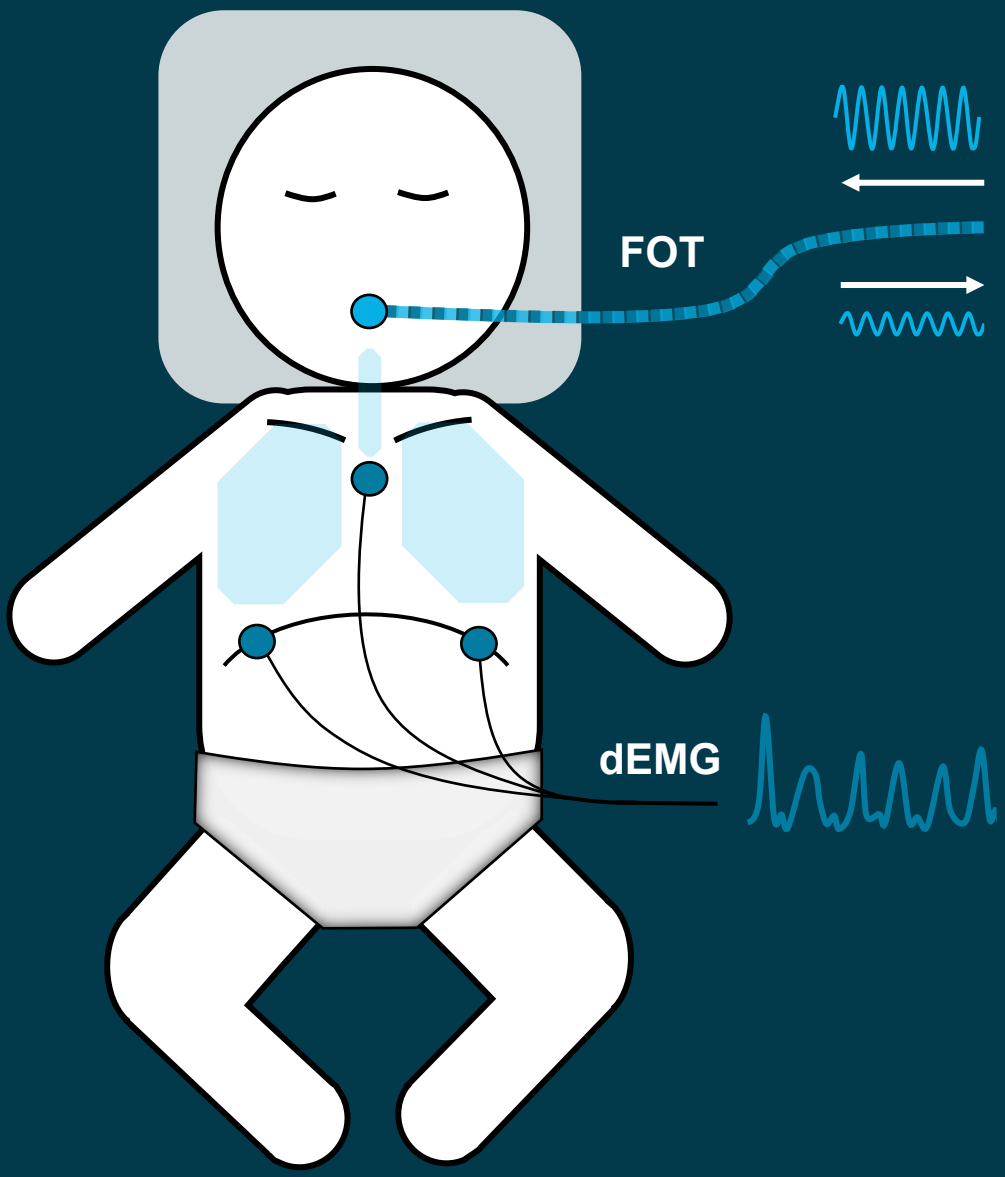
Thesis for the Degree of Master of Science

Optimizing Pressure Settings in Mechanically Ventilated Infants Utilizing Forced Oscillation Technique and Transcutaneous Diaphragmatic Electromyography



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Master's Thesis

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Voorwoord

Voor u ligt de scriptie ‘Optimizing Pressure Settings in Mechanically Ventilated Infants Utilizing Forced Oscillation Technique and Transcutaneous Diaphragmatic Electromyography’. Het onderzoek voor deze scriptie naar de combinatie van forced oscillation technique en elektromyografie om beademingsinstellingen te optimaliseren voor pasgeborenen, is uitgevoerd op de neonatologie afdeling van het Emma Kinderziekenhuis te Amsterdam. Deze scriptie is geschreven in het kader van mijn afstuderen aan de opleiding Technical Medicine aan de Universiteit Twente. Vanaf mei 2021 tot en met juni 2022 ben ik bezig geweest met het onderzoek en het schrijven van deze scriptie.

Door het schrijven van deze scriptie is er een einde gekomen aan zeven leerzame studiejaren. Het waren jaren waarin ik niet alleen vanzelfsprekend medische als technologische kennis heb opgedaan, maar deze kennis ook heb leren combineren en toepassen in de praktijk. Verder zijn ook mijn wetenschappelijke kennis, academische bedrevenheid en praktische vaardigheden verbreed en verrijkt. Daarnaast heb ik afgelopen jaren ook veel over mezelf mogen leren, waar ik mijn hele leven profijt van zal hebben. Al deze aspecten zijn in het afgelopen jaar samengekomen, waardoor het een volmaakte afsluiting van een geweldige tijd is geworden.

Samen met mijn stagebegeleiders werd de onderzoeksvraag voor deze scriptie bedacht. Om deze vraag te beantwoorden, werden er twee onderzoeken opgesteld die eerst goedkeuring van de Medisch Ethische Toetsingscommissie vereisten. Na dit akkoord ontvangen te hebben was ik enthousiast om de metingen van deze onderzoeken uit te voeren. Het vinden van geschikte kandidaten voor deelname aan deze onderzoeken was echter moeilijker dan gehoopt. Om de hierdoor overblijvende tijd nuttig te besteden, heb ik een aanvullend verkennend onderzoek uitgevoerd over de toegevoegde waarde van variabiliteitsanalyses op elektromyografie data. Al deze beschreven onderzoeken zijn met bijbehorende achtergrondinformatie verwerkt in deze scriptie waardoor het een uitgebreid verslag is geworden. Ik wens u veel leesplezier toe.

Jorrit van Poelgeest

Amsterdam, juni 2022

Abstract

Rationale:

Due to underdevelopment of the respiratory system, preterm infants may need mechanical ventilation (MV) to facilitate gas exchange. Nowadays, the titration of MV is done based on peripheral oxygen saturation (SpO_2), oxygen demand (FiO_2) and transcutaneous carbon dioxide ($tcCO_2$). However, a dedicated lung function measurement, which is not influenced by the circulatory condition, is currently lacking. In this research the Forced Oscillation Technique (FOT) and the use of transcutaneous electromyography of the diaphragm (dEMG) are investigated as candidate techniques to fill this gap. FOT measures reactance (X_{rs}) providing information about lung mechanics while dEMG can express the respiratory effort of the patient. Incorporating this information in practice might aid optimization of MV settings.

Methods:

First, exploratory research was done about the added value of three different variability analyses on dEMG data: Poincaré, detrended fluctuation- and entropy analysis. If one showed promising results, it was applied in the main research.

Next, a clinical pilot (*FOT study)* was initiated to compare optimal pressure as determined by FOT measurements (P_{FOT}) and by clinicians (P_{CLIN}) during lung recruitment manoeuvres.

In the main research (*Gemini study*), dEMG and FOT measurements were performed regularly during the period of MV, to acquire knowledge on development of these parameters over time. SpO_2, FiO_2 and $tcCO_2$ values were noted for correlation analysis between clinical parameters and the FOT and dEMG parameters. Lastly, correlation analysis was done between X_{rs} and dEMG to investigate whether these techniques might be complementary.

Results:

Poincaré seemed to be the most useful variability analysis on dEMG data and was used in subsequent analyses. Eight infants were included in the *FOT study* which showed that P_{FOT} and P_{CLIN} are correlated. However, P_{FOT} was lower than P_{CLIN} for some patients. The *Gemini study* included six infants so far and showed various correlations between the two techniques and clinical parameters (e.g. correlation X_{rs} vs tonic diaphragm activity: r =-0.63, p=0.03). Inclusion is ongoing to increase the power of the analysis.

Conclusion:

Our study shows that both FOT and dEMG can provide interesting information on the lung function of ventilated preterm infants. The *FOT study* suggests that lower pressure settings might be sufficient as well, while the *Gemini study* is the first study combining techniques striving for a combination of techniques to get a full picture of the patient's condition. The studies are ongoing and more data on these outcomes is required to draw firm conclusions.

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List of Abbreviations

ApEn	Approximate entropy
AUC	Area under the curve
CDP	Continuous distending pressure
dEMG	Transcutaneous electromyography of the diaphragm
$dEMG_{AUC}$	Area under the dEMG curve
$dEMG_{peak}$	Peak (end-inspiratory) diaphragm activity
$dEMG_{tonic}$	Tonic (end-expiratory) diaphragm activity
DFA	Detrended Fluctuation Analysis
ECG	Electrocardiography
ETT	Endotracheal tube
FiO2	Fraction of inspired oxygen
FOT	Forced oscillation technique
FRC	Functional residual capacity
GA	Gestational age
HFOV	High frequency oscillation ventilation
IBI	Inter-breath interval
METC	Medisch Ethische Toetsingscommissie (Dutch; Medical Ethics Review Committee in English)
MV	Mechanical Ventilation
NICU	Neonatal intensive care unit
PEEP	Positive end-expiratory pressure
PIP	Peak inflation pressure
RMS	Root-mean-square
RR	Respiratory rate
SampEn	Sample entropy
SD1	Short-term variability
SD2	Long-term variability
ShEn	Shannon entropy
SpO_2	Peripheral oxygen saturation
$tcPCO_2$	Transcutaneous carbon dioxide
VILI	Ventilation induced lung injury
WMO	Wet Medisch-wetenschappelijk Onderzoek met mensen (Dutch; Medical Research Involving Human Subjects Act in English)
X_{rs}	Respiratory system reactance

1. Introduction

Preterm infants are born before a gestational age (GA) of 37 weeks. Globally, more than 10% of all infants are born prematurely [1]. A wide range of organ systems is affected by the incomplete maturation in preterm infants. One of the major consequences of preterm birth is the underdevelopment of the respiratory system [2]. Besides the immaturity of the lung itself, the respiratory control centre (located in the brainstem) has not yet been adapted to extrauterine life as well [3]. As a result of these immaturities in preterm infants, their lung function is compromised. The primary adverse effect of the underdevelopment of the respiratory system is an impaired gas exchange due to the lack of matured alveoli. Moreover, the underdeveloped control centre causes flaws in breathing regulation that can lead to apnoea's, which are often observed in preterm infants [4]. The frequency of apnoea is inversely proportional to the GA. It is often seen that incidents of apnoea are accompanied by hypoxemia and bradycardia [5]. If adequate oxygenation is maintained, short respiratory pauses are deemed not to be harmful. However, if these pauses are associated with prolonged hypoxic episodes, there is an increased risk of an impaired neurodevelopment [6]. Both the underdeveloped respiratory system and control centre in preterm infants raise the need for cardiorespiratory monitoring and adequate respiratory support.

