923 (-3.608, 55.909)		
	Failure ΔHRV(%)	357.0 (-5.6, 1730.6)		
RMSSD (ms)	Success ΔHRV	6.523 (-53.604, 26.190)	0.073	0.048*
	Success ΔHRV(%)	19.4 (-69.6, 297.6)		
	Failure ΔHRV	24.906 (-3.604, 55.871)		
	Failure ΔHRV(%)	357.0 (-5.6, 1730.5)		
pNN50 (%)	Success ΔHRV	1.280 (-5.238, 2.688)	0.202	0.267
	Success ΔHRV(%)	28.3 (-79.9, 565.7)		
	Failure ΔHRV	2.385 (0.717, 3.689)		
	Failure ΔHRV(%)	379.6 (29.7, 499.9) [n=3]		

*Legend: values presented as median (min, max), * = p <0.05*

Table 13. Group comparisons of NIPPV20 and CPAP for frequency domain HRV parameters (Analysis #2).

HRV Frequency Domain Parameter	Group & Mode	Values	P-values			
			NIPPV20 vs. CPAP Success	NIPPV20 vs. CPAP Failure	Success vs. Failure CPAP	Success vs. Failure NIPPV20
TP (ms^2)	Success NIPPV20	1233.000 (102.086, 2299.380)	0.688	0.063	0.343	0.530
	Success CPAP	976.508 (70.309, 4501.860)				
	Failure NIPPV20	1754.980 (185.709, 11515.800)				
	Failure CPAP	526.053 (24.105, 1440.980)				
VLF (ms^2)	Success NIPPV20	356.554 (72.482, 1339.820)	0.031*	0.063	0.755	0.343
	Success CPAP	241.521 (12.611, 517.825)				
	Failure NIPPV20	990.657 (54.274, 7171.930)				
	Failure CPAP	204.544 (17.510, 416.315)				
LF (ms^2)	Success NIPPV20	293.932 (2.012, 691.993)	0.688	0.063	0.432	0.639
	Success CPAP	223.509 (3.468, 1387.120)				
	Failure NIPPV20	468.748 (62.722, 1671.200)				
	Failure CPAP	179.932 (1.501, 390.864)				
HF (ms^2)	Success NIPPV20	148.576 (3.804, 485.928)	0.688	0.063	0.268	0.755
	Success CPAP	183.586 (13.062, 1023.430)				
	Failure NIPPV20	177.573 (26.401, 907.166)				
	Failure CPAP	82.711 (0.245, 272.701)				
LF/HF Ratio	Success NIPPV20	1.978 (0.529, 3.849)	0.156	0.813	0.268	0.639
	Success CPAP	1.164 (0.265, 2.590)				
	Failure NIPPV20	2.376 (1.534, 2.640)				
	Failure CPAP	1.953 (0.823, 13.052)				

*Legend: values presented as median (min, max), and * = $p < 0.05$*

Table 14. Group comparisons of ΔHRV and ΔHRV(%) for frequency domain HRV parameters (Analysis #2).

HRV Frequency Domain Parameter	Group & Variable	Values	P-values	
			Success vs. Failure ΔHRV	Success vs. Failure ΔHRV(%)
TP (ms^2)	Success ΔHRV	256.492 (-2528.520, 1040.050)	0.106	0.030*
	Success ΔHRV(%)	26.3 (-56.2, 1144.2)		
	Failure ΔHRV	1228.927 (161.604, 10547.110)		
	Failure ΔHRV(%)	670.4 (233.6, 2263.2)		
VLF (ms^2)	Success ΔHRV	186.320 (-26.467, 821.995)	0.149	0.030*
	Success ΔHRV(%)	158.7 (-10.8, 693.7)		
	Failure ΔHRV	288.816 (61.221, 1280.336)		
	Failure ΔHRV(%)	1070.4 (210, 1622.7)		
LF (ms^2)	Success ΔHRV	14.225 (-1093.188, 597.532)	0.149	0.073
	Success ΔHRV(%)	3.5 (-78.8, 1348.5)		
	Failure ΔHRV	288.816 (61.221, 1280.336)		
	Failure ΔHRV(%)	490.9 (160.5, 4668.6)		
HF (ms^2)	Success ΔHRV	-9.258 (-874.854, 399.631)	0.106	0.030*
	Success ΔHRV(%)	-44.0 (-85.5, 694.8)		
	Failure ΔHRV	85.445 (24.982, 824.455)		
	Failure ΔHRV(%)	996.8 (92.7, 26068)		
LF/HF Ratio	Success ΔHRV	0.623 (-1.398, 2.515)	0.530	0.343
	Success ΔHRV(%)	82.3 (-54, 488.8)		
	Failure ΔHRV	0.687 (-10.674, 1.317)		
	Failure ΔHRV(%)	35.2 (-81.8, 124.4)		

*Legend: values presented as median (min, max), * = p <0.05*

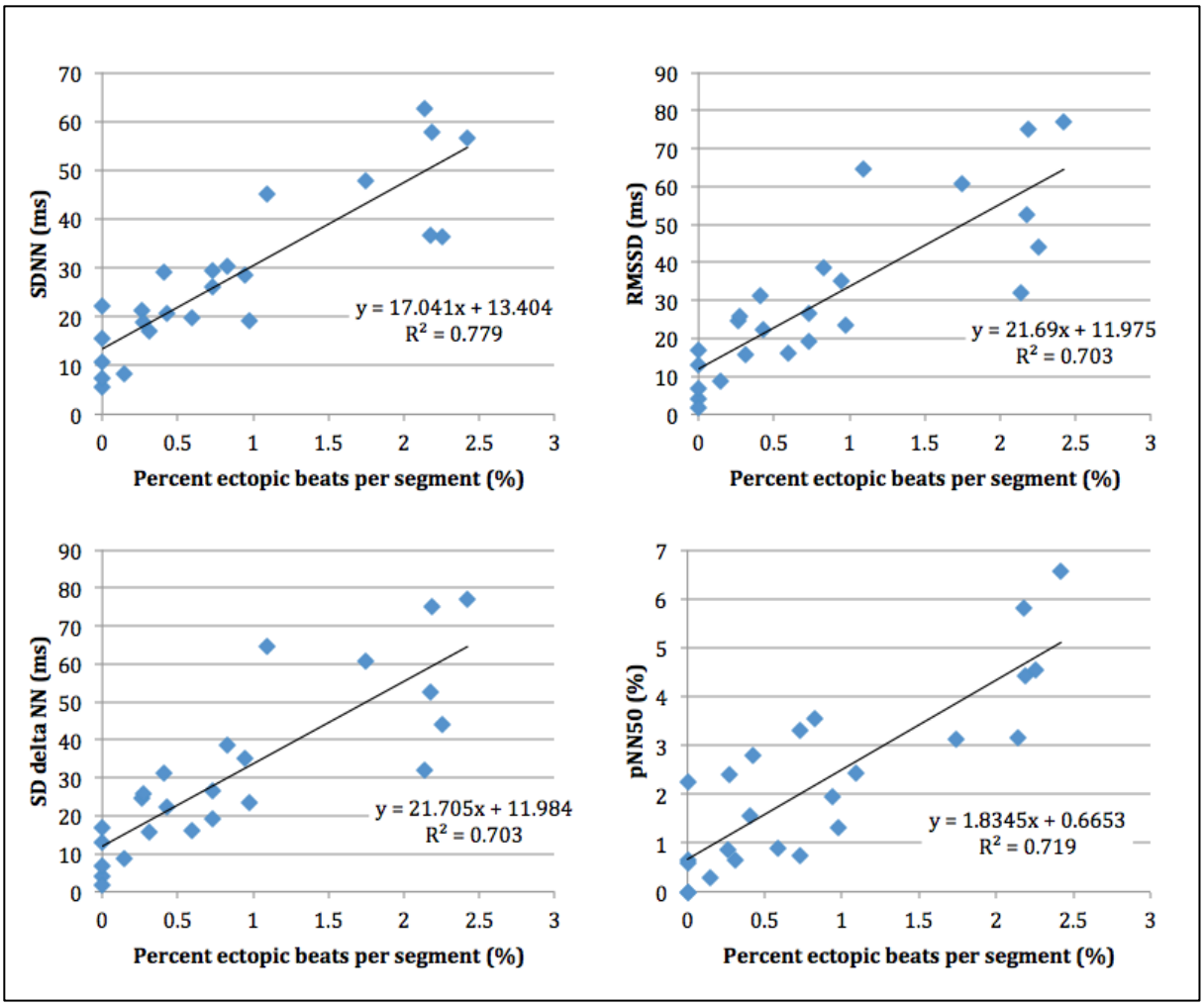

Figure 11. Effect of percent ectopics per segments on time domain HRV parameters for all patients' segments (blue diamonds), including both modes (Analysis #2). The linear fit (black line), its equation, and coefficient of determination (R^2) were obtained for each parameter.

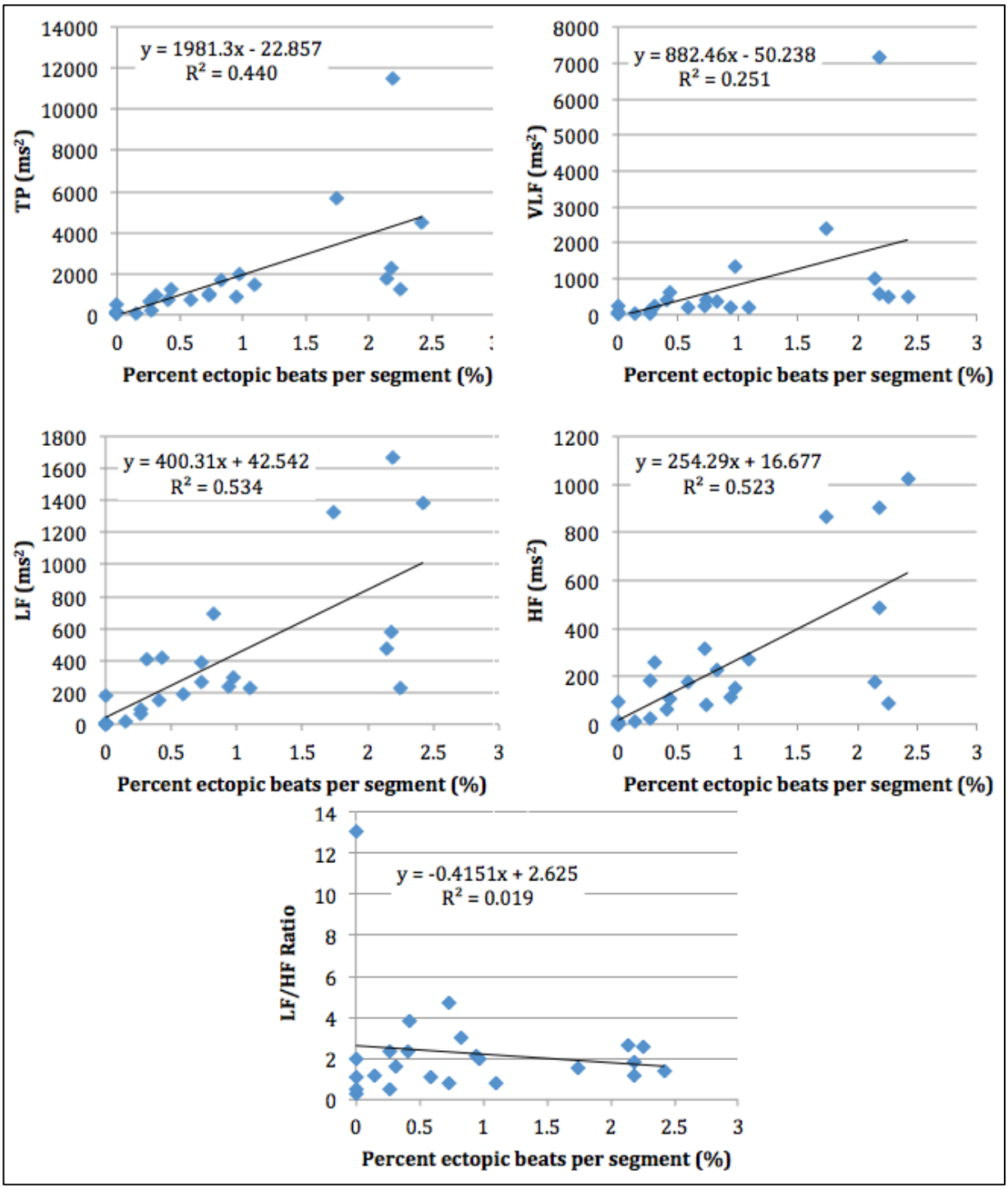

Figure 12. Effect of percent ectopics per segments on frequency domain HRV parameters for all patients' segments (blue diamonds), including both modes (Analysis #2). The linear fit (black line), its equation, and coefficient of determination (R^2) were obtained for each parameter.

3.5 Results of Analysis #3

There were no statistically significant differences between NIPPV20 and CPAP for all patients across all HRV parameters (Table 15). During NIPPV20, HRV values were significantly higher in the failure group when compared to the success group only for the time domain parameter SDNN (14.688 vs. 23.901 ms, $p = 0.005$; Table 16). However, ΔHRV values were significantly higher in the failure group for almost all the time and frequency domain parameters (Tables 17 and 19): SDNN (-3.908 vs. 13.154 ms, $p = 0.018$), SD delta NN (-5.690 vs. 18.230ms, $p = 0.010$), RMSSD (-5.686 vs. 18.219 ms, $p = 0.010$), pNN50 (-0.410 vs. 2.083%, $p = 0.010$), TP (-60.994 vs. 406.392 ms^2, $p = 0.010$), and VLF (0.999 vs. 295.608 ms^2, $p = 0.018$). Furthermore, the ΔHRV(%) values were significantly increased in the failure group for all time domain parameters (Table 17): SDNN (-18.4 vs. 164.0%, $p = 0.030$), SD delta NN (-33.9 vs. 261.0%, $p = 0.048$), RMSSD (-33.9 vs. 261.0%, $p = 0.048$), and pNN50 (-68.3 vs. 315.4, $p = 0.036$). There were no statistically significant differences for the comparison NIPPV20 with CPAP in the success or failure groups and the comparison of success and failure groups while on CPAP for any time or frequency domain HRV parameter (Tables 16 and 18).