In this report it is investigated to what extent two medical technologies can aid respiratory support titration in mechanically ventilated preterm infants. As lung function test, forced oscillation technique detects mechanical properties of the respiratory system. The addition of diaphragm electromyography provides information of the inspiratory muscle activity. With the combination of these medical technologies, the respiratory system can be described as a whole. Furthermore, it was thought that variability analyses may provide additional relevant information. Therefore, the added value of three different variability analyses on electromyography data is investigated in a brief exploratory research.

2. Rationale

Most premature born infants need respiratory support to improve their oxygenation and/or ventilation [7]. When non-invasive support is not sufficient, the infant will be mechanically ventilated. At the Neonatal Intensive Care Unit (NICU) of the Emma children's hospital, Amsterdam UMC, two mechanical ventilation (MV) methods are commonly used for respiratory support. For both methods, a maximally protective ventilation strategy is aimed for. The major principles of lung-protective ventilation in premature born infants are to minimize the presence of atelectasis (alveoli collapse) and to avoid usage of high tidal volumes. In addition, high oxygen exposure, which may lead to oxidative stress, is avoided [8]. The conventional method provides the patient a positive end-expiratory pressure (PEEP), which prevents atelectasis (especially during expiration) and therefore enables oxygenation. To ensure ventilation, a peak inflation pressure (PIP) is applied above the PEEP.

The second method uses continuous distending pressure (CDP) to prevent atelectasis and is called high frequency oscillation ventilation (HFOV). In HFOV, ventilation is ensured by oscillating volumes (equal to or) less than the anatomical dead space of the infant. These oscillating volumes can be generated by for example a moving membrane, of a back-and-forth striking piston or a flow interrupting technique, creating respectively negative and positive pressures. This reciprocation generates tiny alternating flows with a range of three to fifteen Hertz. As a result, HFOV maintains alveolar inflation at a constant, less variable airway pressure. Therefore, ventilation induced lung injury (VILI) is possibly prevented by reducing the risks of volutrauma and barotrauma. The open lung strategy is applied, where low tidal volumes are combined with CDP to maximize alveolar recruitment. In addition, recruitment manoeuvres and efforts to minimize derecruitment are performed. The appropriate ventilation settings for a patient depend on its own lung function, physiology and pulmonary condition.

Since there is no standard technique to monitor an infant's lung function, the optimal level of respiratory support is hard to ascertain. Moreover, whilst respiratory support is often lifesaving, it also carries the risk of ventilation induced lung and brain injury and subsequent development of bronchopulmonary dysplasia [9]. Therefore, pressure settings should be carefully considered before application. To date, the individually most favourable PEEP and CDP settings are determined by the clinician utilizing three parameters. The first parameter is the peripheral oxygen saturation (SpO_2) which is measured by a non-invasive flexible wrap sensor around the foot or hand of the patient. As second parameter, oxygen demand, expressed as the required fraction of inspired oxygen (FiO_2) to achieve adequate saturation is used. At last, the concentration of carbon dioxide is determined by a blood test. However, transcutaneous sensors can be utilized as well to determine the concentration of carbon dioxide ($tcPCO_2$) in the peripheral circulation.

In case of conventional MV, an average PEEP value suitable for the infant is set at initiation. Subsequently, this pressure setting is adjusted based on the aforementioned three clinical parameters. In order to find an adequate CDP setting, a lung recruitment manoeuvre is performed. During this manoeuvre, CDP is gradually increased first to recruit lung volume until the oxygenation no longer improves, which is known as the opening pressure. During this process, the FiO_2 is reduced and set to preferably below 0.3, while maintaining adequate SpO_2 and $tcPCO_2$ levels. For SpO_2 a value of 90-95% is aimed for in premature infants and 92-98% for term infants. For $tcPCO_2$, a value between 6.5 and 9 kPa is usually aimed for. Nonetheless, in case of persistent pulmonary hypertension (PPHN), a value between 9 and 15 kPa is targeted. If the oxygenation (SpO_2) is stable, CDP is decreased until a drop in SpO_2 and/or rapid rise in $tcPCO_2$ is noticed. This final level of CDP indicates atelectasis and is determined as the closing pressure.

The optimal level of CDP is then construed as two to three cmH_2O above the closing pressure, which is applied after the lung volume has been recruited on the opening pressure again. This method ensures adequate gas exchange and theoretically prevents lung injury as the patient is ventilated at the lowest possible pressure level [10].

Although the adjustment of ventilation settings to clinical parameters sounds convenient, this method carries a substantial disadvantage. As the pressure level is based on FiO_2, SpO_2 and $(tc)PCO_2$ it does not only depend on the lung function, but on the circulatory condition of the patient as well. As just mentioned, some new-borns for example suffer PPHN. The circulatory system of infants with this condition did not adapt well for extrauterine life. After birth, the resistance in the blood vessels around the lungs remains relatively high, which impedes the blood flow. As a result, the gas exchange is impaired, causing too less oxygen and carbon dioxide to be diffused over the capillary walls.

Besides the possible influence of circulatory conditions, physiological changes are not immediately reflected by these parameters. It may take at least several minutes before responses in these parameters can be noted. These disadvantages facilitate the desire for an alternative approach identifying optimal pressure levels in a way that is more direct and primarily dependents on the lung function and condition. Furthermore, this approach should meet the requirements of being quick to use, applicable at bedside, preferably non-invasive and not requiring patient cooperation in order to be used at the NICU.

Forced Oscillation Technique

Forced oscillation technique (FOT) is a non-invasive method for investigation of respiratory mechanics in clinical practice [11]. During FOT measurements external driving signals (forced oscillations) are applied to the airway opening of the patient. The impedance of the respiratory system can be derived from the difference between the inserted and returned superimposed oscillatory signal. This impedance exists of two components: resistance and reactance. The resistance, i.e. airway resistance, reflects the resistance of the respiratory tract to airflow during breathing. The reactance of the respiratory system reveals both elastic and inertial properties of the lung [12]. Initial studies in animals show FOT to be a useful tool during lung recruitment [13].

In addition, the first studies proofing the potential of FOT in clinical practice at the NICU, are published as well [14],[15]. In one of these studies they found that the optimal PEEP during mechanical ventilation of extremely preterm infants, as evaluated by FOT in the first week of life, is lower than the clinically set PEEP [14]. Besides, this technique does not require any active cooperation from the patient, which makes it applicable for the NICU population. Additional FOT research should show the feasibility of this technique in clinical practice and may add information of preterm lung mechanics which eventually could contribute to improved respiratory support.

Electromyography

The respiratory muscles undertake the work of breathing and consist of the diaphragm, intercostal muscles and abdominal muscles. Whereas the latter mentioned group of muscles are only activated in case of increased respiratory needs to facilitate expiration, the main inspiratory muscle is the diaphragm [16]. Preterm infants, however, have a lower mass of the diaphragm and due to the large angle of insertion on the rib cage both the area of apposition and range of displacement are reduced [17]. Due to both an affected diaphragm and respiratory control system, preterm infants are prone to respiratory muscle dysfunction [18]. Therefore, aside from mechanical lung properties, assessment of respiratory muscle activity is important to identify the required support for a patient as well.

The activity of the diaphragm could provide information about the breathing effort and can non-invasively be monitored using transcutaneous electromyography (EMG). Although EMG of the diaphragm (dEMG) does not provide a direct lung function parameter, it gives information on a crucial part of the respiratory system, namely the inspiratory muscle effort [19],[20].

By combining the information acquired from FOT and dEMG measurements, it is expected to obtain a complete and objective picture of an infant's respiratory condition. This combination could therefore add to the proper titration of the current respiratory support of the individual patient. Thus, dEMG would be a befitting addition in supplementary FOT research on (preterm) infants.

Variability Analyses

Frequently used dEMG parameters are peak activity ($dEMG_{peak}$), tonic activity ($dEMG_{tonic}$) and the area under the curve ($dEMG_{AUC}$). These parameters can be extracted from each breath observable in the dEMG signal. While $dEMG_{peak}$ represents the end-inspiratory diaphragm activity, $dEMG_{tonic}$ corresponds to its end-expiratory activity. The $dEMG_{AUC}$ is often used as a measure for the power of the dEMG signal. An overview of these dEMG parameters can be observed in *Figure 1*. Usually, individual or group characteristics are described by means of the median or mean value of the described dEMG parameters. However, these parameters might be only a poor representation of the entire spectrum of behavioural information that may be hidden in the data.

Some natural processes do not have a specific scale to fit in. Such (physiological) processes are broadly referred to as "scale-free" [21]. Multiple publications engender the evidence that physiological processes can reveal fluctuations without a specific scale, while its scale-free nonlinear dynamics are important for its function [22][23][24]. Therefore, the mentioned generally used characteristics may only provide a limited description the true properties of such physiological signals.

An example of such a scale-free physiological entity is the dEMG signal. There are no reference values of dEMG representing normal breathing nor to distinguish between disorders. In order to compare a set of EMG signals, patients are their own control. In other words, only the percentual differences of the described parameters are appropriate to be investigated, as there are no longer scales involved.

It is presumed that additional analyses providing outcomes of dimensionless quantity (no assigned physical dimension/scale) may be valuable while investigating dEMG signals. Especially analyses which focus on the scale-free nonlinear dynamics are thought to be effective for dEMG research. For example, the variability of the inter-breath intervals and $dEMG_{peak}$ outcomes over time may provide relevant information of the inspiratory muscle condition.

This mathematical approach may bring to light some additional dEMG signal characteristics favourable for future research. Therefore, next to those generally used parameters in the scientific field of dEMG, variability analyses are performed in order to investigate their potential added value.

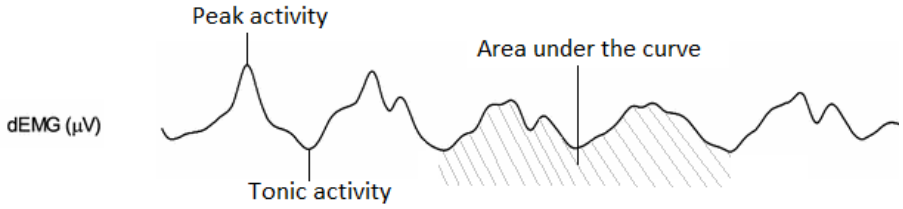

***Figure 1**: Representative example of a transcutaneous diaphragmatic EMG signal. The peak activity, tonic activity and area under the curve are indicated.*

3. Research Question

With the combination of both respiratory muscle activity and mechanical lung properties the respiratory system can be described as a whole. It is suggested that this combination of techniques, together with the previously described generally used and additional variability analyses may provide yet unknown knowledge about the interaction between lung mechanics and respiratory muscle activity in developing preterm infants. Since to our knowledge no literature on variability analyses on dEMG had been published yet, exploratory research on previously collected dEMG data was performed first in order to investigate its potential clinical relevance, investigating the following research question:

- To what extent can the addition of variability analyses advance the investigation of diaphragm activity measured by transcutaneous EMG?

Additionally, the combined FOT and dEMG approach may potentially contribute to the optimization of respiratory support settings and therefore lower the risk of adverse effects of MV. This latter presumption was ground for the initial main research question. As this research question led to a legitimate approved scientific study, most of the time was invested in the proposal and execution of this research. This proposal had been set up to better define the clinical utility of FOT in ventilated neonates. In order to investigate the possible clinical added value of FOT, the following main research question was set up:

- To what extent can forced oscillation technique in combination with electromyography aid respiratory support titration in mechanically ventilated infants in clinical practice at the NICU?

To be able to answer this main research question, the following sub-questions will be addressed:

- Are the optimal pressure settings as determined with FOT and as defined in clinical practice, correlated?

- Does the reactance of the respiratory system determined with FOT (X_{rs}) correlate with parameters used by clinicians (SpO_2, FiO_2 and $tcPCO_2$) for respiratory support titration?

- Does respiratory system reactance determined with FOT (X_{rs}) correlate with diaphragm activity measured by transcutaneous EMG?

- Does respiratory system reactance determined with FOT (X_{rs}) correlate with variability analyses outcomes of diaphragm activity measured by transcutaneous EMG?

4. Background

Although the exploratory investigation of the potential value of variability analyses is provided before the elaboration of the initial main research question, the background information is given in a constructive order. First, knowledge about the respiratory system and its development is shared. In the sections hereafter, technical deepening increases gradually when explaining the utilized medical technologies and variability analyses.

Medical Background

In comparison to the development of the other organs, the prenatal development of the lungs occupies a special position. As respiratory organs, the lungs are unnecessary for intrauterine gas exchange. However, they should develop to such an extent that they are ready to function immediately following birth. The next section briefly explains the development and physiology of these vital organs.

Lung Development

During the first minutes of extrauterine life, foetal airway fluid has to be cleared before gaseous distention of the lungs can occur. The first amount of this fluid is removed by thoracic squeeze resulting from vaginal delivery. Second, in case of both vaginal delivery and caesarean section, the gasps as part of the initiation of the own respiration generate deep negative pressures that are responsible for fluid clearance. In addition, the clearance is accomplished by triggered fluid absorption into the pulmonary interstitium as a result of (maternal) stress hormones [25]. In this way the lungs are gradually cleared of the amniotic fluid and filled with air for the first time to take over the respiratory function from the placenta [26].

Normal lung development during pregnancy can be characterized by five stages. The first stage is the embryonic stage which takes place in the third to sixth week of pregnancy. Hereafter the pseudoglandular stage (7-17 weeks), canalicular stage (17-27 weeks), saccular stage (27-36 weeks) and alveolar stage (36 weeks to 8 years after birth) come to pass consecutively [27]. During the first developmental stages, the airways continuously subdivide into new branches until the latter mentioned stages are reached where alveoli flourish around the 23^{th} generation in the adolescent lung [28]. In this way the trachea bifurcates into two main bronchi, which further bifurcates into bronchioles, terminal bronchioles, respiratory bronchioles, alveolar sacs and finally into individual alveoli to complete the bronchial tree. A schematic overview of these developments can be seen in *Figure 2*.

In more detail, the first generations of the respiratory region start to develop at the canalicular stage. During this period the respiratory bronchioles, alveolar ducts and primitive alveoli will bifurcate as well. Finally, the first region of gas exchange, the alveolar-capillary barrier, develops at this stage while epithelial cells differentiate to surfactant producing pneumocytes type II [29]. This surfactant (surface active agent) is needed to decrease the surface tension within the eventually flourished alveoli. Due to this decreased surface tension, the alveoli - and therefore the lungs in general - have an increased compliance. This compliance (C) is defined as the elastic deformation as a result of volume change (ΔV) when a certain pressure (ΔP) is applied [30], which can be described by the following equation:

Equation 1: $C = \frac{\Delta V}{\Delta P}$

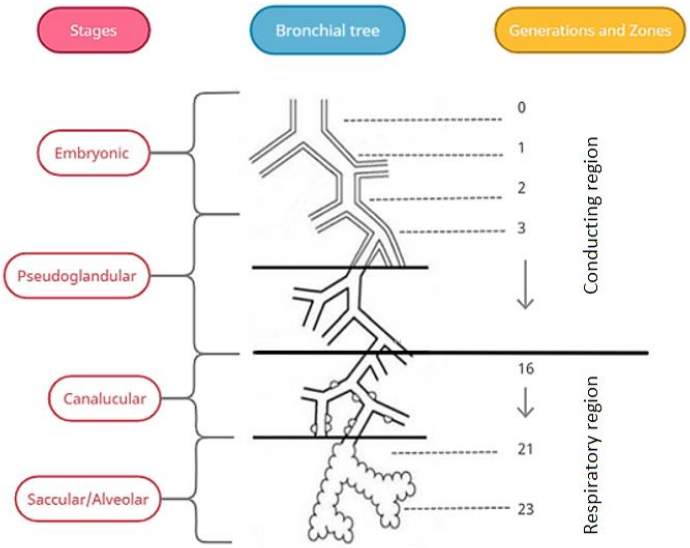

***Figure 2**: Schematic representation of the development of the bronchial tree during pregnancy. A rough rendering of the developmental branches associated with the consecutive stages is shown. This figure was acquired and edited from Chakraborty et al.* [74].

When fully developed, the airway generations can roughly be divided into two regions: the first region exists of the conducting airways while the combined transitional and respiratory airways are called the respiratory region. The conducting region consists of all generations up to the terminal bronchioles at around the 16th bifurcation, which can be seen in *Figure 2* as well. As one would guess, the conducting airways are responsible for the conduction of the air (by convection) to those regions of the lungs responsible for the main purpose of the lungs, gas exchange with the blood. At the alveolar membrane, carbon dioxide (CO_2) is exchanged for oxygen (O_2) to remain homeostatic (and prevent hypoxemia and hypercarbia). The concept of gas exchange is schematically shown in *Figure 3*.

Due to inspiration (grey arrow facing inwards the alveolar space), oxygen rich air flows into the alveolar space. The outflux of CO_2 (blue triangle) will be reciprocated by the influx of O_2 (red triangle) as a result of diffusion at the surface of the alveoli, which are surrounded by capillaries [30]. In this way, deoxygenated blood (blue arrow facing inwards the capillary) is oxygenated to provide oxygen to the rest of the body (red arrow facing outwards the capillary). Hereafter, carbon dioxide is removed from the alveolar space by next exhalation (grey arrow facing outwards the alveolar space).

Premature infants are frequently born within the saccular stage of lung development, or even within the canalicular stage as seen more often in extremely preterm born infants (GA <28 weeks). Since premature birth interrupts intrauterine development, it leads to incomplete foetal growth and organ maturation. Unfortunately, the respiratory system is one of the organ systems to be highly affected by preterm birth [27],[31]. As a result, extremely preterm infants have an increased diffusion barrier for gas exchange at birth [32], which hinders this main purpose of the lungs.

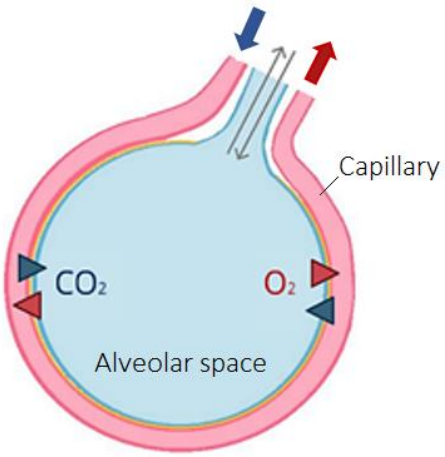

***Figure 3**: Schematic representation of alveolar gas exchange. Deoxygenated blood (blue arrow inwards capillary) is oxygenated by diffusion of O_2 from the alveolar space to the erythrocytes in the blood (red triangle) while CO_2 diffuses in opposite direction (blue triangle).*

Infants born at the saccular stage (GA 27-36 weeks) usually have more differentiated cells to produce surfactant and developed an increased gas exchange area in comparison to those born extremely preterm. Nevertheless, the overall synthesis of surfactant is still far from optimal at the saccular stage, causing an impaired lung compliance which may lead to atelectasis [33]. To prevent this from happening, adequate lung volume at the end of expiration should be maintained, which is called the functional residual capacity (FRC). This FRC ensures the patency of the (majority of the) alveoli during and at the end of expiration, which aids inspiration. In order to accomplish this, preterm infants actively maintain their FRC by preserving inspiratory muscle activation throughout expiration [29]. This end-expiratory activation is called tonic activity and can be measured with electromyography.