Given that the comparison between the success and failure groups for ΔHRV and ΔHRV(%) yielded multiple significant results, ROC curves were developed for each significant HRV parameter (Figures 13 and 14). For ΔHRV, the areas under the ROC curves were high (≥0.914), with high sensitivities (≥80.0), specificities (≥85.7), PPVs (≥83.3), and NPVs (≥87.5), and for ΔHRV(%), the areas under the ROC curves were also high (≥0.886), with high sensitivities (≥80.0), specificities (≥85.7), PPVs (≥80.0), and NPVs (≥85.7)(Table 20).

As expected by using Analysis #3, the percentage of ectopics was considerably reduced (Figures 15 and 16). Although lower, a linear association between time domain parameters and the percentage of ectopic NN intervals was still observed (R^2 values > 0.6; Figure 15), while frequency domain parameters exhibited much lower values (R^2 values between 0.019 and 0.737; Figure 16).

Table 15. NIPPV20 and CPAP for all patients (Analysis #3).

HRV Parameter	NIPPV20	CPAP	P-value
SDNN (ms)	18.390 (6.775, 66.819)	12.234 (5.284, 45.913)	0.380
SD delta NN (ms)	14.506 (2.065, 28.181)	7.37061 (1.44996, 57.1179)	0.791
RMSSD (ms)	14.496 (2.064, 28.159)	7.366 (1.449, 57.081)	0.791
pNN50 (%)	0.520 (0, 4.827)	0.499 (0, 5.236)	0.520
TP (ms^2)	240.070 (60.489, 1794.450)	143.819 (21.411, 1844.620)	0.569
VLF (ms^2)	126.811 (23.124, 1133.460)	39.798 (12.487, 422.305)	0.110
LF (ms^2)	49.407 (2.038, 514.760)	33.889 (1.182, 388.610)	0.677
HF (ms^2)	41.358 (1.642, 151.517)	24.540 (0.158, 358.768)	0.622
LF/HF Ratio	3.149 (0.500, 14.224)	1.685 (0.265, 23.043)	0.733

Legend: values presented as median (min, max)

Table 16. Group comparisons of NIPPV20 and CPAP for time domain HRV parameters (Analysis #3).

HRV Time Domain Parameter	Group & Mode	Values	P-values			
			NIPPV20 vs. CPAP Success	NIPPV20 vs. CPAP Failure	Success vs. Failure CPAP	Success vs. Failure NIPPV20
SDNN (ms)	Success NIPPV20	14.688 (6.775, 19.648)	0.469	0.125	0.876	0.005*
	Success CPAP	13.721 (5.999, 45.913)				
	Failure NIPPV20	23.901 (19.440, 66.819)				
	Failure CPAP	10.747 (5.284, 26.702)				
SD delta NN (ms)	Success NIPPV20	11.578 (2.065, 25.785)	0.156	0.063	0.202	0.202
	Success CPAP	17.062 (1.450, 57.118)				
	Failure NIPPV20	24.749 (4.767, 28.181)				
	Failure CPAP	5.785 (1.495, 11.889)				
RMSSD (ms)	Success NIPPV20	11.570 (2.064, 25.769)	0.156	0.063	0.202	0.202
	Success CPAP	17.050 (1.449, 57.081)				
	Failure NIPPV20	24.733 (4.764, 28.159)				
	Failure CPAP	5.782 (1.494, 11.880)				
pNN50 (%)	Success NIPPV20	0.298 (0, 4.827)	0.219	0.063	0.318	0.194
	Success CPAP	0.603 (0, 5.236)				
	Failure NIPPV20	2.229 (0.141, 2.743)				
	Failure CPAP	0.245 (0, 0.661)				

*Legend: values presented as median (min, max), and * = p <0.05*

Table 17. Group comparisons of ΔHRV and ΔHRV(%) for time domain HRV parameters (Analysis #3).

HRV Time Domain Parameter	Group & Variable	Values	P-values	
			Success vs. Failure ΔHRV	Success vs. Failure ΔHRV(%)
SDNN (ms)	Success ΔHRV	-3.908 (-26.265, 8.689)	0.018*	0.030*
	Success ΔHRV(%)	-18.4 (-57.2, 144.8)		
	Failure ΔHRV	13.154 (-1.628, 44.617)		
	Failure ΔHRV(%)	164.0 (-6.1, 284.8)		
SD delta NN (ms)	Success ΔHRV	-5.690 (-31.333, 7.011)	0.010*	0.048*
	Success ΔHRV(%)	-33.9 (-73.4, 483.5)		
	Failure ΔHRV	18.230 (3.272, 25.359)		
	Failure ΔHRV(%)	261.0 (38.0, 898.8)		
RMSSD (ms)	Success ΔHRV	-5.686 (-31.313, 7.005)	0.010*	0.048*
	Success ΔHRV(%)	-33.9 (-73.4, 483.3)		
	Failure ΔHRV	18.219 (3.270, 25.339)		
	Failure ΔHRV(%)	261.0 (38.0, 898.7)		
pNN50 (%)	Success ΔHRV	-0.410 (-1.687, 0.455)	0.010*	0.036*
	Success ΔHRV(%)	-68.3 (-100.0, -7.8) [n=5]		
	Failure ΔHRV	2.083 (0.141, 2.229)		
	Failure ΔHRV(%)	315.4 (73.3, 872.7) [n=3]		

*Legend: values presented as median (min, max), * = p <0.05*

Table 18. Group comparisons of NIPPV20 and CPAP for frequency domain HRV parameters (Analysis #3).

HRV Frequency Domain Parameter	Group & Mode	Values	P-values			
			NIPPV20 vs. CPAP Success	NIPPV20 vs. CPAP Failure	Success vs. Failure CPAP	Success vs. Failure NIPPV20
TP (ms^2)	Success NIPPV20	179.144 (60.489, 593.175)	0.297	0.063	0.755	0.073
	Success CPAP	137.335 (27.444, 1844.620)				
	Failure NIPPV20	712.411 (118.123, 1794.450)				
	Failure CPAP	150.302 (21.411, 877.865)				
VLF (ms^2)	Success NIPPV20	100.241 (24.876, 143.165)	1.000	0.063	0.639	0.106
	Success CPAP	47.6041 (12.487, 297.911)				
	Failure NIPPV20	355.015 (23.124, 1133.460)				
	Failure CPAP	31.991 (17.648, 422.305)				
LF (ms^2)	Success NIPPV20	41.713 (2.038, 106.764)	0.469	0.313	0.876	0.106
	Success CPAP	37.275 (3.481, 230.584)				
	Failure NIPPV20	251.620 (14.128, 514.760)				
	Failure CPAP	30.504 (1.182, 388.610)				
HF (ms^2)	Success NIPPV20	11.178 (1.642, 100.540)	0.156	0.438	0.639	0.343
	Success CPAP	13.134 (0.158, 358.768)				
	Failure NIPPV20	56.183 (10.117, 151.517)				
	Failure CPAP	35.945 (0.219, 92.452)				
LF/HF Ratio	Success NIPPV20	1.062 (0.540, 14.224)	0.297	0.625	0.149	0.876
	Success CPAP	0.643 (0.265, 23.043)				
	Failure NIPPV20	5.140 (0.500, 9.162)				
	Failure CPAP	5.396 (0.849, 14.256)				

Legend: values presented as median (min, max).

Table 19. Group comparisons of ΔHRV and ΔHRV(%) for frequency domain HRV parameters (Analysis #3).

HRV Frequency Domain Parameter	Group & Variable	Values	P-values	
			Success vs. Failure ΔHRV	Success vs. Failure ΔHRV(%)
TP (ms^2)	Success ΔHRV	-60.994 (-1251.445, 151.701)	0.010*	0.073
	Success ΔHRV(%)	-50.2 (-69.4, 552.8)		
	Failure ΔHRV	406.392 (96.713, 1268.336)		
	Failure ΔHRV(%)	374.0 (41.0, 1350.4)		
VLF (ms^2)	Success ΔHRV	0.999 (-177.677, 85.420)	0.018*	0.149
	Success ΔHRV(%)	4.2 (-59.6, 576.3)		
	Failure ΔHRV	295.608 (5.476, 886.356)		
	Failure ΔHRV(%)	358.7 (26.7, 1252.5)		
LF (ms^2)	Success ΔHRV	-12.054 (-123.820, 47.291)	0.202	0.106
	Success ΔHRV(%)	-37.3 (-55.2, 1049)		
	Failure ΔHRV	58.447 (-136.990, 334.951)		
	Failure ΔHRV(%)	898.5 (-35.3, 1832.6)		
HF (ms^2)	Success ΔHRV	-10.991 (-258.228, 54.310)	0.106	0.149
	Success ΔHRV(%)	-71.5 (-96.9, 34473.0)		
	Failure ΔHRV	23.317 (-36.269, 90.281)		
	Failure ΔHRV(%)	147.4 (-39.2, 12794.0)		
LF/HF Ratio	Success ΔHRV	0.419 (-22.277, 11.405)	0.530	0.639
	Success ΔHRV(%)	103.8 (-96.7, 2383.8)		
	Failure ΔHRV	-4.685 (-8.163, 7.217)		
	Failure ΔHRV(%)	-57.3 (-90.7, 505.7)		

*Legend: values presented as median (min, max), * = $p < 0.05$.*

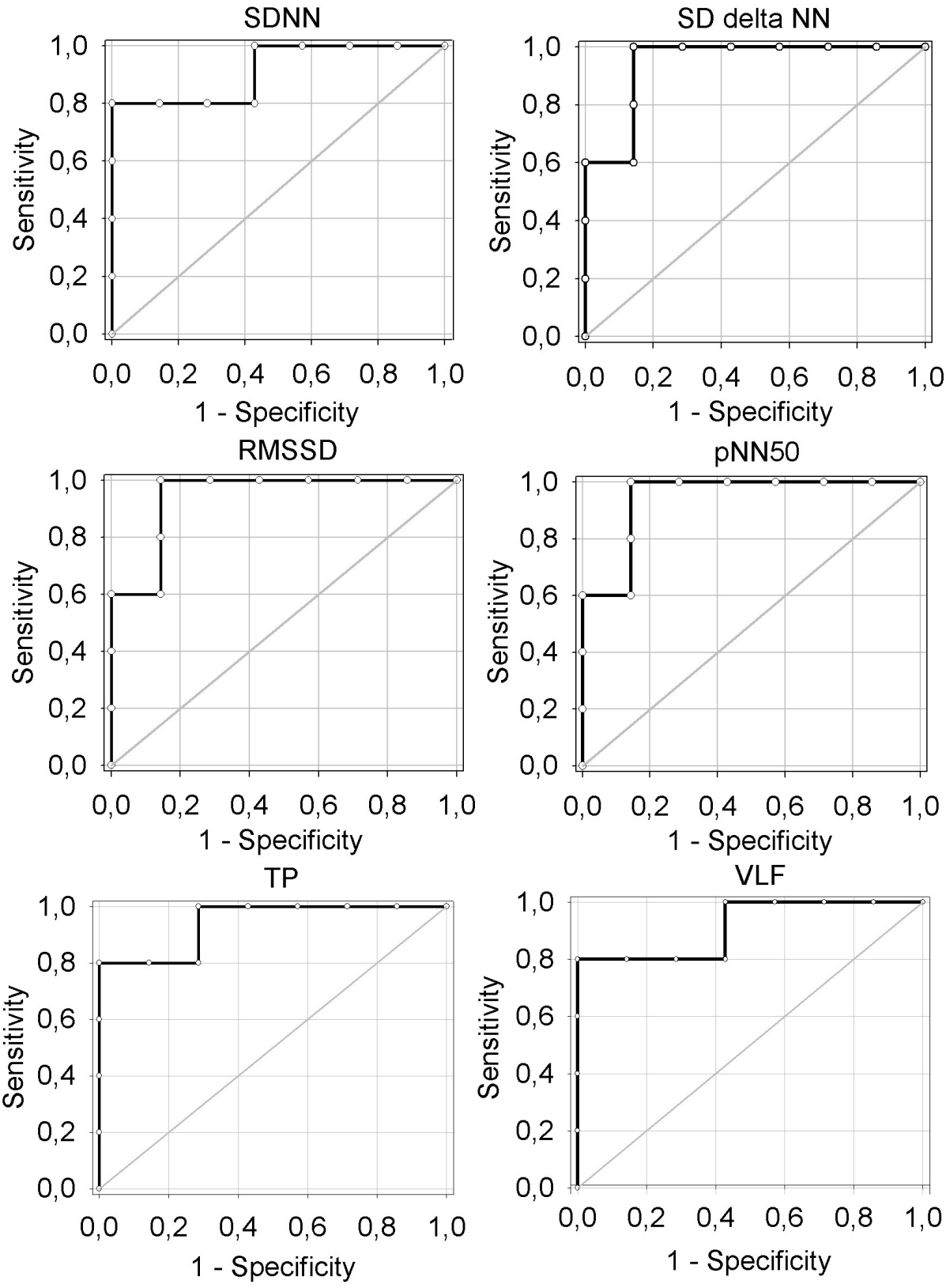

Figure 13. ROC curves for the HRV parameters that had statistically significant differences in ΔHRV values between success and failure groups (Analysis #3).