In case of the whole respiratory system, the compliance depends on both the lungs and the chest wall. Whereas the lung compliance in infants is decreased due to the limited amount of surfactant, the chest wall compliance is increased due to a more cartilaginous thorax in comparison to adults. Along with the increased anatomical and physiological dead space, these compliances are responsible for the relatively high respiratory rate (RR) in infants as the tidal volume depends on both compliances. Whereas the RR at rest in adults is around 12 to 16 breaths per minute, normal infant's RR range from 30 to 60 breaths per minute [34].

Control of Breathing

In a healthy situation, respiration is well regulated by the respiratory centre located in the pons and medulla in the brainstem. This regulation is called control of breathing and operates by a feedback system to maintain adequate partial pressures of both gasses involved in gas exchange, oxygen (PaO_2) and carbon dioxide ($PaCO_2$), and the concentration of hydrogen molecules (pH) [35]. This feedback system can be observed in *Figure 4*.

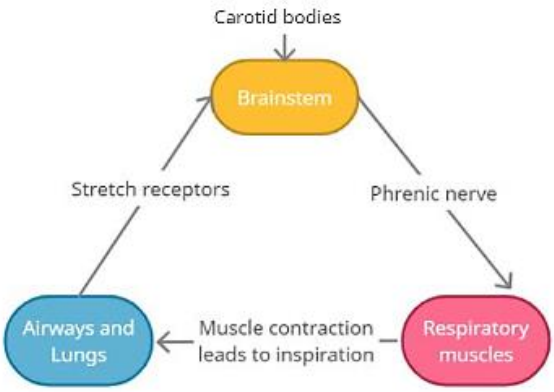

***Figure 4**: Simplified model of the feedback system describing the respiratory control mechanism.*

During breathing, the brainstem receives chemical and mechanical input. Chemoreceptors for PaO_2, $PaCO_2$ and pH, provide information on the effectiveness of the gas exchange to the control centre in the brainstem. For example, chemoreceptors of the respiratory centre that detect pH levels in the blood send signals to the respiratory centres of the brain to adjust the respiratory rate to change acidity by increasing or decreasing the degree of carbon dioxide removal. The medulla is the primary respiratory control centre of which the main function is to innervate respiratory muscles by two different regions. The ventral respiratory group (VRG) of the medulla stimulates expiratory muscles, while the dorsal group (DRG) is responsible for the inspiration. Aortic and carotid bodies have a similar chemoreceptor function as the medulla, which are located in the adventitia around the bifurcation of the common carotid artery and aortic arch respectively.

The pons is located above the medulla and its main function is to control the involuntary respiration rate. The pons has two functional regions as well, the apneustic and pneumotaxic centre, which together are called the pontine respiratory group (PRG). The apneustic centre controls the intensity of breathing and is inhibited by stretch receptors when the maximum depth of inspiration is reached. The pneumotaxic centre finetunes the respiratory rate by inhibiting inspiration. Therefore it can work against apneustic centre overactivity and is able to limit the activity of the phrenic nerve [35]. An overview of the respiratory groups can be observed in *Figure 5*.

The phrenic nerve is responsible for the innervation of the diaphragm, which causes the membrane potential of the muscle to depolarize [36]. This depolarization eventually results in an action potential that induces muscle contraction. More detailed information about muscle contraction is provided in the *Electromyography* section. However, contraction of the diaphragm results in flattening of this dome-shaped muscle, resulting in a drop in intrathoracic pressure. This pressure drop causes influx of air, which actually is inspiration [30].

In contrast to the previously described active expiration of neonates in order to maintain an adequate FRC, normal expiration is usually a passive process. This process is accomplished by relaxing the respiratory muscles after inspiration. Due to its elastic properties, lung tissue recoils when the diaphragm relaxes. This relaxation results in a decrease in intrathoracic volume and thus an increase in pressure which causes expiration. To complete the cycle of *Figure 4*, mechanical feedback from stretch receptors in the smooth muscles of bronchi and bronchioles is send to the brainstem in return. These pulmonary stretch receptors are part of the Hering-Breuer reflex and respond to excessive stretching of the lung in order to prevent overdistention [37].

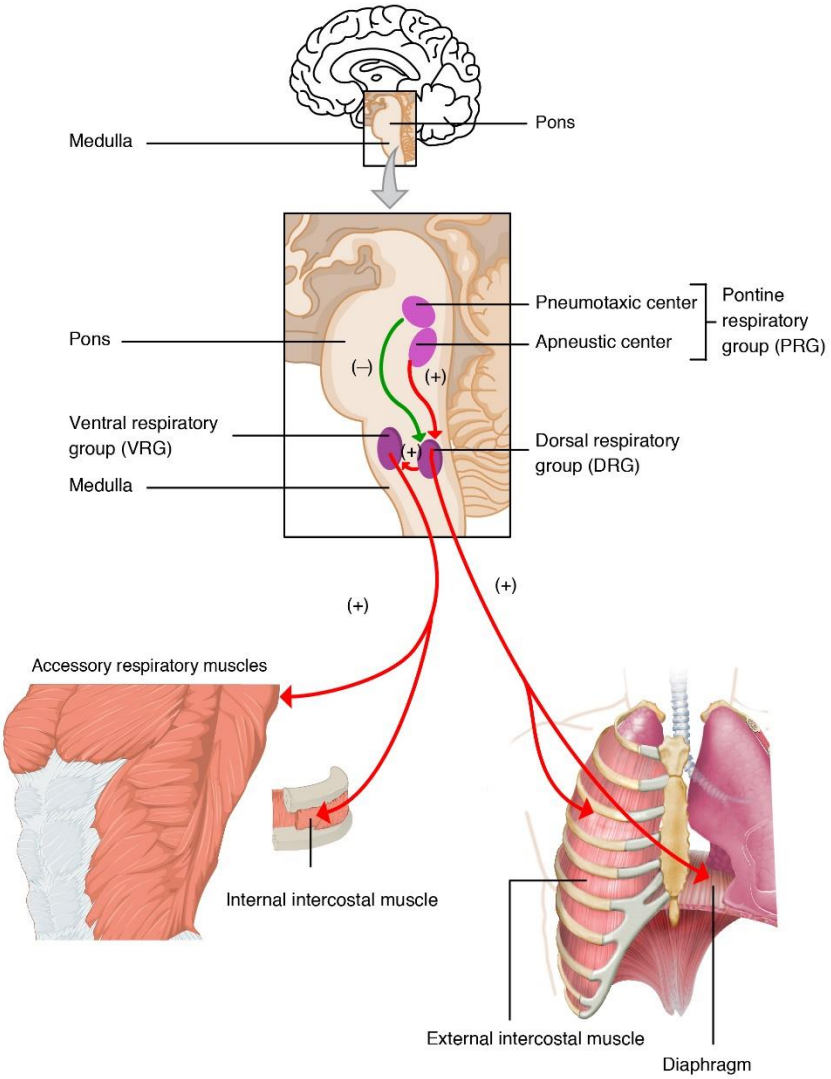

***Figure 5**: The respiratory centre in the brainstem, with the respiratory groups and their influence. This figure was acquired from OpenStax* [75].

In preterm infants however, there is an immature respiratory control due to underdeveloped brain and functioning of the chemoreceptors. Whereas term born neonates (and adults) increase ventilation in response to an increased pCO_2, in preterm infants this capability is impaired. The underdeveloped control of breathing in preterm infants predisposes them to apnoea of prematurity and bradycardia, which can have serious consequences [38]. Both the immature respiratory system and its control centre facilitate the requirement for pharmacological treatment, respiratory support and cardiorespiratory monitoring [39][40].

Medical Technology

Vital signs, such as heart rate, respiratory rate, oxygen saturation and temperature are physical parameters that can be measured to assess the physiological state and functioning. Monitoring of such vital parameters is crucial in neonatal intensive care. Continuous monitoring of the respiration of preterm infants is critical to detect and prevent abnormalities in breathing [39]. The respiratory muscles and its innervations play an important role in the respiration of preterm infants [18]. Monitoring diaphragm function gives insight in breathing effort and might provide information on the effect of prematurity, critical illness, respiratory diagnoses and their treatments [41][42].

Electromyography

The function of the diaphragm can be monitored with three techniques: transdiaphragmatic pressure measurements, ultrasound and EMG. However, the first two mentioned techniques are not convenient for this research. As the study population individuals might be critically ill, it is desired to prevent any additional load. Therefore, only non-invasive techniques are suitable, causing transdiaphragmatic pressure measurements to be incompatible. Ultrasound is a non-invasive technique, however, it can only be used for short term registrations and is therefore unsuited for this research as well. Therefore, EMG is the only applicable technique for diaphragm monitoring in this research.

EMG uses electrodes to measure electrical muscle activity. During muscle contraction, electrical activity results from the depolarization of muscle fibres, which produces an action potential (AP) that can be detected by the electrodes as a change in voltage over time. Muscle fibres are organized into motor units, the smallest functional muscle units involved in contraction, resulting in a motor unit action potential (MUAP). Muscle contraction is the result of simultaneous contractions of numerous motor units. The total electrical activity measured with EMG is the summation of all MUAPs and depends on the number of activated motor units as well as the shape and phase of the APs [41].

Electrical activity of the diaphragm can be measured with EMG, which is a representative of muscle contraction and a measure of respiratory effort. Moreover, since the electrical diaphragm activity is directly related to the stimulation of the phrenic nerves, dEMG reflects the neural output of the respiratory control centre as well. An alteration in signal amplitude can therefore indicate a change in neural drive or conduction [43]. EMG of the diaphragm can be obtained by three different approaches: intramuscular, transoesophageal and transcutaneous. The first mentioned approach uses needles for direct measures of muscle fibre activity, however, this method is not used in preterm infants because of its invasive nature and risk of complications.

Transoesophageal dEMG can be used in preterm infants [44]. For this approach dEMG electrodes are integrated into a feeding tube or placed on a catheter to be inserted in the oesophagus. The electrodes will then be positioned at the level of the diaphragm to measure this muscle's activity. Both treatment effects and breathing patterns can be obtained in premature infants by this approach [44], nevertheless, this method is undesired in this research due to its invasive nature (and cost effectiveness) as well. Thus, in order to measure diaphragmatic muscle function non-invasively, only one technique remains: transcutaneous dEMG.