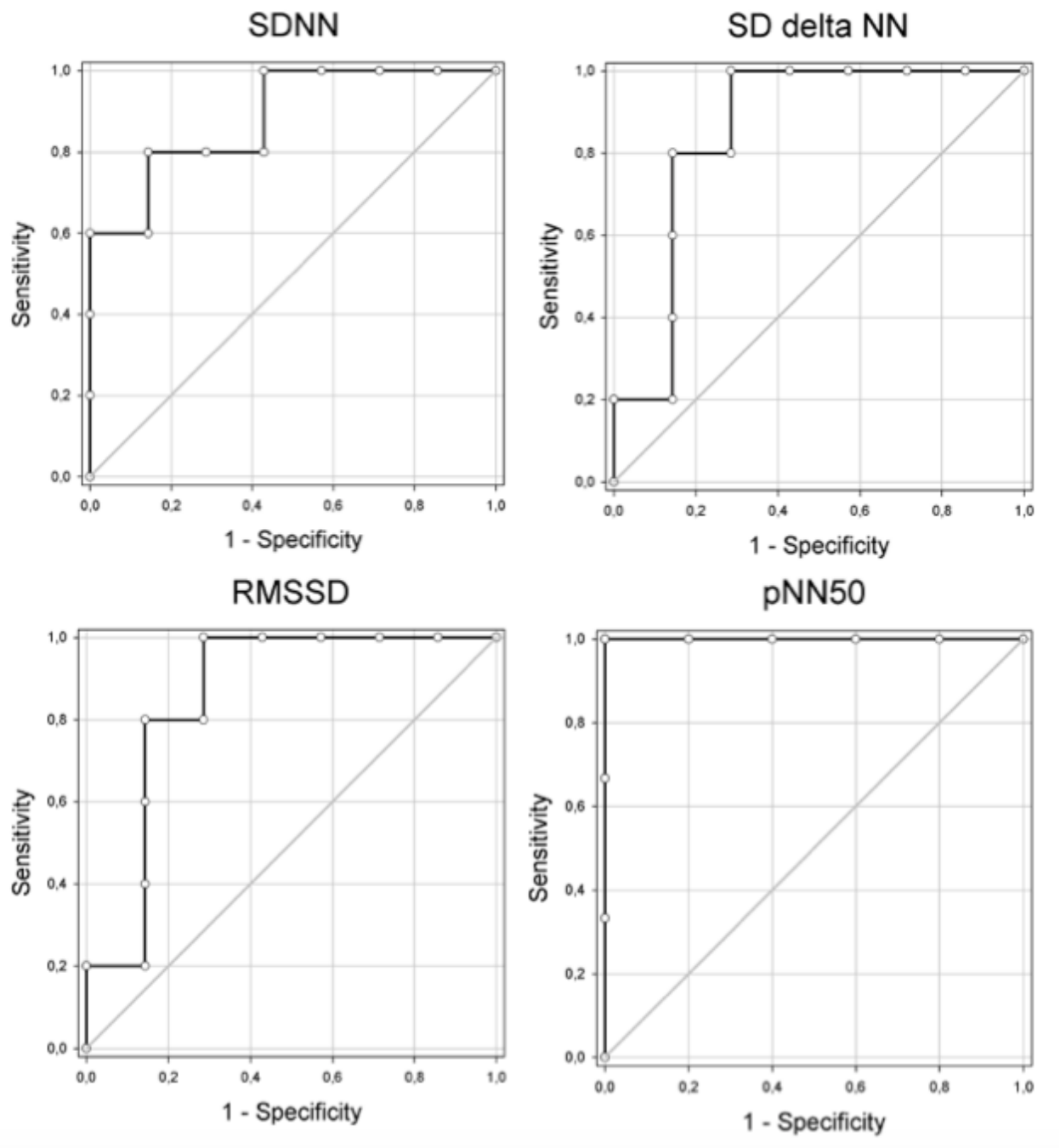

Figure 14. ROC curves for the HRV parameters that had statistically significant differences in ΔHRV(%) values between success and failure groups (Analysis #3).
Note: pNN50 ROC curve is generated with 5 success and 3 failure values.

Table 20. Predictive values for extubation failure for the HRV parameters that had statistically significant differences in ΔHRV and ΔHRV(%) values between success and failure groups (Analysis #3).

HRV Parameter	Cut-off value	Area under ROC curve	Sensitivity	Specificity	PPV	NPV
ΔHRV						
SDNN (ms)	10.380	0.914	80.0	100	100	87.5
SD delta NN (ms)	2.786	0.943	100	85.7	83.3	100
RMSSD (ms)	2.784	0.943	100	85.7	83.3	100
pNN50 (%)	0.071	0.943	100	85.7	83.3	100
TP (ms^2)	255.700	0.943	80.0	100	100	87.5
VLF (ms^2)	99.130	0.914	80.0	100	100	87.5
ΔHRV(%)						
SDNN (%)	83.99	0.886	80.0	85.7	80.0	85.7
SD delta NN (%)	137.1	0.886	80.0	85.7	80.0	85.7
RMSSD (%)	137.1	0.886	80.0	85.7	80.0	85.7
pNN50[a] (%)	32.72	1.000	100	100	100	100

Legend: [a] = values only available for 5 success infants and 3 failure infants.

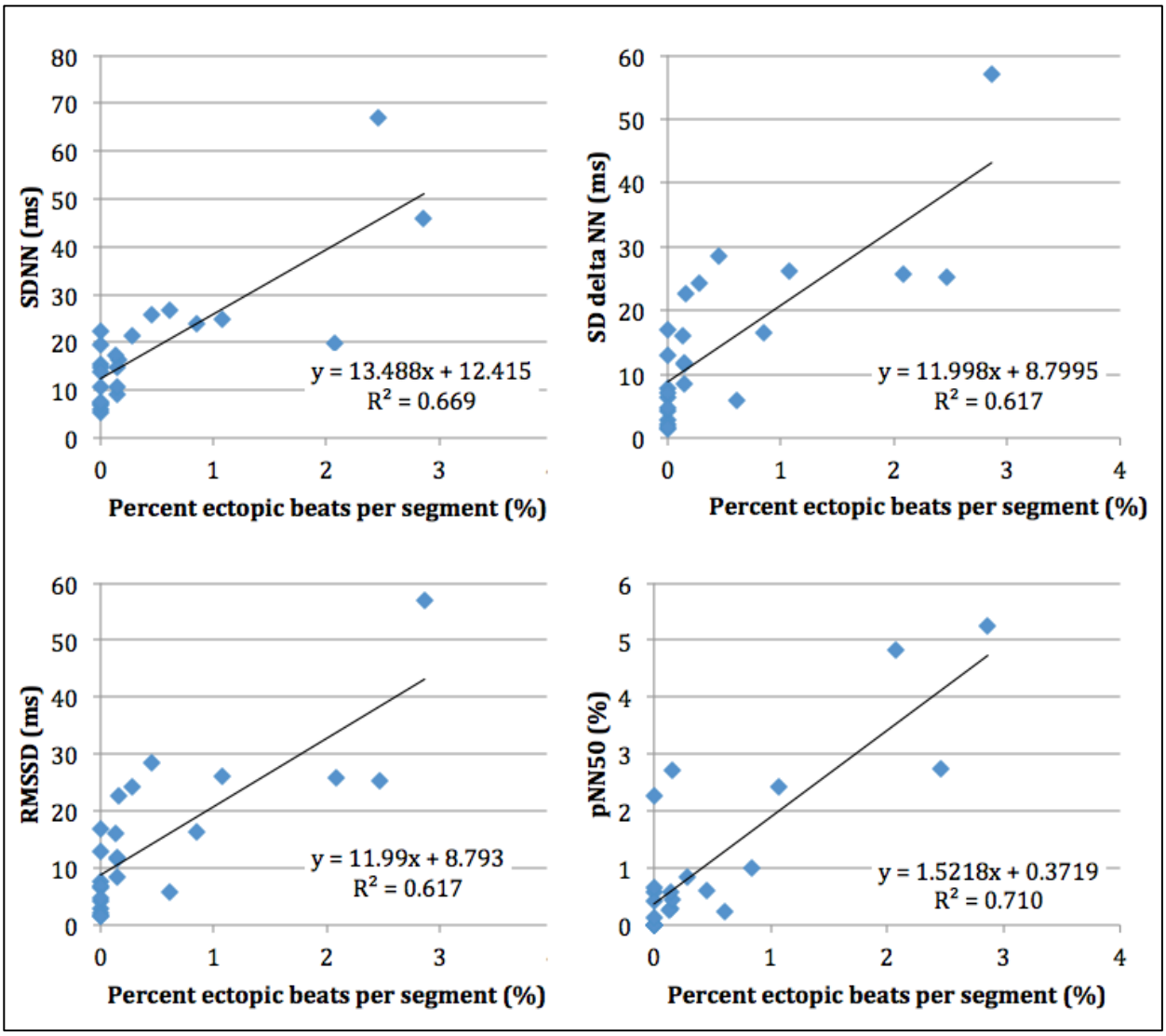

Figure 15. Effect of percent ectopics per segments on time domain HRV parameters for all patients' segments (blue diamonds), including both modes, from Analysis #3. The linear fit (black line), its equation, and coefficient of determination (R^2) were obtained for each parameter.

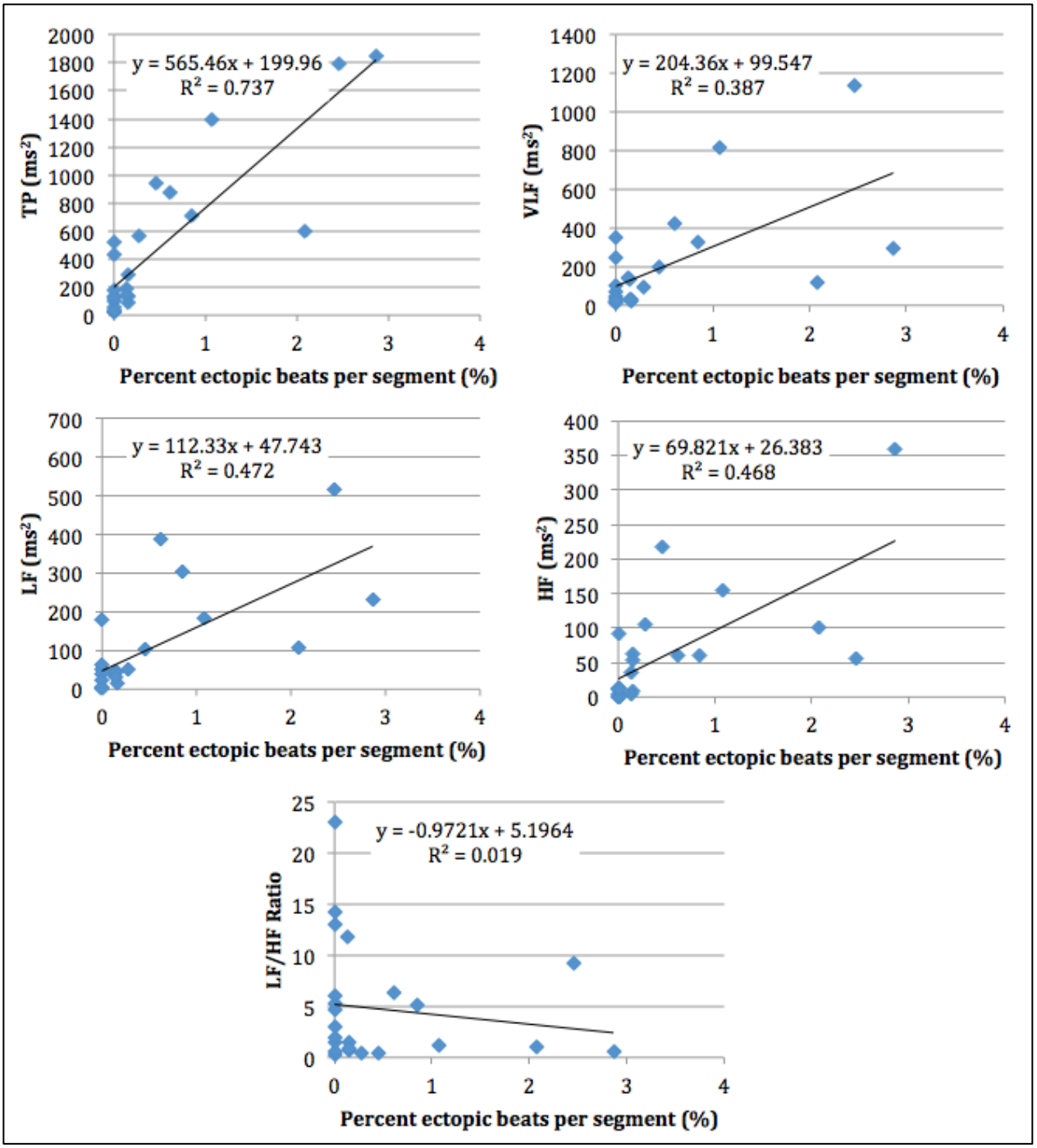

Figure 16. Effect of percent ectopics per segment on frequency domain HRV parameters for all patients' segments (blue diamonds), including both modes, from Analysis #3. Linear fit (black line), its equation, and coefficient of determination (R^2) were obtained for each parameter.

3.6 Results of Analysis #4

There were no significant differences between NIPPV20 and CPAP for all patients in any HRV parameters (Table 21). Group comparisons between extubation success and failure groups yielded no significant differences in any time domain (Tables 22 and 23) or frequency domain (Tables 24 and 25) parameters. While the trend of increased ΔHRV and ΔHRV(%) values in the failure group while on NIPPV20 remained, the effect is considerably attenuated.

3.7 Lengths of the data recordings and segment selection for all analyses.

The lengths of the data recordings were highly variable between patients and modes, with lengths ranging between 23 to 65 minutes (Figure 17). Furthermore, the time of the segments selected for analyses #2, #3 and #4 vary considerably from one another, with half of the segments having partial or complete overlap in time and the other half having variable distances from each other, with gaps ranging from 1 to 22 min (Figure 17).

Table 21. NIPPV20 and CPAP for all patients (Analysis #4).

HRV Parameter	NIPPV20	CPAP	P-value
SDNN (ms)	3.284 (1.494, 6.456)	2.764 (1.450, 10.037)	0.424
SD delta NN (ms)	3.302 (2.189, 4.866)	3.360 (1.443, 4.935)	0.424
RMSSD (ms)	3.282 (1.493, 6.452)	2.762 (1.449, 10.029)	0.424
pNN50 (%)	0 (0, 0.153)	0 (0, 0.299)	1.000
TP (ms^2)	135.294 (10.789, 436.487)	33.106 (20.120, 236.250)	0.176
VLF (ms^2)	96.660 (9.011, 355.015)	25.606 (14.821, 163.939)	0.110
LF (ms^2)	14.375 (0.690, 61.636)	6.540 (1.185, 76.863)	0.677
HF (ms^2)	1.833 (0.049, 11.178)	1.255 (0.083, 6.810)	0.677
LF/HF Ratio	7.823 (2.823, 24.120)	10.023 (1.254, 26.659)	0.266

Legend: values presented as median (min, max)

Table 22. Group comparisons of NIPPV20 and CPAP for time domain HRV parameters (Analysis #4).

HRV Time Domain Parameter	Group & Mode	Values	P-values			
			NIPPV20 vs. CPAP Success	NIPPV20 vs. CPAP Failure	Success vs. Failure CPAP	Success vs. Failure NIPPV20
SDNN (ms)	Success NIPPV20	9.850 (4.426, 14.688)	0.938	0.125	0.876	0.530
	Success CPAP	7.155 (5.999, 16.844)				
	Failure NIPPV20	12.972 (6.973, 19.440)				
	Failure CPAP	7.371 (5.290, 11.366)				
SD delta NN (ms)	Success NIPPV20	3.024 (1.494, 6.456)	1.000	0.313	0.639	0.343
	Success CPAP	3.006 (1.450, 10.037)				
	Failure NIPPV20	4.112 (2.113, 4.767)				
	Failure CPAP	2.665 (1.545, 3.586)				
RMSSD (ms)	Success NIPPV20	3.022 (1.493, 6.452)	1.000	0.313	0.639	0.343
	Success CPAP	3.004 (1.449, 10.029)				
	Failure NIPPV20	4.109 (2.112, 4.764)				
	Failure CPAP	2.663 (1.544, 3.584)				
pNN50 (%)	Success NIPPV20	0 (0, 0.153)	1.000	1.000	1.000	1.000
	Success CPAP	0 (0, 0.299)				
	Failure NIPPV20	0 (0, 0.141)				
	Failure CPAP	0 (0, 0)				

Legend: values presented as median (min, max)

Table 23. Comparisons of ΔHRV and ΔHRV(%) for time domain HRV parameters (Analysis #4).

HRV Time Domain Parameter	Group & Variable	Values	P-values	
			Success vs. Failure ΔHRV	Success vs. Failure ΔHRV(%)
SDNN (ms)	Success ΔHRV	-2.415 (-6.898, 8.689)	0.106	0.202
	Success ΔHRV(%)	-19.7 (-50.3, 144.8)		
	Failure ΔHRV	1.912 (-0.364, 12.062)		
	Failure ΔHRV(%)	36.1 (-5.0, 163.5)		
SD delta NN (ms)	Success ΔHRV	-0.185 (-6.492, 2.299)	0.432	0.530
	Success ΔHRV(%)	-11 (-64.7, 55.3)		
	Failure ΔHRV	1.101 (-0.551, 3.069)		
	Failure ΔHRV(%)	30.7 (-20.7, 180.8)		
RMSSD (ms)	Success ΔHRV	-0.185 (-6.487, 2.298)	0.432	0.530
	Success ΔHRV(%)	-11 (-64.7, 55.3)		
	Failure ΔHRV	1.100 (-0.550, 3.067)		
	Failure ΔHRV(%)	30.7 (-20.7, 180.8)		
pNN50 (%)	Success ΔHRV	0 (-0.299, 0.153)	0.939	n/a
	Success ΔHRV(%)	n/a		
	Failure ΔHRV	0 (0, 0.141)		
	Failure ΔHRV(%)	n/a		

Legend: values presented as median (min, max)

Table 24. Group comparisons of NIPPV20 and CPAP for frequency domain HRV parameters (Analysis #4).

HRV Frequency Domain Parameter	Group & Mode	Values	P-values			
			NIPPV20 vs. CPAP Success	NIPPV20 vs. CPAP Failure	Success vs. Failure CPAP	Success vs. Failure NIPPV20
TP (ms^2)	Success NIPPV20	105.579 (10.789, 201.515)	1.000	0.063	0.343	0.530
	Success CPAP	35.920 (27.444, 236.250)				
	Failure NIPPV20	165.009 (45.075, 436.487)				
	Failure CPAP	30.291 (20.120, 114.122)				
VLF (ms^2)	Success NIPPV20	93.079 (9.011, 166.278)	0.813	0.063	0.639	0.268
	Success CPAP	30.150 (14.821, 163.939)				
	Failure NIPPV20	136.256 (36.889, 355.015)				
	Failure CPAP	24.957 (17.648, 62.169)				
LF (ms^2)	Success NIPPV20	8.885 (0.690, 51.832)	0.813	0.438	0.432	0.530
	Success CPAP	8.540 (3.026, 76.863)				
	Failure NIPPV20	19.865 (5.662, 61.636)				
	Failure CPAP	3.199 (1.185, 44.554)				
HF (ms^2)	Success NIPPV20	1.388 (0.049, 11.178)	0.578	0.313	0.343	0.268
	Success CPAP	2.970 (0.158, 6.810)				
	Failure NIPPV20	3.253 (1.088, 10.117)				
	Failure CPAP	0.245 (0.083, 3.631)				
LF/HF Ratio	Success NIPPV20	13.982 (2.823, 24.120)	0.813	0.063	1.000	0.202
	Success CPAP	7.775 (1.254, 26.659)				
	Failure NIPPV20	6.092 (4.090, 9.540)				
	Failure CPAP	12.270 (5.251, 14.327)				

Legend: values presented as median (min, max)

Table 25. Group comparisons of ΔHRV and ΔHRV(%) for frequency domain HRV parameters (Analysis #4).

HRV Frequency Domain Parameter	Group & Variable	Values	P-values	
			Success vs. Failure ΔHRV	Success vs. Failure ΔHRV(%)
TP (ms^2)	Success ΔHRV	2.180 (-175.361, 151.701)	0.073	0.073
	Success ΔHRV(%)	1.5 (-74.2, 552.8)		
	Failure ΔHRV	50.887 (3.611, 406.196)		
	Failure ΔHRV(%)	130.2 (8.7, 1634.7)		
VLF (ms^2)	Success ΔHRV	8.750 (-119.844, 136.128)	0.106	0.106
	Success ΔHRV(%)	13.4 (-77.5, 576.3)		
	Failure ΔHRV	74.087 (11.933, 328.760)		
	Failure ΔHRV(%)	119.2 (47.8, 1546.2)		
LF (ms^2)	Success ΔHRV	-2.940 (-53.495, 47.291)	0.639	0.202
	Success ΔHRV(%)	-50.6 (-81.0, 1041.4)		
	Failure ΔHRV	7.081 (-24.689, 58.438)		
	Failure ΔHRV(%)	597.7 (-55.4, 3271.4)		
HF (ms^2)	Success ΔHRV	-0.208 (-3.097, 7.989)	0.268	0.106
	Success ΔHRV(%)	-45.4 (-82.2, 256.6)		
	Failure ΔHRV	1.796 (-0.963, 9.872)		
	Failure ΔHRV(%)	795.9 (-47.0, 4963)		
LF/HF Ratio	Success ΔHRV	-2.540 (-11.667, 9.452)	0.755	0.343
	Success ΔHRV(%)	-9.5 (-63.7, 753.8)		
	Failure ΔHRV	-4.787 (-6.960, -0.796)		
	Failure ΔHRV(%)	-33.4 (-53.3, -13.3)		

Legend: values presented as median (min, max)

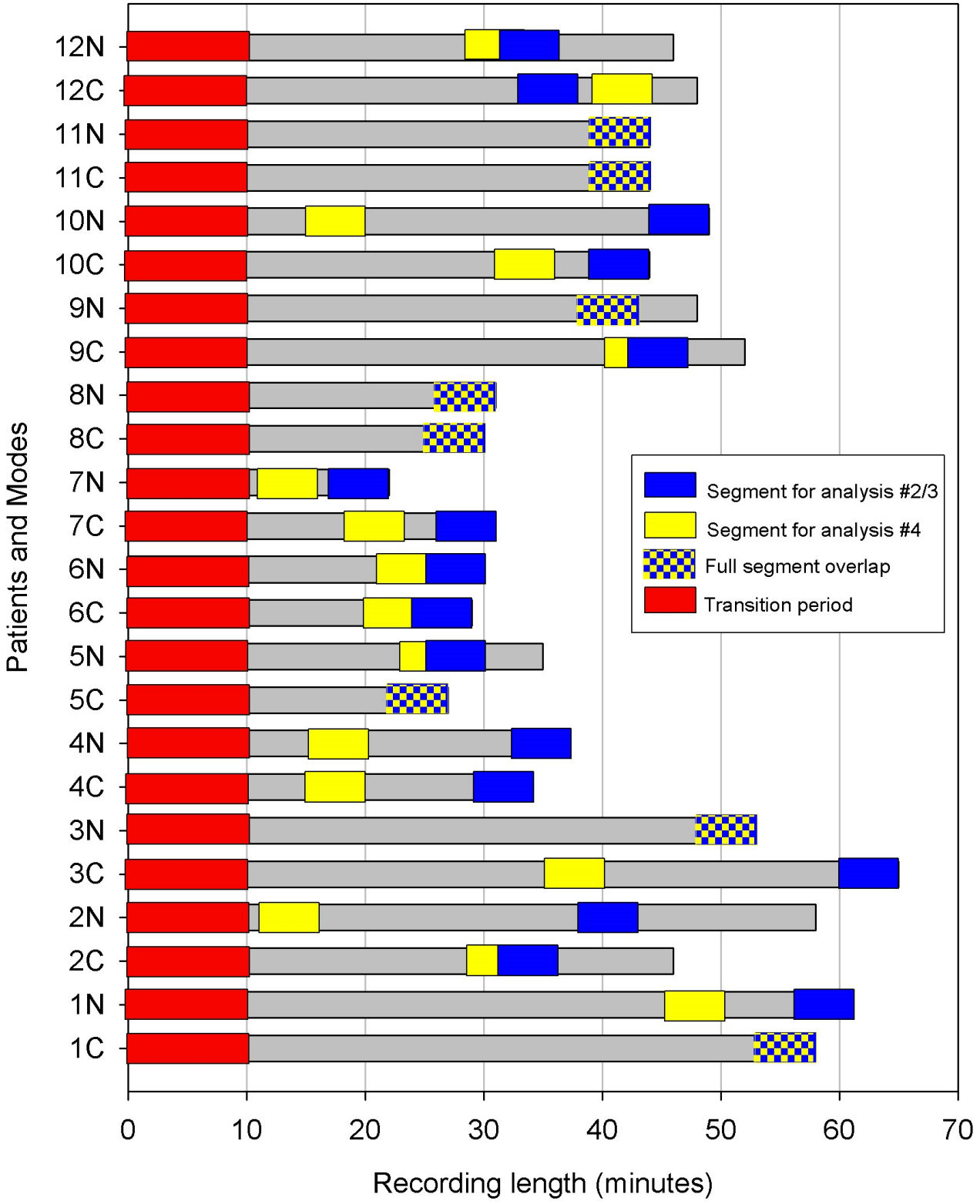

Figure 17. Length of data recordings and segment selections for individual patients on CPAP (C) and NIPPV20 (N).

Section 4 – Discussion

In this prospective observational study, we investigated EPT infants during the immediate post-extubation period while receiving NIPPV20 or CPAP in a random order. Using HRV as a measure of wellbeing, no differences were noted between those most commonly used modes of non-invasive respiratory support. Interestingly, infants that went on failing their first extubation attempt exhibit a significant change in HRV when switched from one mode to another, which was not present in infants successfully extubated. In the new era of precision medicine, HRV measurements may be a useful tool for early identification of infants at higher risks of extubation failure, which could allow targeted interventions or investigations with the aim to prevent this undesirable outcome.

4.1 Non-synchronized NIPPV and CPAP

Nasal CPAP has been shown to reduce the risk of extubation failure in EPT infants. Over the last years, non-synchronized NIPPV has been used as an alternative to conventional CPAP but clear evidence of its mechanisms of action and clinical benefits for immediate support after extubation is lacking [36]. Indeed, there are only a few physiological studies comparing the effects of non-synchronized NIPPV and CPAP within the entire preterm population. *This was the first study to investigate for differences between NIPPV and CPAP in EPT infants during the immediate post-extubation period using HRV*. In EPT infants under unstable conditions such as during the immediate post-extubation phase, any additional benefit of NIPPV by delivering positive inflation pressures above the CPAP level at a predetermined rate would be reflected by differences in HRV. No statistically significant differences in HRV were observed between the modes. However, despite no statistical differences, higher HRV values were noted during

NIPPV20 when compared to CPAP, which may be due to individual differences between infants studied. Such differences may be reflected by the fact that despite similar management, some infants were successfully extubated whereas others failed.

Given the lack of clear guidelines on how to select and analyze ECG signals for calculation of HRV in EPT infants, a major component of this thesis was dedicated to investigate the effects of different methodologies used upon the final results of HRV measurements, as discussed below.

4.2 Methodologies

As detailed previously, four types of methods were used. To compare these four methodologies, first each analysis will be summarized individually. The strengths and limitations of the method used will be discussed leading to why a subsequent methodology was undertaken. Finally, once the rationale behind each method is elucidated, a comparison of the four methodologies will follow.

4.2.1 Analysis #1

In this analysis, as much of the available ECG signal as possible was used by averaging *all acceptable 5-minute segments*. By averaging several segments the final result should, ideally, be less affected by the presence of artifacts. Unfortunately, there were not very many segments to average, as some infants had only 2 acceptable segments that could be obtained. Overall, the median number of acceptable segments was only 5 and the effect of artifacts could still be an issue. Furthermore, taking the average of non-stationary signals, i.e. signals where statistical properties change over time, is not appropriate. Most biological signals are non-stationary, and this applies to ECG signals [38]. Indeed, graphical depictions of the acceptable segments over

time (data not shown) showed mixed results with increasing and decreasing linear trends, or no change over time; all with highly variable and inconsistent R^2 values between patients and HRV parameters.

In order to avoid the potentially erroneous averaging of segments and the non-stationarity of the ECG signals, a more systematic approach was taken to obtain a single segment using Analysis #2.

4.2.2 Analysis #2

ECG segments from Analysis #1 were used to select the first acceptable segment nearest to the end of the mode. Using this segment would ideally allow the infant as much time as possible on a given mode to stabilize beyond the 10 minute transition period allotted. Furthermore, using only one segment that is systematically obtained would address the issue of stationarity by avoiding any erroneous use of averaging and using segments that are obtained at a similar point in time.

Unfortunately, the single segments obtained were widely variable from each other in time, as can be seen from Figure 17, with distances between selected segments up to 37 minutes long. While it seems unlikely that this time difference would be clinically relevant, from a physiological standpoint this could have a considerable effect. If being on a given mode for an extended period of time is beneficial, as it may allow more time for patients to adapt, then having some patients with less time to adapt could put those patients at a disadvantage. In addition, if these patients are still adapting, they could be experiencing more stress. By contrast, if the patient was becoming unstable on a given mode, such a difference could be significant where those who had been exposed to a given mode longer would be at a greater disadvantage. In the

case of lung derecruitment, for example, the longer the patient is subjected to inadequate support could worsen the degree of derecruitment and overall instability. To our knowledge, there is no literature on the degree of lung derecruitment between NIPPV and CPAP in preterm infants over time. However, one study examined 32 preterm infants of birth weight <1800g on different CPAP devices and exposed them to different levels of PEEP to observe lung derecruitment. By reducing the PEEPs from 8 to 6, 4, and zero, they observed the subsequent reduction in lung volume through RIP signals. While they did not examine these changes as a function of time, patients were subjected to each PEEP for only 3-5 minutes each, indicating that lung derecruitment may occur rapidly [58]. Although derecruitment appears to be rapid, such abrupt reductions in PEEP did not occur in our study, therefore the derecruitment process, if any, would likely be slower.