As EMG recordings display a potential difference, at least two electrodes are needed. In transcutaneous dEMG, one of these electrodes should be placed above the muscle of interest and is called the active electrode. The second electrode serves as a ground for the signal and is called the reference electrode, which is usually placed on a bony or other electrically neutral structure. The potential difference between the active and reference electrode is measured, known as a unipolar derivation. However, to reduce common signal noise, frequently two active electrodes are utilized. In this 'bipolar' approach, the difference between the two unipolar derivations is used [45].

For dEMG measurements in infants, the active electrodes are often placed on both the left and right midclavicular line intersections of the costo-abdominal margin, while the reference electrode is placed on the sternum [41]. Schematic dEMG electrode placement in infants can be observed in *Figure 6*. With dEMG, the breathing effort can be recorded. This breathing effort often is increased in prematurely born infants as a result of impaired control of breathing or a compromised lung function. In an attempt to restore the lung function and reduce the breathing effort, infants often receive respiratory support.

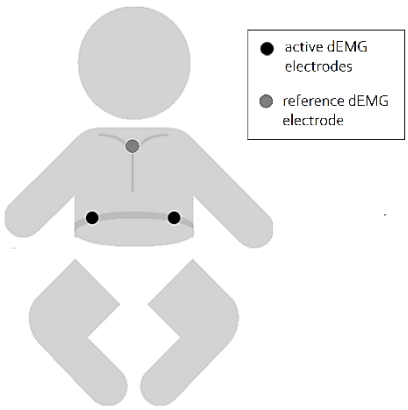

***Figure 6**: Placement of transcutaneous dEMG electrodes in preterm infants. Two active electrodes (black) are placed bilaterally at the costo-abdominal margin in the midclavicular line. The reference electrode (grey) is placed on the sternum. This figure was acquired and edited from Leuteren et al.* [76].

Forced Oscillation Technique

Respiratory support settings are now based on clinical parameters such as breathing pattern (e.g. dyspnoea, tachypnoea), FiO_2, SpO_2 and $tcPCO_2$. However, most of these parameters are not only contingent on the respiratory condition of the patient, but on the circulatory state as well. Therefore, it is thought that objective measurements, completely reliant on lung function, may provide better insight in the respiratory condition. FOT is a long existing technique to measure respiratory system mechanics. The technique was first described in 1956 by DuBois er al. [12], but the technique was not generally accepted at that time due to its technical difficulties and the greater attractiveness of the body plethysmography. Modern microprocessors, however, can deal with this technical complexity and FOT is now widely studied in both adults and children [46]-[48].

During FOT measurements, external pressure oscillations are delivered at the airway opening by a loudspeaker, while flow and pressure changes are measured continuously. The forced oscillations are superimposed on the tidal breathing of the subject. As a result, FOT characterizes mechanical properties of the respiratory system in a non-invasive and effort-independent way.

The key concept of FOT relies on the relationship between pressure and airflow throughout the respiratory cycle: respiratory system impedance (Z_{rs}). This impedance exists of two components, resistance and reactance. The relation between Z_{rs} and these two components is comparable to Ohm's law:

Equation 2: $(Zrs)^2 = (Rrs)^2 + (Xrs)^2$

This equation can eventually be converted to:

Equation 3: $Zrs = R\mathrm{rs} + iX\mathrm{rs},$

In the latter equation, i covers the phase shift, i.e. the imaginary component in the relationship.

Due to the phase-shift, Z_{rs} can be split into its in-phase and out-of-phase components. The out-of-phase component covers the reactance (X_{rs}) and reflects the elastic and inertial properties of the system and is also referred as the imaginary part of the signal. The in-phase component reflects the dissipative mechanical properties of the lung, known as the resistance (R_{rs}) and is called the real part [11]. An overview of the concept of FOT can be seen in *Figure 7*.

Since the extent to which flow and pressure are out of phase differs with frequency, both components appear as functions of the frequency of the oscillation. However, in this research a fixed frequency is used. Therefore, this frequency dependence will no longer be mentioned.

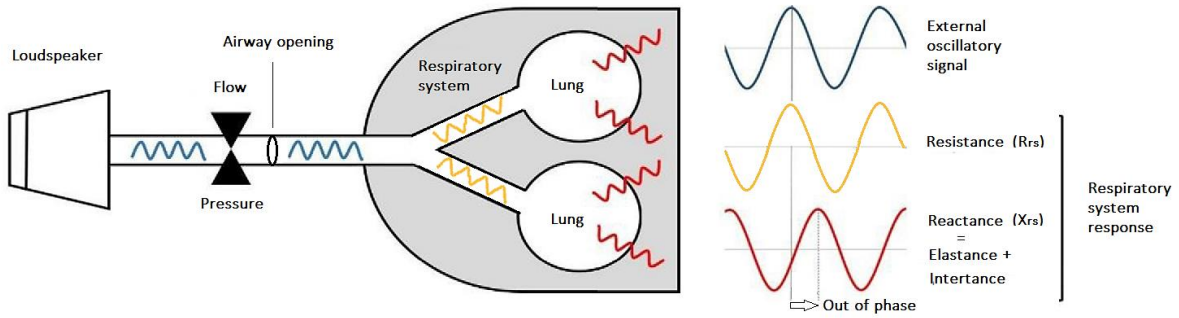

***Figure 7**: A schematic representation of an input oscillatory setup can be seen on the left. Oscillatory signal is delivered by a loudspeaker at the airway opening while flow and pressure changes are registered. The respiratory system response signals are shown op the right. This figure was acquired and edited from Skylogianni et al.* [77].

As the X_{rs} determined by FOT measurements does involve elastic properties, there is a considerable relation to the pressure setting of the respiratory support. Due to the relation between compliance, which is the reciprocal of elastance, and pressure, a change in ventilatory pressure settings should result in a respiratory system reactance change, if the lung is recruitable and thus not optimally aerated. Utilizing this technique, an optimal pressure setting might objectively be determined. This optimal pressure setting is defined as the lowest pressure level corresponding to the highest X_{rs} value [14].

Since this approach is only based on lung function (no circulatory component involved) it should contribute to an optimal ventilatory pressure setting. Therefore, it is thought that FOT might be of added value in optimizing respiratory support. Moreover, this technique seems to fit in well at the NICU. Besides obtaining mechanical lung properties, most important advantages of FOT are that it is both quick and demands no active cooperation [48]. In addition, a FOT modality is already built within the neonatal ventilator used in standard care at the NICU, which makes this technique even more applicable as a candidate technology to optimize respiratory support.

Technical Background

Next to the usual dEMG features, such as peak activity, tonic activity and area under the curve, it is thought that variability analyses may reveal some additional useful signal characteristics. These analyses provide information about the complexity, regularity and predictability of the data. Therefore, these additional parameters may provide information in a more sophisticated technical level.

Variability Analyses

In literature, various variability parameters on (arm and leg muscle surface) EMG signals have been reported such as correlation dimension, Lyapunov exponent and Lempel-Ziv complexity [49]-[51]. Although these parameters have proven EMG signals to be nonlinear and exhibit stationarity, they do not seem to be useful as parameters for individual data. In this study the variability properties of EMG signals are analysed by the α-exponent and the width of the multifractal spectrum derived from the detrended fluctuation analysis approach. Besides, Poincaré analyses are applied to calculate the spatial variability in inter-breath intervals by parameters $SD1$, $SD2$ and the $SD\ ratio$. At last, three different (information) entropy analyses are performed: Shannon Entropy, Approximate Entropy and Sample Entropy.

Detrended Fluctuation Analysis

One scale-free approach is the analysis of the scaling behaviour in time series data. This approach has been described first in 1994 by Peng et al. [52] and is called Detrended Fluctuation Analysis (DFA). Nowadays, the DFA algorithm is a frequently used variability analysis method. The algorithm is able to detect long-range correlations in noisy signals. The output of the algorithm, the fractal scaling exponent α, has shown its usefulness in several fields such as rainfall and weather forecast, earthquakes and economic predictions [53][54]. Additionally, DFA seems to be useful in cardiac and blood pressure dynamics as well as in electrocardiography (ECG) time series [55][56]. In the field of electromyography, DFA has been investigated to be used as a muscle fatigue index, a parameter for pregnancy progress and control signals for prosthetic arm development [57][58]. No research on DFA features for dEMG signals seems to be performed yet as no literature on this subject is available. However, given the mentioned scientific publications, it is suggested DFA could be valuable for this field as well.

The DFA algorithm is based on the root-mean-square analysis of a random walk. As a first step the detrended version of the input signal should be established. Therefore, the time series data is integrated first to achieve the cumulative sum $y(k)$. The time series data is denoted as $\{x(t)\}$, where t is the time in range $[1, N]$ with sample length N. Next, the mean of the time series data ($\overline{x(t)}$) is subtracted from the obtained cumulative sum, resulting in the so-called profile of the input signal.

Equation 4: $y(k) = \sum_{t=1}^{k}[\{x(t)\} - \overline{x(t)}]$, where $k = 1, 2, \dots, N$.

Second, the profile is divided into equal non-overlapping segments or windows n. Per window, the profile is fitted, which results in a local trend. As a next step, the root-mean-square (RMS) fluctuation of the coefficients of all local trends is computed by *Equation 5.*

Equation 5: $F(n) = \sqrt{\frac{1}{N}\sum_{t=1}^{N}[\{y(k)\} - \overline{y_n(k)}]^2}$

This process is repeated for each window size of interest. As a result, each window size corresponds to a certain fluctuation rate.

Finally, the linear relationship between the window sizes and fluctuation rates are plotted into a log-log graph. The subsequent coefficient of the plotted linear relationship is known as the α-exponent and is used as a signal property, which is calculated by:

Equation 6: $\alpha = [\Delta \log_{10}(F(n))] \ / \ [\Delta \log_{10}(n)]$

A visualisation of the mathematical steps can be observed in *Figure 8*. Usually, the calculation time of this analysis ranges from a few seconds to a few minutes, depending on the chosen amount and sizes of applied windows.

For uncorrelated data (white noise), the value of the slope will be exactly 0.5. If there are only short-term correlations, this slope will be slightly different, but α will tend to 0.5 when the window size increases. On the other side of the spectrum, a value of 1.5 corresponds to Brownian noise (integration of white noise). If $0 < \alpha < 0.5$, correlations alternate, i.e. large and small values of time series take turns. When $0.5 < \alpha < 1$, persistent long-range correlations are present in the data, i.e. small values are more likely to co-occur with other small values. Therefore, this parameter can be interpret as fluctuation/predictability indicator [55].

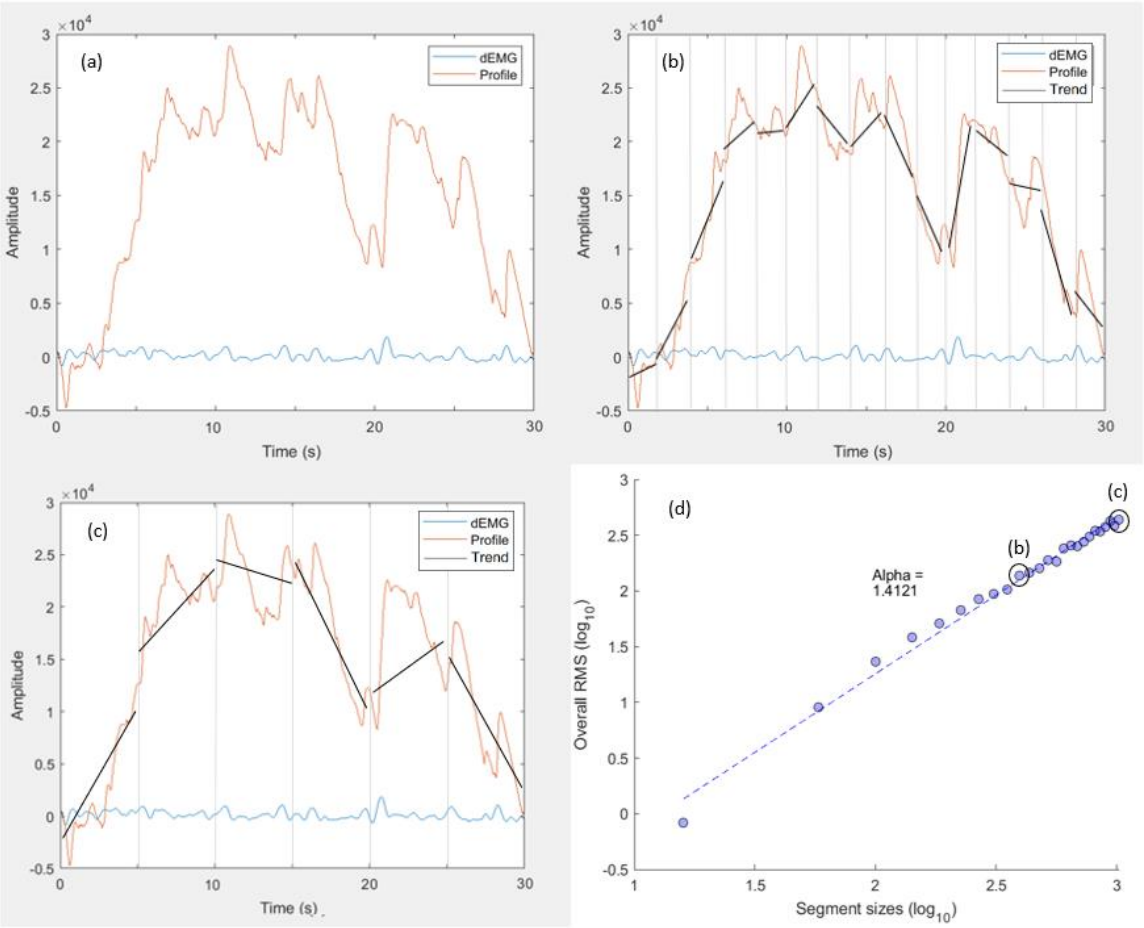

***Figure 8**. **(a):** The mean value of the dEMG data is subtracted from the cumulative sum to obtain the profile. The vertical lines in **(b)** and **(c)** indicate window/segment sizes of respectively two and five seconds. The straight lines represent the estimated local trend of each window by least-squares fit. **(d)** The linear relationship between the window sizes (in samples (fs=200)) and corresponding RMS is plotted into a log-log graph. The results of examples **(b)** and **(c)** are presented in the latter subfigure.*

Multifractal Spectrum

The multifractal spectrum identifies the deviations in fractal structure within time periods of large and small fluctuations. In 2007, Wang et al. showed that the width of the multifractal spectrum of ECG signals can differentiate between several hearth diseases [59]. Ten years later Ghosh et al. succeeded to do the same with neurological disorders as measured with EMG [60]. Given this utility for EMG signals, the width of the multifractal spectrum is investigated in this research on dEMG as well.

For multifractal time series, the local fluctuation can be an exceptionally large magnitude for windows within periods of large fluctuations. This will be the other way around within periods of small fluctuations. Thus, multifractal time series are not normally distributed. Therefore, the described DFA method should be extended to order dependent DFA. In literature these orders are referred to as q, which weight the influence of segments with large and small fluctuations.

For each q-order the α-exponent can be calculated. In literature this α-exponent is also known as the Hurst-exponent, hence the q-order dependent α-exponent is denoted by $h(q)$. Consecutively, $h(q)$ is converted into the q-order mass exponent ($\tau(q)$) by *Equation 7*.

Equation 7: $\tau(q) = q * h(q) - 1$

This $\tau(q)$ is needed to compute the singularity spectrum or so-called dimension $f(\alpha)$, for which *Equation 8* is used.

Equation 8: $f(\alpha) = \mathrm{q} * \alpha - \tau(q)$

In *Equation 8*, $\alpha = \tau'(q)$, which is not the same α as calculated in the previous DFA approach.

Using *Equation 9* and a Legendre transform [61], both $f(\alpha)$ and α can now be related to $h(q)$ by *Equation 10* and *Equation 11*.

Equation 9: $\tau(q) = q * h(q) - 1$

Equation 10: $\alpha = h(q) + q * h'(q)$

Equation 11: $f(\alpha) = q[\alpha - h(q)] + 1$

Finally, the multifractal spectrum arises when $f(\alpha)$ is plotted against $h(q)$. In this research, the width of the resulting parabola in the multifractal spectrum is used as a signal characteristic. An example of a multifractal spectrum is shown in *Figure 9*. This multifractal width can be calculated by *Equation 12*:

Equation 12: $width = h(q)_{max} - h(q)_{min}$.

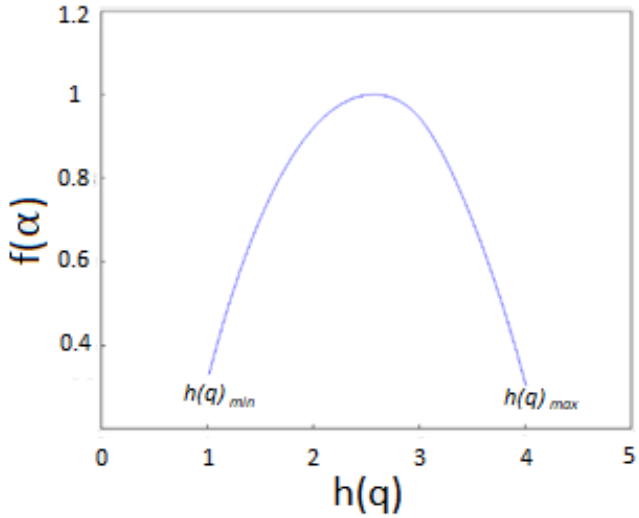

***Figure 9**: Plot of the multifractal spectrum of a dEMG measurement. The width can be determined by subtraction of $h(q)_{min}$ from $h(q)_{max}$.*

Poincaré

Another commonly used variability assessment method is even a bit older, yet more simple to both compute and interpret. This method gives a graphical representation of the correlation between successive intervals and is known as the Poincaré plot, named after the mathematician Henri Poincaré [62]. This method is considered to be nonlinear as it identifies the nonlinear dynamics of covert correlation patterns of time series data. In this research, the inter-breath intervals (IBIs), derived from the time in between peak values in the dEMG signals, are analysed.

An example of a Poincaré plot can be observed in *Figure 10*. Each point in the plot represents the duration of a certain interval, with respect to the one next in line. To characterize the shape of the plot, a fitted ellipse is added to the plot. This ellipse is orientated along the line $y = x$, or more descriptively, where a certain interval and the next one would have been exactly the same.

The dispersion of the dots around this line then represents the data characteristics. The standard deviation of the distance of the dots to the line-of-identity describes the long-term respiratory rate variability, which is noted as $SD2$. The standard deviation of the distance of the dots to the line perpendicular to the line-of-identity is known as $SD1$ and represents the short-term respiratory rate variability.

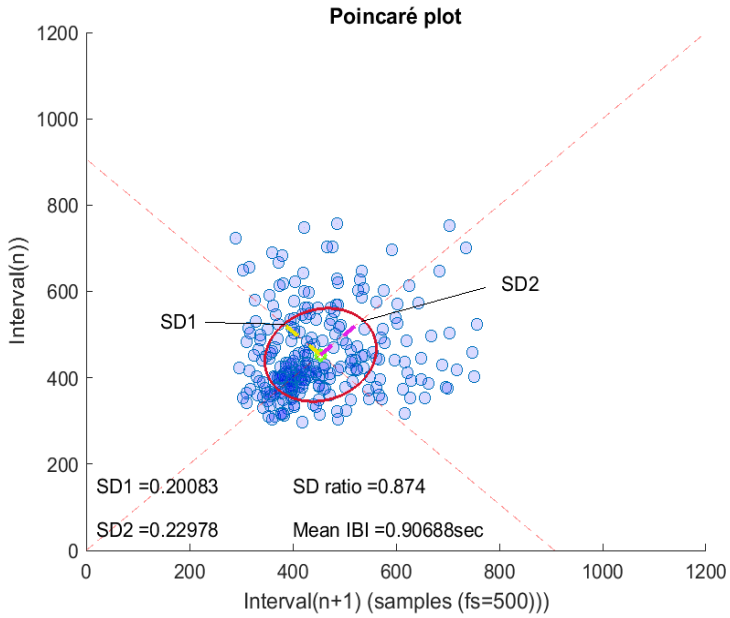

***Figure 10**: Example of a Poincaré plot including both the short-(SD2) and long-term variability (SD2) and corresponding parameter outcomes.*

For the Poincaré plot calculation, a vector containing all (N) timestamps of the peaks of the diaphragm activity corresponding to each breath is created: $x = x_1,\ x_2, \ldots x_N$. In order to compute the Poincaré parameters, two auxiliary vectors are defined: x^+ and x^-. x^+ (*Equation 13*) exists of all described chorological timestamps except the last one, while x^- (*Equation 14*) only misses the first timestamp.