By analyzing only a single segment, there is greater potential for artifacts to influence the results. While this is avoided by setting the acceptable limit of ≥80% normal beats and excluding artifacts within the range of <60 or >300 BPM, ectopic beats between 60-100 and 200-300 BPM are still included in the analysis. It is important to recall that beat detection occurs by setting a voltage threshold to detect R-waves in the ECG signal. Therefore, any noise or patient movement that pushes the signal above this threshold will be detected as a beat, and if it occurs within the ectopic range, it will be included in the HRV analysis by the software. The issue with these beats is that it becomes impossible to know if these are true events without visualization of each detected beat. A long interval could represent a true bradycardia or a missed beat, while a short interval could represent a true tachycardia or an artifact that occurred between beats. Moreover, if the ECG is of poor quality, the QRS complexes may not be easily discernable from noise. The visual inspection of the alleged ectopic beats detracts from the systematic approach of segment

selection. Given the clear linear relationship between ectopic beats and HRV values (Figures 11 and 12), it would be important to ensure that all ectopic beats included in the analysis are true events and not due to movement artifacts or noise. This concern prompted the following methodology in order to reduce such an effect.

4.2.3 Analysis #3

Analysis #3 uses the same single segments from Analysis #2 subjected to a band-pass filter prior to HRV analysis. The filter (5 - 80 Hz) was empirically chosen, as it seemed to have the best removal of noise and signal drift while leaving the R-waves intact for beat detection. Ideally, this would reduce the risk of erroneously detecting a noisy peak as an R-wave and reduce the number of ectopic beats. Indeed, the percentage of ectopic beats per segment was reduced overall, with 83% of segments having less than 1% ectopics (Figures 15 and 16), which is an improvement from the 71% from Analysis #2. While we can see that those with no ectopics still have considerable variability, the range is larger as the percent of ectopics increases. Therefore, although there is a noteworthy reduction in ectopics, their presence still influences the HRV results. Since it is difficult to obtain a segment completely without artifacts using this systematic approach, a methodology using a selective approach was tested (Analysis #4).

4.2.4 Analysis #4

This analysis abandoned the systematic approach of obtaining segments used previously, and instead a single segment was selected based on the visual inspection of *best possible quality of ECG for 5 minutes to ensure 100% normal beats*. Briefly, in this analysis the ECG signal was first band-pass filtered as in Analysis #3 before the segment selection process, if needed. Through visual inspection, a segment was selected if it had no movement artifacts or other noise.

If no such segment could be obtained, then the segment that appeared to have the least amount of noise was chosen. All segments below 100% normal beats were subjected to other LabChart techniques, such as inversion of the signal, derivative of the signal, as well as manual deletion of falsely detected beats in order to remove all ectopics and artifacts. The manual deletion of beats is inherently flawed, given that movement artifacts can be difficult to distinguish from noisy QRS complexes, as previously discussed above for Analysis #2. Therefore, it is possible that true beats were deleted while false beats were included for analysis. Under certain circumstances, other cues that movement occurred can be used to assist in the cleanup of ECG signals, such as comments added to the recordings during data acquisition or simultaneous recordings of respiratory (RIP) signals as done during sleep studies. Nevertheless, even with this information, the cleanup of the ECG signals is still prone to errors given the rapid heart rate (140-160 bpm) of this preterm population where even an abrupt and quick noisy event can obscure a QRS complex. As such, the problem of distinguishing true beats from artifacts persists. Indeed, an example of how difficult this can be is found in Figure 8. Furthermore, recording RIP signals would require additional instrumentation in this fragile population during the critical period following extubation whereas 3-lead ECG is a simple bedside tool.

Similar to Analysis #2 and #3, the issue of varying lengths of exposure is also present in this methodology. Differences between segments for this analysis vary up to a maximum of 37 minutes. In essence, the highest quality sections of the ECG signals vary greatly between patients and between modes. With the variable total exposure times, in order for all segments to occur after the same amount of exposure, all segments would need to be taken from 15-20 minutes of exposure, which may not be of adequate quality for analysis. It would be difficult to obtain acceptable segments that occur at the same time, especially from the preterm population. In

adults, it would be simple to tell the patient not to move to ensure quality ECG signals are acquired, but this cannot be achieved with infants.

4.2.5 Methodology comparisons

Determining which of these methods is best or most appropriate is difficult, given that there is no "gold standard" method to compare the results against. These four analyses are different attempts at coping with certain issues that arise while obtaining data from a challenging population to study; noise, movement, and any other sources of artifacts are difficult to avoid even during short periods of recordings. Even during the selection of the best possible segment (Analysis #4), a perfect segment was not always obtainable and some manipulations were still required. Moreover, comparing single-segment analyses is difficult given that not all segments within or between analyses occurred at the same time, raising again the issue of varying lengths of mode exposure.

Considering only these four methodologies, Analysis #3 is likely to be the most appropriate method, since it lacks any subjectivity in segment selection and has reduced the effects of ectopics. The reduction of ectopics, though not complete, is also done systematically by applying the same band-pass filter to all segments, as opposed to manual deletion of artifacts that is inherently subjective.

4.2.6 HRV analysis

Aside from the previously discussed issues, another problem regarding HRV analysis are the ranges for VLF, LF, and HF that were set according to Task Force guidelines, as it currently remains the only standardization guidelines for HRV analysis [39]. These recommendations are for adults, and while it is unlikely to be appropriate for neonates, there are no standardized

guidelines for frequency ranges in this population. EPT infants have high respiratory rates, which would theoretically correspond to a higher RSA frequency than in adults. RSA is the normal fluctuations in heart rate that occur in synchrony with respiration, where the heart rate increases during inspiration and decreases during expiration. Therefore, as described in Section 1.5.1, these rapid, high frequency changes of heart rate would be represented in the HF band of the frequency domain analysis. A commentary published in the American Journal of Cardiology has addressed this problem, stating that the frequency ranges in the Task Force guidelines are too low for neonates, and thus studies that use these ranges may be underestimating the power in the HF range [59]. With a newborn breathing normally at approximately 50 breaths per minute, the RSA peak would occur around 0.83 Hz, which is more than twice the limit of the Task Force guidelines. Furthermore, preterm infants have higher respiration rates than term infants, thus the RSA peak would theoretically be even higher. As such, an HF range of 0.24 to 1.04 has been mentioned as a potentially appropriate range, but the range should perhaps be even higher for preterm infants [59]. However, the commentary has since been cited 14 times, with only 3 of those articles using the proposed range and over 300 articles investigating HRV in neonates having been published since this commentary. Furthermore, as discussed in Section 1.5.2.2, the presence of an RSA peak in the HF band in preterm infants does not appear within the first five days of life [48]. With the median age of extubation for all infants in this study at 3.25 days, the presence of the RSA peak is unlikely to be a major concern. Moreover, recent work from our research group has demonstrated that RSA is detectable only after 32 weeks of GA, with EPT infants having negligible RSA [60]. Therefore, the power in the HF band is unlikely to be grossly underestimated, and given that all infants were compared using the same HF frequency ranges,

their comparisons are still valid. For the same reasons, comparisons of the LF/HF ratio are also still valid.

4.2.7 Improvements for future HRV analyses

Improvements to these methodologies could be made for any future HRV analyses in this complex population. Ideally, the selection process should be as systematic as possible to avoid any bias or subjectivity, and the segment selection should be completely free of artifacts. With this in mind, I propose the following changes to be taken into consideration for any further HRV analyses:

1. *Set an established time frame for segment selection*

   To prevent any differences in the length of exposure time, each patient's segments should be obtained within a pre-defined period of time (e.g. within 40-60 minutes after beginning the new mode). Furthermore, all total exposure times should be similar.

2. *Obtain a clean, artifact-free segment*

   Certain efforts could be made to reduce the presence of artifacts, including minimizing nursing/parental contact during the recording, potentially swaddling the infant to minimize movement artifacts, and ensuring proper equipment set-up to reduce electrical noise or physiological noise (e.g. proper placement of leads that are well distanced from other electrodes and have good skin contact, and wires should have no contact with other wires or metal surfaces). Furthermore, the range of ectopics could be adjusted to allow only typical ranges for bradycardias and tachycardias (e.g. it is unlikely to have a tachycardia that reaches 300 BPM), or ectopics could be excluded from the analysis altogether.

3. *Better beat detection*

   The LabChart software has an updated HRV analysis module (HRV module 2.0, LabChart 8, ADInstruments, Colorado, U.S.A) where beat detection uses both acceptable NN interval ranges as well as the complexity of the beat in order to specifically detect the R-waves of QRS complexes and not other artifacts, both of which can be preset. This additional form of beat detection will help to minimize the degree of artifact pollution without requiring visual inspection. Additionally, the new software also provides some non-linear analyses for a more in-depth analysis of HRV.

4. *Adjust the frequency domain range*

   Consider adjusting the upper limit of HF to include potential RSA peaks appropriate for a preterm infant's respiration rate (e.g. up to 1.2 Hz, rather than 0.4 Hz).

4.3 HRV and extubation outcome

As Analysis #3 was presumed to be the most appropriate method, only its results will be discussed. Infants that went on to fail extubation had higher HRV values while on NIPPV20 for most parameters than infants that succeeded extubation. Furthermore, these values on NIPPV20 were also higher than CPAP. While these differences alone were not statistically significant, by investigating the absolute and relative changes through ΔHRV and ΔHRV(%), a significant difference between the success and failure groups was found. The high area under the ROC curves, sensitivity, specificity, NPV and PPV values for those parameters with significant results indicate that this physiological response could identify infants at risk of failing as soon as 2 hours after extubation, with the time domain parameters showing the greatest potential. Similar to results found by Kaczmarek *et al.*, changes in HRV may be able to predict extubation failure [25].

A possible explanation for the observed difference between groups could be linked to the greater stability of infants within the success group. If these successfully extubated infants are presumed to be healthier and are coping with the extubation well, then perhaps the lessened HRV change between modes reflects this stability, with ΔHRV values ranging around zero. In these infants, it appears that the modality does not overly affect them and they would be fairly stable on either mode. By contrast, the infants in the failure group that were not truly ready to be extubated were therefore unstable in the post-extubation period, with certain modes causing greater instability. It is difficult to determine from these results which of the modalities is better for the failing infant, as the results can be interpreted in two opposing fashions. The first focuses on the reduction in HRV, while the alternative focuses on the increase in HRV. These opposing views will be discussed below.

4.3.1 Reduced HRV

The first interpretation follows the results that have been previously demonstrated in the HRV and lung function literature (Section 1.5.2), which concludes with a reduction in HRV as an indication of poor health. If this approach were true within the context of this study, then the results would indicate that the infants within the failure group should be on NIPPV20. Since the HRV levels are lower on CPAP than on NIPPV20 within this group, then CPAP should be avoided. Placing these at-risk infants on NIPPV20 would theoretically be providing a higher level of support as it provides intermittent breaths in addition to the constant PEEP. By providing this additional support to infants that will subsequently fail extubation, these infants may become more stable and this could be reflected in their increased HRV while on NIPPV20.

4.3.2 Increased HRV

The alternative interpretation relates to the issue of artifact-contamination that can falsely increase the HRV values. If the presence of ectopics is due to artifacts and not true bradycardic/tachycardic events as previously discussed, then these artifacts are normally due to patient movement. Thus, if the increase in HRV observed during NIPPV20 in the failure group is solely due to movement artifacts, then it is the increase in patients' movement that is the indication of failure. An increased amount of movement in preterm infants can indicate agitation or discomfort; therefore, perhaps NIPPV20 is actually creating greater discomfort in the failure group that is not observed in the healthier success group.

In order to understand why these infants would be more agitated on NIPPV20 than on CPAP, we need to understand the ventilatory and physiological effects of NIPPV. A few studies by the same research group have investigated the effects of non-synchronized NIPPV (as in our present study) on preterm infants. The first studied NIPPV intra-prong pressure in 11 preterm infants less than 30 weeks GA. The results revealed that the pressures delivered by NIPPV were highly variable around the set PIP, with 12.7% of the mechanical inflations exceeding the set PIP. Furthermore, they found that increased PIP did not result in increased pressures delivered, likely due to the increase in leak, and delivered pressures were found to be higher during patient movement [61]. In their subsequent study, they examined the effects of NIPPV on the spontaneous breathing cycle in 10 preterm infants. When inflations coincided with the patient's spontaneous inspiration, their inspiratory time increased by 21%, tidal volume increased by 15%, with expiratory time unchanged compared to their baseline spontaneous breathing. When inflations coincided with spontaneous expiration, the expiratory time increased by 13%. When inflations were delivered during a period of apnea, the inflations only produced a chest rise 5%

of the time and with small tidal volumes at 26.7% of the patient's spontaneous volume. Furthermore, patients did not entrain to NIPPV and remained unsynchronized throughout the 30-minute study period [62]. These studies demonstrate that NIPPV may not be performing as well as expected, thus not as beneficial to these high-risk infants.

In order to assess the physiological effects of non-synchronized NIPPV, another research group used preterm lambs as animal models for preterm infants. They conducted two studies investigating the glottic response during the delivery of the positive inflation pressures. The first study assessed the electromyographic activity of the thyroarytenoid (TA) muscle responsible for glottal constriction. They found increased TA activity during the inspiratory phase of a delivered breath, when the TA should normally activate during the expiratory phase to limit expiratory flow. Additionally, when the PIP was increased, the TA activity was also increased. Furthermore, this increased TA activity was correlated with an increased trans-upper airway pressure. Occasionally, NIPPV was completely blocked by TA constriction [63]. This active narrowing of the glottis limits lung ventilation, thus infants would not be receiving the amount that is expected. The second study continued this investigation, and ultimately found that glottal closure is initiated by the lung bronchopulmonary receptors, which are responsible for protecting the lungs from overinflating [64].

These studies indicate potential reasons for infants to become agitated while on NIPPV. With glottal constriction preventing ventilation, the highly variable pressures delivered, and the high degree of patient-ventilator asynchrony, it is plausible that these fragile, high-risk infants would be more uncomfortable on non-synchronized NIPPV than on CPAP. If the success group is inherently more stable, then they may have greater tolerance for these effects and thus do not show any changes in HRV between modes. Of course, this interpretation has its own limitations,

as one infant within the failure group did not have any ectopics present during NIPPV20, and it cannot be confirmed that all ectopics are solely due to movement artifacts. Regardless, the potential influence of movement artifacts cannot be easily dismissed.

4.4 HRV as a predictive tool

While the increased benefit of either mode remains unclear, the use of HRV as a predictive tool for extubation failure was evident. The difference between the two groups cannot be discerned only through HRV on a single mode, but requires that the infant be placed on both modes. It is the change in response between modes (ΔHRV and ΔHRV(%)) that has proven to be a useful predictive tool. The ability to determine which infants are at risk of failing confers some benefits, as early identification of these infants could mean the implementation of intervention protocols to support these high-risk patients. The identification of these infants could prompt for simple solutions such as stricter monitoring in order to reduce the risk of reintubation, or actual interventions such as medication or early reintubation to prevent respiratory failure. For research purposes, this could allow us to target a specifically fragile population for other physiological studies, in order to better understand the progression of respiratory failure, or intervention studies, for potentially beneficial medications or techniques.

4.5 Strengths and limitations

The study was conducted in a sample size commonly used in physiological studies including the crossover methodology, where each infant acts as their own control. Rather than comparing two different infants on different modes, we are able to see the true difference between these modes by submitting each infant to both. Furthermore, infants were placed on the three original modalities in a random order, preventing any one mode from influencing the other.

ECG and clinical data were prospectively collected by a group of researchers experienced with the methodology. The most notable limitation of this thesis is the small sample size for analysis of differences between success and failure groups. Furthermore, the lack of specific guidelines for HRV analysis in this population prompted the use of many methodologies in an attempt to elucidate the best possible one but each method has its own limitations. For the secondary analysis, the study by design did not have control over the mode of ventilation received outside of the recordings period, as the attending staff made this decision. However, all infants received NIPPV or CPAP, which is the standard of care for these infants following extubation. In addition, infants were exposed to these modes of support for relatively short durations (30-60 min), and it is unclear if the HRV results may change with longer lengths of exposure.

Section 5 - Conclusion

5.1 Conclusion

In this prospective observational study we compared for the first time the effects of the two most commonly used modes of non-invasive respiratory support used in EPT infants after extubation. Using measurements of HRV as an indicator of wellbeing, no differences were noted between NIPPV20 and CPAP in the overall study population. Interestingly, in a sub-analysis of the collected data, changes in the time domain analysis of HRV between those two modes was significantly different in infants that went on failing extubation when compared to infants successfully extubated. Indeed, those HRV changes demonstrated a high accuracy in predicting these outcomes. Therefore, these results indicates that HRV is a promising tool to evaluate these EPT infants following disconnection from mechanical ventilation while receiving some type of

non-invasive support. Given the small sample size, the results of this study must be cautiously interpreted as hypothesis generating data.

The study also highlighted the difficulties in obtaining high quality, artifact-free biological signals from this fragile population, as abrupt movements cannot be avoided. In this thesis we attempted to deal with the influence of these artifacts in a variety of ways, from averaging to systematic approaches to the manual selection of the ECG signals. By comparing the different analysis methods used here and their results, it became clear that the approach used to select and process ECG segments for HRV analysis does affect the results. Therefore, this thesis also demonstrates the need for detailed standardized guidelines for neonatal HRV analyses; otherwise, results cannot be compared to those of other research studies and reference values cannot be generated.

5.2 Future work

For my future research, I am planning to expand on the topics of this thesis. With respect to the methodological concerns, I will conduct a large systematic review of all published studies analyzing HRV in preterm infants. This will provide some insight into the applications of neonatal HRV and how researchers are conducting the analysis. Moreover, this review may lead to a proposal for standardization of HRV analysis in the preterm population, in a model similar to the Task Force guidelines for adults.

As part of my future research work, I will start my PhD studies on measurements of HRV in neonates of different gestational ages (term, late preterm and extremely preterm infants) under several clinical conditions (asphyxia and cooling, neurophysiological maturation, and HFNC vs. CPAP and CPAP vs. NIPPV vs. NIV-NAVA during the immediate post-extubation period). The

results of some of these pilot studies may help in the planning of a large, multicenter study comparing post-extubation modes of respiratory support in a higher risk population. In this large study, I will investigate the accuracy of cardiorespiratory analysis for the Prediction of Reintubation and Mechanical ventilation in Extremely Preterm Infants (PRIMEX study).

Addendum – RIP bands

A.1 Introduction

During my Master's training, I was involved in multiple other studies aside from my analysis of NIPPV vs. CPAP. I obtained informed consent, collected clinical data and acquired physiological data for three studies: the automated prediction of extubation readiness (APEX) study, the HFNC vs. CPAP study, and the neurophysiological maturation (NEMO) study. Each of these studies acquires the same cardiorespiratory data as follows:

1) ECG: measured from 3 ECG leads placed on the infant's chest or limbs, positioned at least 1cm away from pre-existing leads.
2) RIP: chest and abdominal movements are measured using RIP bands with the Respitrace QDC system (Viasys Healthcare, CA, U.S.A.). One RIP band is placed around the infant's chest at the level of the nipple line. The other band is placed around the infant's abdomen, half a centimeter above the umbilicus.
3) Oxygen saturation (SpO_2) and photoplethysmograph signals will be measured with a pulse oximeter (Radical, Masimo Corp, CA, U.S.A.) placed on the infant's hand or foot. Signals will be amplified, anti-alias filtered, and sampled at 1 kHz by a portable data-acquisition system mounted in a battery powered laptop computer.

The APEX study is a multicenter international study, collaborating with two other sites in the U.S.A. (Brown University in Providence, Rhode Island, and Wayne State University in Detroit, Michigan). The target sample size is 200 infants with birth weight <1250g and undergoing their first extubation attempt. Cardiorespiratory data is acquired for 1 hour

immediately preceding extubation, with an additional 5-minute SBT. The goal of the study is to develop an automated way to predict extubation readiness, using collaborative efforts with biomedical engineers and computer scientists. The HFNC vs. CPAP study is a pilot study similar to NIPPV vs. CPAP, with a convenience sample size of 30 infants. The eligibility criteria are the same for the APEX study, and so the infants are typically enrolled in both. The infants' cardiorespiratory data is acquired 30 minutes after extubation, when placed on HFNC or CPAP in a random order for 45 minutes each. The NEMO study targets an older population of MPT (GA 32^0 to 32^6 weeks) and LPT (GA 34^0 to 34^6 weeks) infants. Cardiorespiratory data is acquired for one hour during the first 24-96 hours of life, with concurrent recording of amplitude-integrated electroencephalogram lasting 6 hours. The MPT infants are additionally studied at 2 weeks of life. The goal of this study is to determine if the assessment of neurophysiological maturation through aEEG and cardiorespiratory data can improve the correlation between gestational age and the length of hospital stay.

These ongoing studies and other upcoming projects all require RIP signals. However, the company (Viasys Healthcare, USA) that produces the bands used no longer manufactures bands for neonates. As such, we were left with a limited supply of RIP bands, and since we want to continue using the Respitrace QDC machine, we needed to secure a source of RIP bands that function with this machine. As such, I decided to attempt manufacturing my own RIP bands.

A.2 Band development

The bands consist of three basic materials: the elastic material, the snaps, and the wire in a sinusoidal shape. I realized that the elastic material was similar to a cohesive bandage product by Andover (Andover Health Care, Netherlands), and similar sized snaps could be obtained from

any local fabric store. With assistance from my father Mike Latremouille, a technician in electronic repairs, I was able to find a similar wire to those in the bands. Using a simplistic jig, I was able to curve the wire into a sinusoidal shape so that it would fit into half of the cohesive bandage, so that it could be folded over with the wire secured inside. To avoid the closing of the snaps severing the wire, I had to use lead-free solder to connect the wires to the inner edge of the snaps.

With respect to the wire, I learned from Dr. Ross Wagner in biomedical engineering at McGill that the original wire I used is not appropriate as it was made of tin and could be prone to tin leaking. He provided me with an alternative, single-stranded wire that was enamel coated so it would be safe from any metal leaking. Furthermore, Dr. Wagner made a proper, full-sized wooden jig so that I could create a perfect sinusoidal pattern in the wire with ease.

A.3 Validation study

During the development of the bands, a reusable product was made available by SleepSense (S.L.P. Ltd., Tel-Aviv, Israel) as an alternative solution. From our research group, Lara Kanbar, PhD candidate in biomedical engineering, and Dr. Wissam Shalish, neonatologist and PhD candidate in experimental medicine, developed a RIP band validation protocol that would compare the old Viasys bands with the new SleepSense bands and the manufactured bands. Using a mechanically ventilated manikin of a preterm infant, we placed the RIP bands around the chest of the manikin and recorded the RIP signal and the pressure waveform from the ventilator on LabChart, as shown in Figure A1 and A2. The protocol submits each of the three bands to two repeated rounds of testing, where the PEEP remains constant at 5 cmH_2O, but the PIP increases by increments of 10 from 20 to 50 cmH_2O, with each PIP provided at both a rate of

20 and a rate of 60 inflations per minute, for a minimum of 100 inflations for each combination. The outline of this round is as follows:

1) PIP/PEEP 20/5, Rate 20, 5 minutes
2) PIP/PEEP 30/5, Rate 20, 5 minutes
3) PIP/PEEP 30/5, Rate 60, 2 minutes
4) PIP/PEEP 40/5, Rate 60, 2 minutes
5) PIP/PEEP 40/5, Rate 20, 5 minutes
6) PIP/PEEP 50/5, Rate 20, 5 minutes
7) PIP/PEEP 50/5, Rate 60, 2 minutes
8) PIP/PEEP 20/5, Rate 60, 2 minutes
9) PIP/PEEP 20/5, Rate 20, 5 minutes (repeat of 1st run)

Examples of the RIP band comparison are found in Figures A3 to A5, where all RIP signals appropriately follow the pressure waveform from the ventilator, but each band has a different maximum value. All bands have the same baseline value of approximately 625 in arbitrary units (AU), with the manufactured bands reaching the highest value at 660 AU, the Viasys bands reaching 650, and the SleepSense bands reaching 645. It was noted that the SleepSense bands feel more rigid than the other bands, and this is reflected in the lower amplitude values. It may be beneficial to have highly flexible bands, especially in this fragile population of infants. The bands should be the least restrictive as possible to allow the patients to breathe with ease, as any additional force to overcome may be detrimental to these fragile infants.

A.4 Further development

Further analysis on the validation study will be needed to verify the accuracy of these manufactured bands, and to determine how these can be produced in a large-scale fashion. Health Canada approval will need to be obtained if we are to use these bands or make them commercially available.

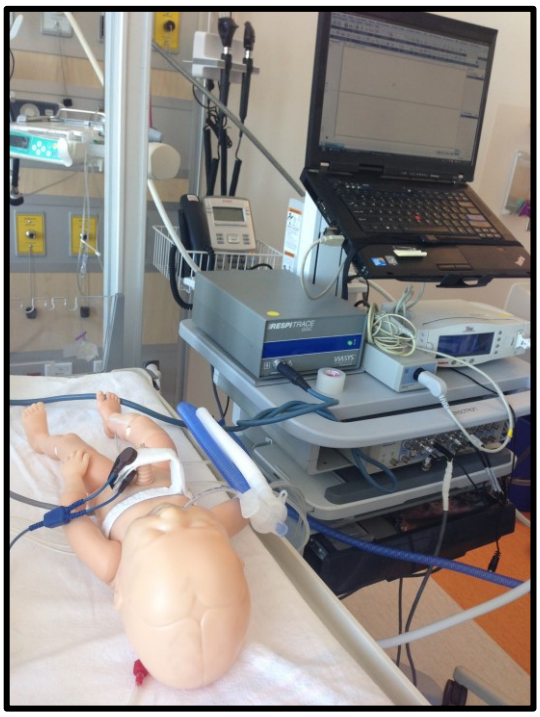

Figure A1. RIP band validation experimental set-up, showing the ventilated manikin, the SleepSense band placed around the chest of the manikin, and the data acquisition cart.

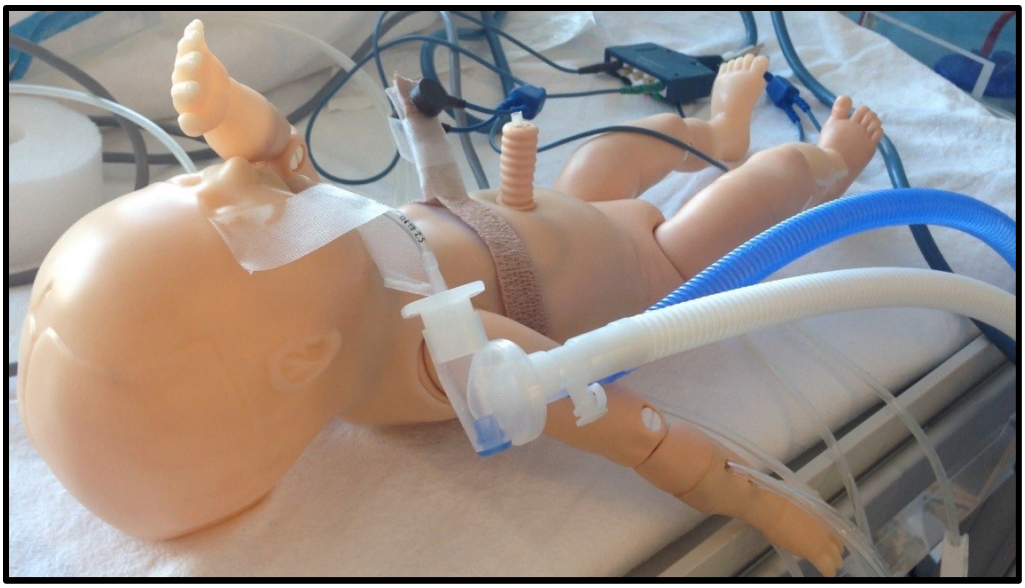

Figure A2. Close-up of the RIP band validation experimental set-up, showing the ventilated manikin and a prototype of the manufactured bands around the chest.