Equation 13: $x^+ = x_1,\ x_2, \ldots x_{N-1}$

Equation 14: $x^- = x_2, x_3, \ldots\ x_N$.

For these standard deviation parameters, first x_1 and x_2 are determined by *Equation 15* and *Equation 16* respectively.

Equation 15: $x_1 = (x^+ - x^-)/\sqrt{2}$

Equation 16: $x_2 = (x^+ + x^-)/\sqrt{2}$

Then $SD1$ is the root of the variance of x_1 (*Equation 17*). Similarly, $SD2$ can be computed from x_2 (*Equation 18*).

Equation 17: $SD1 = \sqrt{Var(x_1)}$

Equation 18: $SD2 = \sqrt{Var(x_2)}$

Usually, the ratio between these two components $(SD1/SD2)$ is taken into account in scientific papers as well. This ratio represents the relationship between the short and long interval variation. As final parameter, the mean inter-breath interval ($\overline{IBI}$) itself is used. Although this parameter is not nonlinear, it is entangled with other Poincaré parameter calculations and may provide some useful information as well.

Entropy Analyses

As additional parameters, multiple entropy analyses were chosen. Entropy is used as a measure of variability as it detects the uncertainty or disorder in a system. Ideally, a (biomedical) system is in complete order, resulting in zero entropy. When stability is lacking, the degree of disorder - and therefore entropy - can be high. In 1949 Shannon et al. introduced this measure of disorder in data, referred as information entropy. Nowadays, diverse types of entropy methods are developed for diverse fields of application. For this research, three suitable entropy analyses were selected.

Shannon Entropy

Since Claude Shannon was the one to propose this measure, this initial analysis of information entropy is called after the mathematician himself. This type of entropy is defined by the degree of uncertainty associated with the incidence of a certain outcome. Therefore, the Shannon Entropy ($ShEn$) of any type variable X is a measure of the expected uncertainty acquired throughout the computation of that variable. When variable X exists of values $x = x_1, x_2, \dots\ x_N$, Sen is defined by *Equation 19*.

Equation 19: $ShEn = \sum_{i=1}^{n} p(x_i) \log_a \frac{1}{p(x_i)} = -\sum_{i=1}^{n} p(x_i) \log_a p(x_i)$

In this formula, $p(x_i)$ represents the probability of acceptance of the random values x_i of variable X. Lower values of $ShEn$ therefore indicate more predictable outcomes and are labelled less uncertain.

Next to $ShEn$, Approximate entropy ($ApEn$) and Sample entropy ($SampEn$) analyses were performed. These two algorithms are used to determine regularity in data, based on the existence of patterns. For these measures, the randomness of the data is estimated without any previous knowledge about the source of the data, which enables limitless applications.

Both $ApEn$ and $SampEn$ were developed for physiological applications, however they have been used in other research fields as well. The more uniform (variable) the distribution, the higher the uncertainty and therefore entropy as well. Hence, time series data containing repetitive patterns has a relatively small entropy.

Approximate Entropy

$ApEn$ was introduced by Pincus et al. in 1991 to avoid some difficulties with the initial information entropy analysis [63]. Although $ShEn$ forms the core for assessing randomness of a series, when it is applied to experimental data series some difficulties are encountered for finite lengths of real time series.

For the calculation of both $ApEn$ and $SampEn$ the same variable X existing of values $x = x_1, x_2, \dots x_N$ is considered. At first, two vectors are constructed in a pseudo-phase:
$y_i = [x_i, \dots, x_{(i+m-1)}]$ and $y_j = [x_j, \dots, x_{(j+m-1)}]$

As a second step, a fixed value of m and r has to be set. The value of m specifies the length of compared runs of data, known as the *embedding dimension*, while r represents a filtering level, referred to as *tolerance*.

In general, given time series data of length N, $ApEn$ is approximately equal to the negative average natural logarithm of the conditional probability that two subseries of length m that are similar, remain similar for subseries of length $m + 1$. This similarity is true within a certain tolerance of usually 20% of the standard deviation of the amplitude values of the time-series data ($r = 0.2$).

Equation 19: $$ApEn = \Phi^m(r) - \Phi^{m+1}(r)$$

Equation 20: $$\Phi^m(r) = \frac{1}{(N-m+1)} \sum_{i=1}^{N-m+1} \ln C_i^m(r)$$

Equation 21: $$C_i^m(r) = \frac{(number\ of\ y(j)\ so\ that\ d[y(i),y(j)] \leq r)}{N-m+1}$$

In these equations, $\ln$ is the natural logarithm and d represents the distance between consecutive data points. This adjustment of information entropy by Pincus et al. led to an improved applicability. The $ApEn$ computation results in a (unit-less) value from zero to two. An outcome equal to zero is consistent with perfectly regular time-series (a true periodic signal), whereas a value equal to two is generated by random time-series (Gaussian noise) [63].

Sample Entropy

Although the $ApEn$ algorithm sounds convenient, it has a disadvantage as well. Due to the $ApEn$ algorithm, unavoidable bias towards regularity is produced since each subseries is counted as self-matching. This shortcoming is counteracted by $SampEn$, which even causes this just mentioned algorithm to slightly reduce the computing time.

Except for this correction, the $ApEn$ and $SampEn$ resemble each other, therefore $SampEn$ is equal to the negative natural logarithm of the conditional probability that two subseries of length m that are similar for subseries of length $m + 1$, where self-matching is not included while the probability is calculated. In addition, $SampEn$ has the advantage to be independent of time-series data length and shows a more consistent behaviour than $ApEn$ [64].

For the computation of $SampEn$ the probabilities $\phi^m(r)$ and $\phi^{m+1}(r)$ need to be reformulated a bit. The probability that two sequences match for m or $m+1$ amount of points is computed by the average counted number of vector pairs for which the distance is smaller than the tolerance in *Equation 22* or *Equation 23* respectively.

Equation 22: $\phi^m(r) = \frac{1}{N-m} \sum_{i=1}^{N-m} C_i^m(r)$

Equation 23: $\phi^{m+1}(r) = \frac{1}{N-m+1} \sum_{i=1}^{N-m+1} C_i^m(r)$

As a final step, $SampEn$ is calculated by *Equation 24.*

Equation 24: $SampEn = \ln \frac{\phi^m(r)}{\phi^{m+1}(r)}$.

The peak values in dEMG signals can be seen as the maximal generated activity needed to breathe (end-inspiratory activity). For each breath, this peak value has been noted. A vector has been made, holding all chronological peak values. The described variability analyses were applied to the full length of original dEMG data. However, even though the 'peak-data' showed similar results for entropy outcomes as the original data in a test setup, the calculation time was significantly reduced. Therefore, the three information entropy analyses were only applied to the chronologically peak value vectors.

5. Exploratory Research

In order to reveal the potential clinical relevance of the described variability analyses on dEMG signals, an exploratory research was performed. It was thought the variability analyses may provide clinically relevant information, yet unrevealed by the usual parameters as used in previous studies. As these variability analyses can reveal details of complexity, regularity and predictability of the dEMG data, these additional parameters can give further insight into the respiratory muscle condition of the patient.

For this exploratory research dEMG data collected during two previous scientific studies at the NICU of the Emma Children's Hospital, Amsterdam University Medical Centre (Amsterdam UMC), was utilized [19][65]. If one of these analyses proves its utility on dEMG data in this exploratory research, it will be applied to answer the last-mentioned sub-question of the main research as well.

Caffeine Effect

The first study was proposed to determine the effect of administration of caffeine on diaphragmatic activity. In the caffeine study of Kraaijenga et al. ([65]) dEMG was measured 30 minutes prior (baseline) to three hours after administration of an intravenous caffeine dose in 30 spontaneously breathing preterm infants (mean GA: 29.1 ± 1.3 weeks), of which most received non-invasive respiratory support. At fixed time points after caffeine administration (15, 30, 60, 90, 120, 150 and 180 minutes), dEMG variables were calculated and compared with baseline.

Extubation Response

The second study was executed by van Leuteren et al. ([18]) and described the diaphragmatic activity before and after extubation in critically ill children. Children and infants who received mechanical ventilation for longer than 24 hours were included. Eventually, the diaphragmatic activity of 147 children (median age: 1.9 (0.9-6.7) weeks) was recorded from 15 minutes before till three hours after extubation. The diaphragmatic activity outcome variables were calculated at the same fixed time points as in the caffeine study, though here after extubation instead of caffeine administration. In addition, the outcome variables of failed and successfully extubated patients were compared.

Method

In this exploratory research on the use of variability analyses the outcomes of the studies were determined at fixed time points as well. The calculations were performed on five minutes of data around each fixed time point. Therefore, at each fixed time point of each dEMG recording, the most stable (least visible noise) five minutes of data were manually selected. Due to the calculation duration of some of these additional parameters, it was chosen not to take over all fixed time points as in the previously described studies. In this exploratory research the calculations were performed around 15 minutes before and repeated around 30, 60, 90 and 120 minutes after the intervention (in both the caffeine and extubation study dEMG data).

Hypothesis

It is known that caffeine is a drug that stimulates breathing by acting on the respiratory centre in the medulla. Therefore, caffeine is administered to reduce the frequency of apnoea's in these infants. Moreover, the mentioned study of Kraaijenga et al. also described a quick and persistent increase in the level of diaphragm activity after caffeine administration in preterm infants. Besides, there was found a peripheral relation, suggesting a direct effect of caffeine on the diaphragm. Therefore, the interest was aroused to perform the variability analyses on this data set.

Simply put, the caffeine stimulatory effect should enhance the regularity of the respiratory system, which in addition should reduce the complexity and increase the predictability of the dEMG data.

It was suggested that children who failed extubation would show similar results in variability outcome measures, as their respiratory system has to be compressed to basic activity in an emergency state in order to survive. Successfully extubated children were thought to have more variance in the dEMG data in comparison to those who failed and would therefore show more complex and less regular and predictable dEMG data.

In order to prove these hypotheses, subsets of data of both described studies are analysed. With this exploratory research, the effectiveness of these analyses on dEMG data will be assessed. If one of the described analyses shows workable results, it will be applied on the own recorded dEMG data of the main research as well.