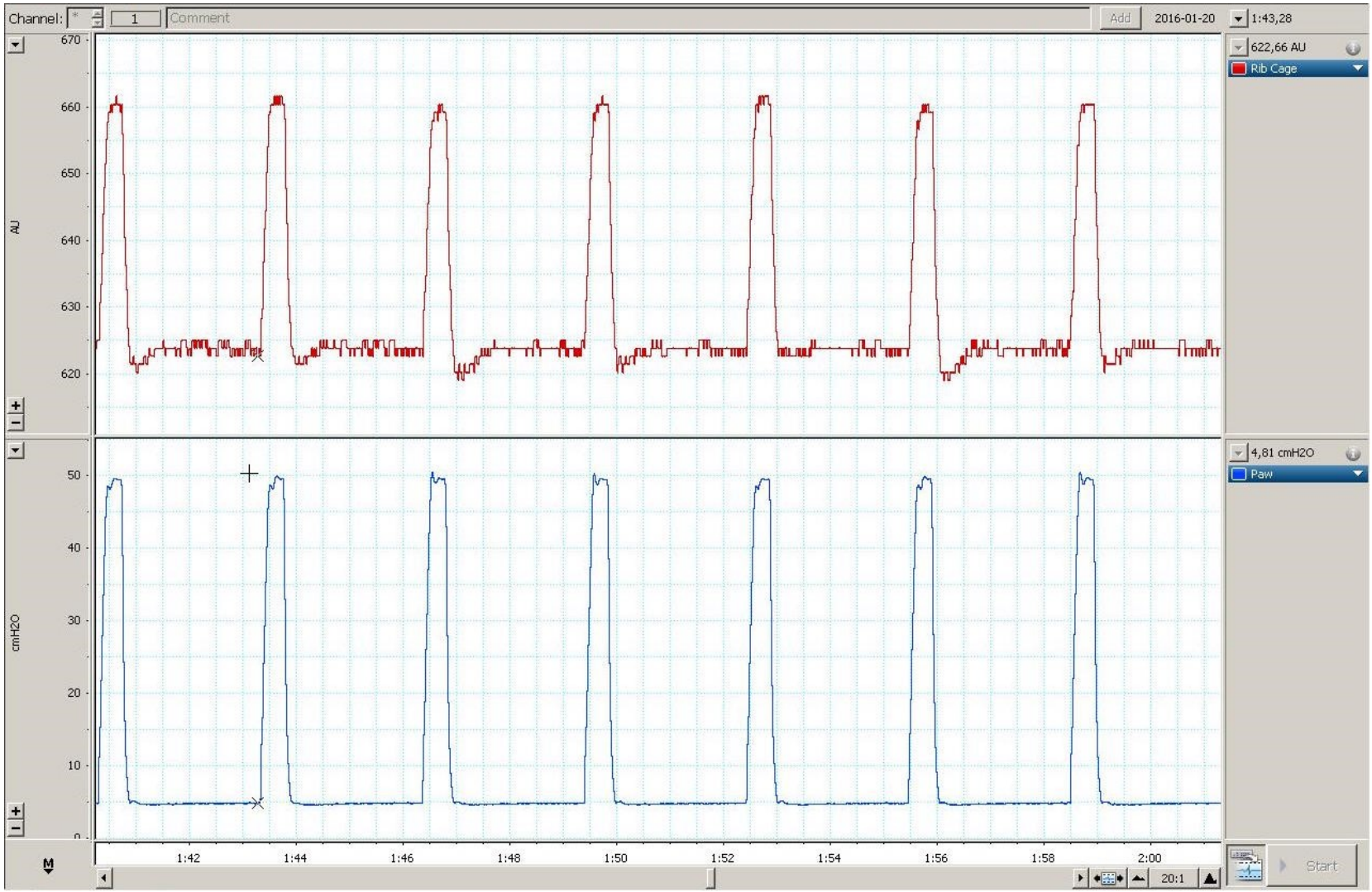

Figure A3. Example of LabChart window for the manufactured band, with PIP of 50, PEEP of 5, and rate of 20 breaths per minute.

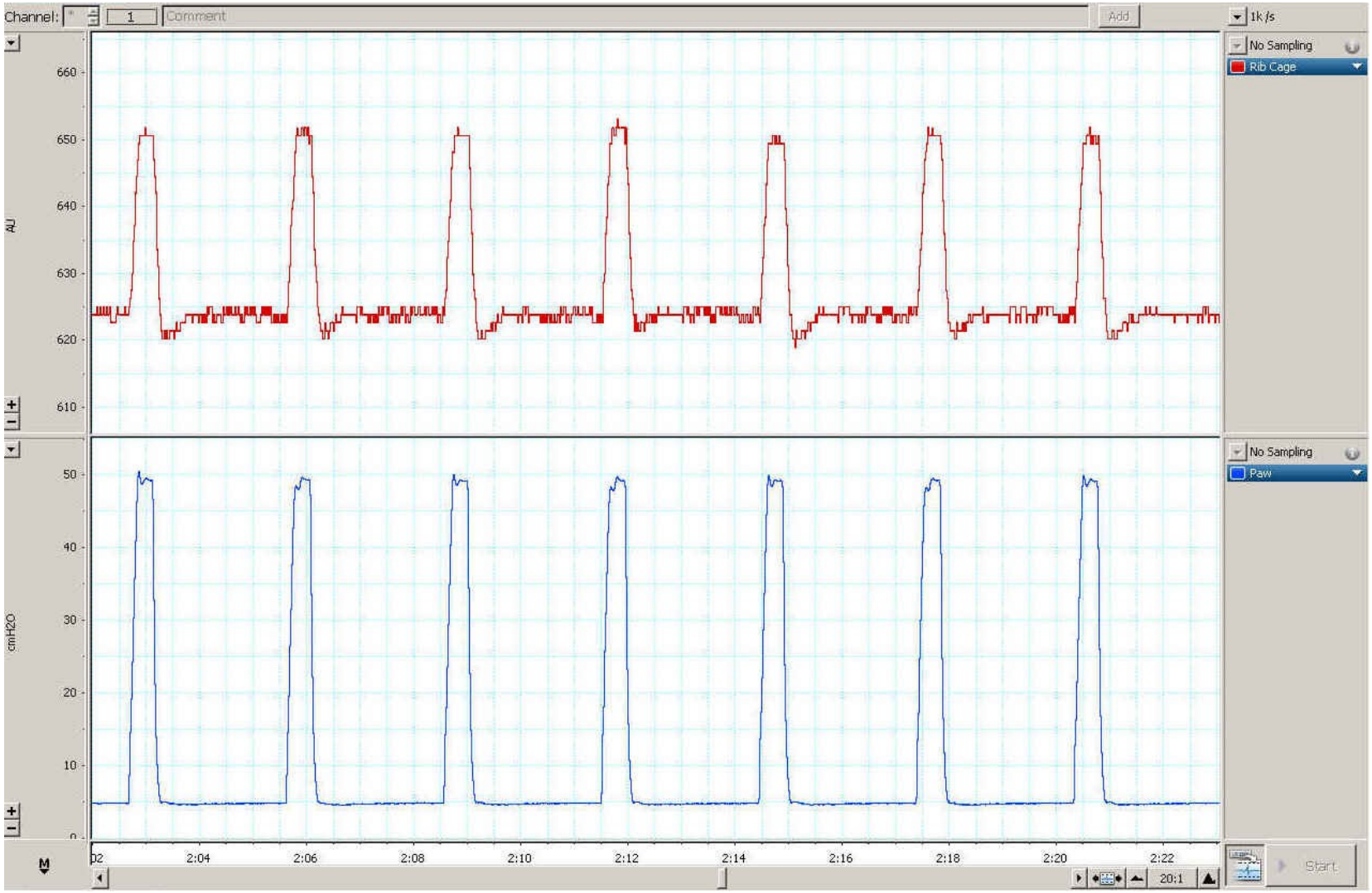

Figure A4. Example of LabChart window for the Viasys band, with PIP of 50, PEEP of 5, and rate of 20 breaths per minute.

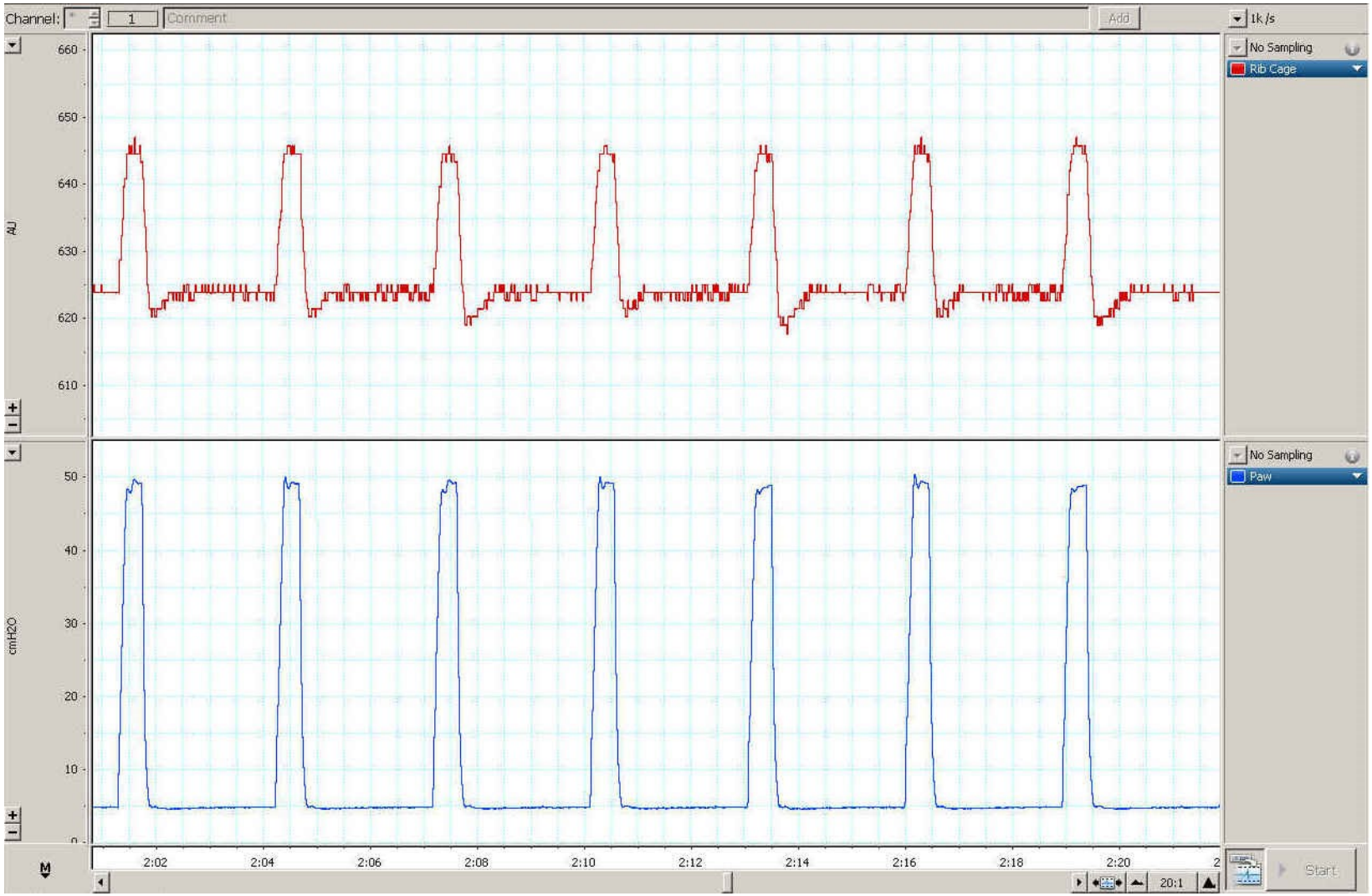

Figure A5. Example of LabChart window for the SleepSense band, with PIP of 50, PEEP of 5, and rate of 20 breaths per minute.

References

1. Canadian Premature Babies Foundation (2014, July). Premature Birth in Canada: An Environmental Scan – Final Report. Retrieved from: http://cpbf-fbpc.org/dev/wp-content/uploads/2014/11/2014-07-23-CPBF-Premature-Birth-environmental-scan_Final.pdf
2. Butler, A. S., & Behrman, R. E. (Eds.). (2007). Preterm Birth: Causes, Consequences, and Prevention. National Academies Press.
3. Wert, S.E. (2004). Normal and abnormal structural development of the lung. In Polin, R. A., Fox, W. W., & Abman, S. H. (Eds.), *Fetal and neonatal physiology* (3rd ed.)(pp. 783-793). Philadelphia, PA: Saunders Elsevier.
4. Whitsett, J.A., & Weaver, T.E. (2008). Hereditary disorders of alveolar homeostasis in the newborn. In Bancalari, E. (Ed.), *The newborn lung: neonatology questions and controversies* (pp. 42-49). Philadelphia, PA: Saunders Elsevier.
5. Burri, P.H. (1999). Lung development and pulmonary angiogenesis. In Gaultier, C., Bourbon, J. R., & Post, M. (Eds.), *Lung development* (pp. 122-151). New York, NY: Oxford University Press.
6. Mai, J. K., & Paxinos, G. (Eds.). (2013). The human nervous system (3rd ed.). Elsevier Academic press.
7. Walsh, M. C., Morris, B. H., Wrage, L. A., Vohr, B. R., Poole, W. K., Tyson, J. E., Wright, L. L., Ehrenkranz, R. A., Stoll, B. J., & Fanaroff, A. A. (2005). Extremely low birthweight neonates with protracted ventilation: mortality and 18-month neurodevelopmental outcomes. *The Journal of pediatrics*, *146*(6), 798-804.

8. Gagliardi, L., Bellù, R., Lista, G., & Zanini, R. (2011). Do differences in delivery room intubation explain different rates of bronchopulmonary dysplasia between hospitals?. Archives of Disease in Childhood-Fetal and Neonatal Edition, 96(1), F30-F35.
9. Rivera, R., & Tibballs, J. (1992). Complications of endotracheal intubation and mechanical ventilation in infants and children. Critical care medicine, 20(2), 193-199.
10. Garland, J. S. (2014). Ventilator-Associated Pneumonia in Neonates: An Update. NeoReviews, 15(6), e225-e235.
11. Apisarnthanarak, A., Holzmann-Pazgal, G., Hamvas, A., Olsen, M. A., & Fraser, V. J. (2003). Ventilator-associated pneumonia in extremely preterm neonates in a neonatal intensive care unit: characteristics, risk factors, and outcomes. Pediatrics, 112(6), 1283-1289.
12. Sant'Anna, G. M., & Keszler, M. (2012). Weaning infants from mechanical ventilation. *Clinics in perinatology*, *39*(3), 543-562.
13. Shalish, W., Sant'Anna, G. M., Natarajan, G., & Chawla, S. (2014). When and How to Extubate Premature Infants from Mechanical Ventilation. Current Pediatrics Reports, 2(1), 18-25
14. Shalish, W., & Sant'Anna, G. M. (2015). The use of mechanical ventilation protocols in Canadian neonatal intensive care units. *Paediatrics & Child Health (1205-7088)*, *20*(4).
15. Hermeto, F., Martins, B. M., Ramos, J. R., Bhering, C. A., & Sant'Anna, G. M. (2009). Incidence and main risk factors associated with extubation failure in newborns with birth weight< 1,250 grams. *Jornal de pediatria*, *85*(5), 397-402.
16. Mehta, P., Berger, J., Bucholz, E., & Bhandari, V. (2014). Factors affecting nasal intermittent positive pressure ventilation failure and impact on bronchopulmonary dysplasia in neonates. *Journal of Perinatology*.

17. Giaccone, A., Jensen, E., Davis, P., & Schmidt, B. (2013). Definitions of extubation success in very premature infants: a systematic review. *Archives of Disease in Childhood-Fetal and Neonatal Edition*, fetalneonatal-2013.
18. Shalish, W., Al Mandhari, H., & Sant'Anna, G.M. (2014). Spontaneous Breathing Tests Prior To Extubation In Preterm Infants: A Systematic Review Of The Literature [Abstract]. *Am J Respir Crit Care Med*, 189(2014), A2597.
19. Veness-Meehan, K. A., Richter, S., & Davis, J. M. (1990). Pulmonary function testing prior to extubation in infants with respiratory distress syndrome. *Pediatric pulmonology*, *9*(1), 2-6.
20. Balsan, M. J., Jones, J. G., Watchko, J. F., & Guthrie, R. D. (1990). Measurements of pulmonary mechanics prior to the elective extubation of neonates. *Pediatric pulmonology*, *9*(4), 238-243.
21. Smith, J., Pieper, C. H., Maree, D., & Gie, R. P. (1999). Compliance of the respiratory system as a predictor for successful extubation in very-low-birth-weight infants recovering from respiratory distress syndrome. *South African medical journal (Suid-Afrikaanse tydskrif vir geneeskunde)*, *89*(10), 1097-1102.\
22. Kavvadia, V., Greenough, A., & Dimitriou, G. (2000). Prediction of extubation failure in preterm neonates. *European journal of pediatrics*, *159*(4), 227-231.
23. Dimitriou, G., Greenough, A., Endo, A., Cherian, S., & Rafferty, G. F. (2002). Prediction of extubation failure in preterm infants. *Archives of Disease in Childhood-Fetal and Neonatal Edition*, *86*(1), F32-F35.
24. Kaczmarek, J., Kamlin, C. O. F., Morley, C. J., Davis, P. G., & Sant'Anna, G. M. (2013). Variability of respiratory parameters and extubation readiness in ventilated neonates. *Archives of Disease in Childhood-Fetal and Neonatal Edition*, *98*(1), F70-F73.

25. Kaczmarek, J., Chawla, S., Marchica, C., Dwaihy, M., Grundy, L., & Sant'Anna, G. M. (2013). Heart rate variability and extubation readiness in extremely preterm infants. Neonatology, 104(1), 42-48.
26. Stein, H., & Firestone, K. (2014). Application of neurally adjusted ventilatory assist in neonates. Seminars in Fetal and Neonatal Medicine,19(1), 60-69.
27. Al-Mandari, H., Shalish, W., Dempsey, E., Keszler, M., Davis, P. G., & Sant'Anna, G. (2015). International survey on periextubation practices in extremely preterm infants. *Archives of Disease in Childhood-Fetal and Neonatal Edition*, *100*(5), F428-F431.
28. Bisceglia, M., Belcastro, A., Poerio, V., Raimondi, F., Mesuraca, L., Crugliano, C., & Corapi, U. P. (2007). A comparison of nasal intermittent versus continuous positive pressure delivery for the treatment of moderate respiratory syndrome in preterm infants. *Minerva pediatrica*, *59*(2), 91-95.
29. Armanian, A. M., Badiee, Z., Heidari, G., Feizi, A., & Salehimehr, N. (2015). Initial treatment of respiratory distress syndrome with Nasal Intermittent Mandatory Ventilation Versus Nasal Continuous Positive Airway Pressure: A Randomized Controlled Trial. *International Journal of Preventive Medicine*, *1*(1).
30. Meneses, J., Bhandari, V., Alves, J. G., & Herrmann, D. (2011). Noninvasive ventilation for respiratory distress syndrome: a randomized controlled trial. *Pediatrics*, *127*(2), 300-307.
31. Sai Sunil Kishore, M., Dutta, S., & Kumar, P. (2009). Early nasal intermittent positive pressure ventilation versus continuous positive airway pressure for respiratory distress syndrome. *Acta Paediatrica*, *98*(9), 1412-1415.
32. Khorana, M., Paradeevisut, H., Sangtawesin, V., Kanjanapatanakul, W., Chotigeat, U., & Ayutthaya, J. K. (2008). A randomized trial of non-synchronized Nasopharyngeal

Intermittent Mandatory Ventilation (nsNIMV) vs. Nasal Continuous Positive Airway Pressure (NCPAP) in the prevention of extubation failure in pre-term< 1,500 grams. *Journal of the Medical Association of Thailand= Chotmaihet thangphaet*, *91*, S136-42.

33. Ramanathan, R., Sekar, K. C., Rasmussen, M., Bhatia, J., & Soll, R. F. (2012). Nasal intermittent positive pressure ventilation after surfactant treatment for respiratory distress syndrome in preterm infants< 30 weeks' gestation: a randomized, controlled trial. *Journal of Perinatology*, *32*(5), 336-343.
34. Kirpalani, H., Millar, D., Lemyre, B., Yoder, B. A., Chiu, A., & Roberts, R. S. (2013). A trial comparing noninvasive ventilation strategies in preterm infants. *New England Journal of Medicine*, *369*(7), 611-620.
35. Lemyre, B., Davis, P. G., De Paoli, A. G., & Kirpalani, H. (2014). Nasal intermittent positive pressure ventilation (NIPPV) versus nasal continuous positive airway pressure (NCPAP) for preterm neonates after extubation. *Cochrane Database Syst Rev*, *9*.
36. Cummings, J. J., & Polin, R. A. (2016). Noninvasive Respiratory Support. *Pediatrics*, 137(1):e20153758.
37. Chang, H. Y., Claure, N., D'Ugard, C., Torres, J., Nwajei, P., & Bancalari, E. (2011). Effects of synchronization during nasal ventilation in clinically stable preterm infants. Pediatric research, 69(1), 84-89
38. Kamath, M.V., Watanabe, M.A., & Upton, A.R.M. (Eds.)(2013). Heart Rate Variability (HRV) Signal Analysis: Clinical Applications. CRC Press, FL.
39. Task Force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology (1996). Heart rate variability standards of measurement, physiological interpretation, and clinical use. *Circulation*, 93(5), 1043-1065.

40. Pöyhönen, M., Syväoja, S., Hartikainen, J., Ruokonen, E., & Takala, J. (2004). The effect of carbon dioxide, respiratory rate and tidal volume on human heart rate variability. *Acta Anaesthesiologica Scandinavica*, *48*(1), 93-101.

41. Shen, H. N., Lin, L. Y., Chen, K. Y., Kuo, P. H., Yu, C. J., Wu, H. D., & Yang, P. C. (2003). Changes of heart rate variability during ventilator weaning. *CHEST Journal*, *123*(4), 1222-1228.

42. Huang, C. T., Tsai, Y. J., Lin, J. W., Ruan, S. Y., Wu, H. D., & Yu, C. J. (2014). Application of heart rate variability in patients undergoing weaning from mechanical ventilation. *Crit Care*, *18*(1), R21.

43. Seely, A. J., Bravi, A., Herry, C., Green, G., Longtin, A., Ramsay, T., Fergusson, D., McIntyre, L., Kubelik, D., Maziak, D. E., Ferguson, N., Brown, S. M., Mehta, S., Martin, C., Rubenfield, G., Jacono, F. J., Clifford, G., Fazekas, A., & Marshall, J. (2014). Do heart and respiratory rate variability improve prediction of extubation outcomes in critically ill patients. *Crit Care*, *18*(2), R65.

44. Cabal, L. A., Siassi, B., Zanini, B., Hodgman, J. E., & Hon, E. E. (1980). Factors affecting heart rate variability in preterm infants. *Pediatrics*, *65*(1), 50-56.

45. Jenkins, J. G., Reid, M., & McClure, B. G. (1980). Study of heart rate variability in sick newborn infants. *Acta Paediatrica*, *69*(3), 393-396.

46. van Ravenswaaij-Arts, C. M., Hopman, J. C., Kollée, L. A., van Amen, J. P., Stoelinga, G. B., & van Geijn, H. P. (1991). The influence of respiratory distress syndrome on heart rate variability in very preterm infants. *Early human development*, *27*(3), 207-221.

47. Prietsch, V., Knoepke, U., & Obladen, M. (1994). Continuous monitoring of heart rate variability in preterm infants. *Early human development*, *37*(2), 117-131.

48. Äärimaa, T., Oja, R., Antila, K., & Välimäki, I. (1988). Interaction of heart rate and respiration in newborn babies. *Pediatric research*, *24*(6), 745-750.

49. Matic, V., Cherian, P. J., Widjaja, D., Jansen, K., Naulaers, G., Van Huffel, S., & De Vos, M. (2013). Heart rate variability in newborns with hypoxic brain injury. In S. Van Huffel, A. Caicedo, G. Naulaers, D. K. Harrison & D. F. Bruley (Eds.), Oxygen Transport to Tissue XXXV (pp. 43-48). 233 Spring Street, New York NY 10013-1578, United States: Springer New York.

50. Vergales, B. D., Zanelli, S. A., Matsumoto, J. A., Goodkin, H. P., Lake, D. E., Moorman, J. R., & Fairchild, K. D. (2014). Depressed heart rate variability is associated with abnormal EEG, MRI, and death in neonates with hypoxic ischemic encephalopathy. American Journal of Perinatology, 31(10), 855-862. doi: http://dx.doi.org/10.1055/s-0033-1361937

51. Weissman, A., Zimmer, E. Z., Aranovitch, M., & Blazer, S. (2012). Heart rate dynamics during acute pain in newborns. Pflugers Archiv - European Journal of Physiology, 464(6), 593-599. doi: http://dx.doi.org/10.1007/s00424-012-1168-x

52. Cong, X., Cusson, R. M., Walsh, S., Hussain, N., Ludington-Hoe, S. M., & Zhang, D. (2012). Effects of skin-to-skin contact on autonomic pain responses in preterm infants. Journal of Pain, 13(7), 636-645. doi: http://dx.doi.org/10.1016/j.jpain.2012.02.008

53. Hambleton, M. T., Reynolds, E. W., Sithisarn, T., Traxel, S. J., Patwardhan, A. R., Crawford, T. N., Mendiondo, M.S., & Bada, H. S. (2013). Autonomic nervous system function following prenatal opiate exposure. Frontiers in Pediatrics, 1, 27. doi: http://dx.doi.org/10.3389/fped.2013.00027

54. Schaffer, L., Burkhardt, T., Tomaske, M., Schmidt, S., Luzi, F., Rauh, M., Leone, A., & Beinder, E. (2010). Effect of antenatal betamethasone administration on neonatal cardiac

autonomic balance. Pediatric Research, 68(4), 286-291. doi: http://dx.doi.org/10.1203/00006450-201011001-00559

55. Yiallourou, S. R., Witcombe, N. B., Sands, S. A., Walker, A. M., & Horne, R. S. (2013). The development of autonomic cardiovascular control is altered by preterm birth. Early Human Development, 89(3), 145-152. doi: http://dx.doi.org/10.1016/j.earlhumdev.2012.09.009
56. Eiselt, M., Zwiener, U., Witte, H., & Curzi-Dascalova, L. (2002). Influence of prematurity and extrauterine development on the sleep state dependent heart rate patterns. Somnologie, 6(3), 116-123. doi: http://dx.doi.org/10.1046/j.1439-054X.2002.02189.x
57. Fairchild, K. D. (2013). Predictive monitoring for early detection of sepsis in neonatal ICU patients. *Current opinion in pediatrics*, *25*(2), 172-179.
58. Courtney, S. E., Pyon, K. H., Saslow, J. G., Arnold, G. K., Pandit, P. B., & Habib, R. H. (2001). Lung recruitment and breathing pattern during variable versus continuous flow nasal continuous positive airway pressure in premature infants: an evaluation of three devices. *Pediatrics*, *107*(2), 304-308.
59. Fortrat, J. O. (2002). Inaccurate normal values of heart rate variability spectral analysis in newborn infants. *The American Journal of Cardiology*, *3*(90), 346.
60. Shalish, W., Rao, S., Sant'Anna, G., and Mortola, J. (2016). Breath-by-Breath Analysis Of Respiratory Sinus Arrhythmia In Preterm Infant [Abstract]. *Am J Respir Crit Care Med*, 193(2016), A3835.
61. Owen, L. S., Morley, C. J., & Davis, P. G. (2010). Pressure variation during ventilator generated nasal intermittent positive pressure ventilation in preterm infants. *Archives of Disease in Childhood-Fetal and Neonatal Edition*, *95*(5), F359-F364.

62. Owen, L. S., Morley, C. J., Dawson, J. A., & Davis, P. G. (2011). Effects of non-synchronised nasal intermittent positive pressure ventilation on spontaneous breathing in preterm infants. *Archives of Disease in Childhood-Fetal and Neonatal Edition*, *96*(6), F422-F428.

63. Moreau-Bussière, F., Samson, N., St-Hilaire, M., Reix, P., Lafond, J. R., Nsegbe, E., & Praud, J. P. (2007). Laryngeal response to nasal ventilation in nonsedated newborn lambs. *Journal of Applied Physiology*, *102*(6), 2149-2157.

64. Roy, B., Samson, N., Moreau-Bussiere, F., Ouimet, A., Dorion, D., Mayer, S., & Praud, J. P. (2008). Mechanisms of active laryngeal closure during noninvasive intermittent positive pressure ventilation in nonsedated lambs. *Journal of applied physiology*, *105*(5), 1406-1412.