6. Exploratory Research - Results

In this section the results of the exploratory research are shown. Most notable results are visualized in *Figures 11* and *12* for respectively caffeine effect and extubation response. An overview of all the results is located in the *Appendix*, *Table 4* and *5*, again for respectively caffeine effect and extubation response. The results are presented by the mean value and the standard error of the mean (SEM).

Caffeine Effect

The described variability analyses were applied to dEMG recordings of 18 infants. First, a baseline measurement (pre) was performed. Then the variability analyses were performed each half an hour after caffeine administration. Only the most relevant results are provided in this section by *Figure 11*. The remaining outcomes can be observed in the *Appendix*, *Table 4*.

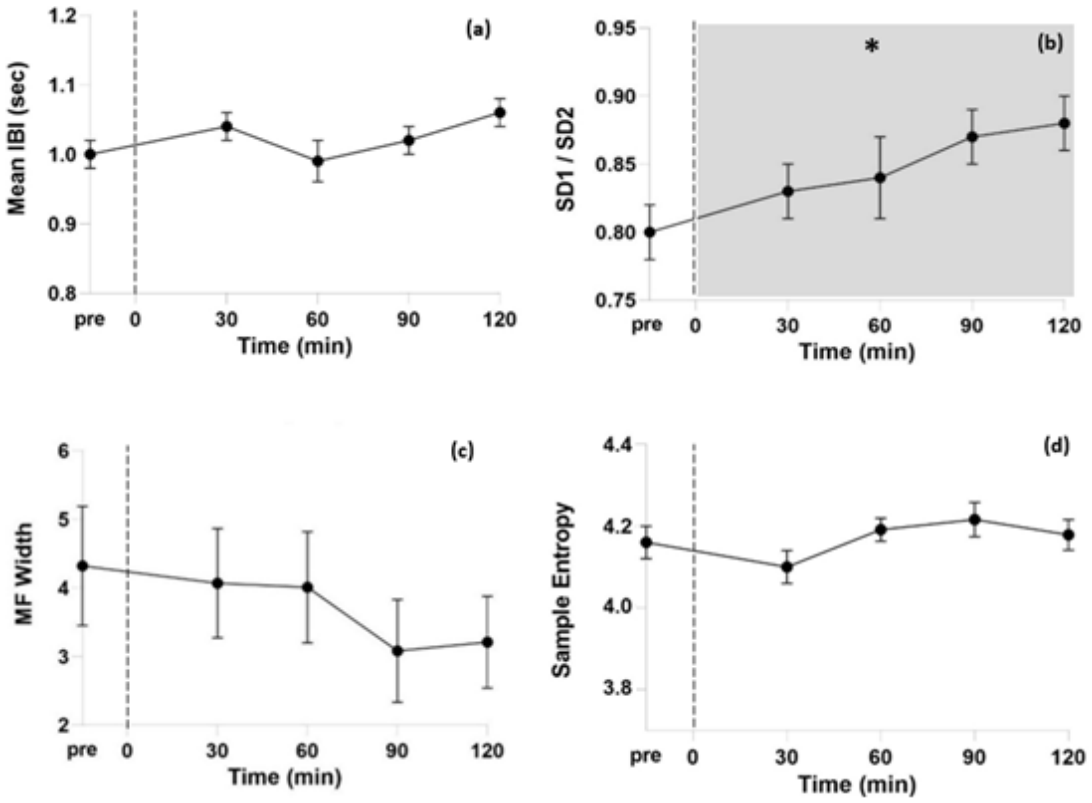

***Figure 11**: Changes over time describing the effect of caffeine on diaphragm activity based on electromyography on four nonlinear variability analysis variables. Results are presented as mean outcome (standard error of the mean (SEM)). (a): SD ratio (SD1/SD2). (b): Mean inter-breath interval (60/RR). (c): Width of the multifractal spectrum parabola. (d): Sample Entropy. *Significant overall mean difference between before and after caffeine administration (grey shaded area) as determined with a paired t-test, p<0.05.*

From the Poincaré analysis, the mean inter-breath intervals were extracted (*Figure 11.a*). By this parameter, we did not notice any remarkable effect of caffeine on the respiratory rate. However, at the $SD\ ratio$, an increase over time after caffeine administration can be observed (*Figure 11.b*).

The detrended fluctuation analyses showed α to remain quite consistent over time. Nonetheless, there arose a small decline in the width of the multifractal dimension, which can be seen in *Figure 11.c*.

None of the three proposed entropy analyses showed a noteworthy pre-post difference nor trend over time. For Sample entropy, this result can be observed in *Figure 11.d*.

Extubation Response

The described variability analyses were applied to dEMG recordings of 42 infants in total of which 10 failed extubation. First, a baseline measurement (pre) was performed. Then the variability analyses were performed each half an hour after the infant was extubated. Only the most relevant results are provided in this section by *Figure 12*. The remaining outcomes can be observed in *Table 5,* located in the *Appendix*.

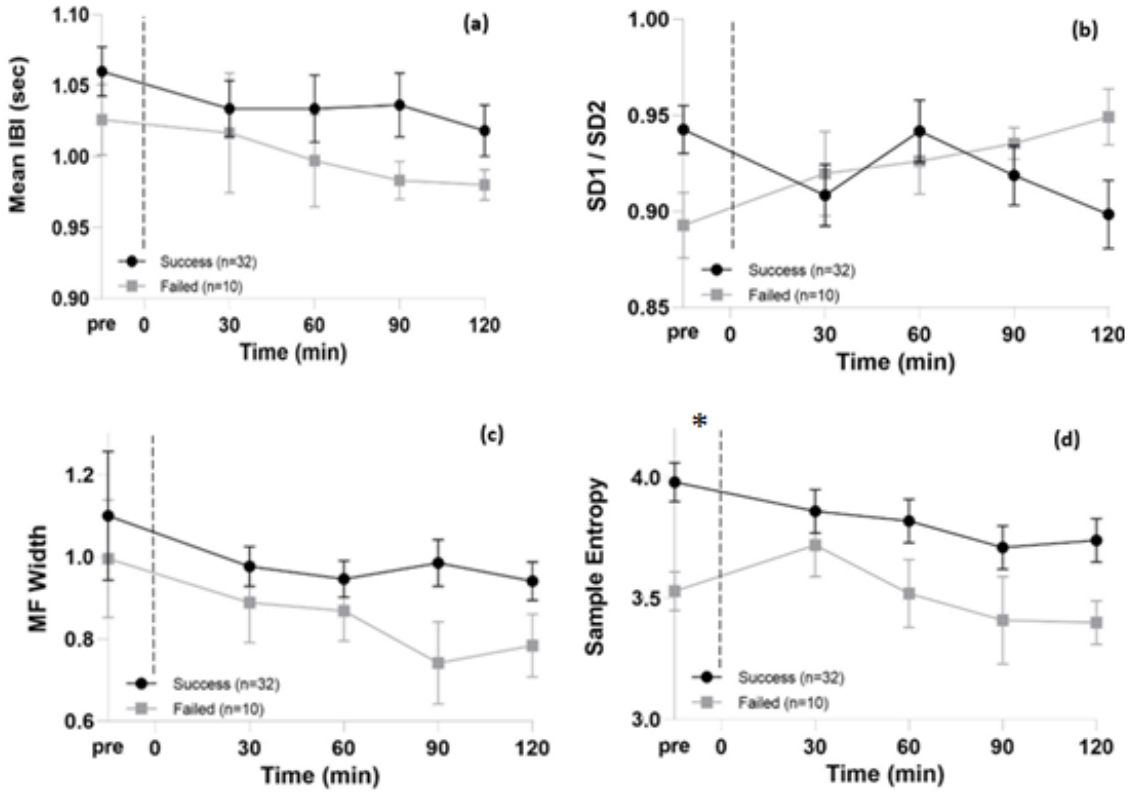

Figure 12*: Changes over time describing the response of extubation on diaphragm activity based on electromyography on four nonlinear variability analysis variables. The difference in outcomes between those who fail and succeed extubation is visualized. Results are presented as mean (SEM). (a): SD ratio (SD1/SD2). (b): Mean inter-breath interval (60/RR). (c): Width of the multifractal spectrum parabola. (d): Sample Entropy. *Significant difference between failed and success patients before extubation as determined with a Mann-Whitney U test, p<0.05.*

Although the respiratory rate in both groups increases after extubation, their mean IBI appears to differ. The IBI of the success group seems quite stable after extubation, while the failed group showed a constant decline in the IBI over time. This result was in line with our hypothesis and is shown in *Figure 12.a*. Remarkably, the SEM of the IBI results of the failed group decreases considerably after an hour. Similar to the caffeine study, both $SD1$ and $SD2$ did not result in large differences. However, the results of the ratio between these variability parameters are strikingly different for each group.

From the detrended fluctuation analyses, α seems to be quite similar for both groups before extubation, which indicates there is no difference in the fluctuation scaling exponent. However, there is a difference in the multifractal width between the two groups, see *Figure 12.c*.

Both the Shannon and Approximate entropy analyses do not seem useful to distinguish between patients who fail and succeed extubation, based on the utilized dEMG data (*Appendix, Table 5*). However, there is a difference between the failure and success groups in Sample entropy (*Figure 12.d*).

7. Exploratory Research - Discussion

As one can observe, only few results are statistically significant different ($p<0.05$). However, it was not meant to prove any statistically significance with this exploratory research, but to investigate which type of variability outcome measure could be most useful. These variability analyses on dEMG are relatively new in the use on dEMG, causing power calculations hard to perform. Moreover, all utilized data was previously acquired. Thus, no new data was collected in order to achieve a certain number of subjects to aim for statistical significance for the investigated outcome measures. Therefore, these statistical findings are only mentioned for interpretation and to support the hypotheses on the use of these signal analysis techniques.

Caffeine Effect

In this section, the outcomes of the effect of caffeine on the dEMG variability analyses are discussed.

Poincaré

In the original publication of the study about the effect of caffeine on dEMG data [64], is was concluded that the respiratory rate was not considerably affected by this medication. In our research, we extracted the mean inter-breath intervals from the Poincaré analysis (*Figure 11.a*) and came to the same conclusion.

Although both $SD1$ and $SD2$ themselves do not develop an impressing trend over time (*Table 1* in the *Appendix*) both these short- and long-term variability parameters seem to decline a little after caffeine administration. This implies that caffeine decreases the dispersion of the data points of the Poincaré plot and therefore causes more regularity in the inter-breath intervals. As caffeine stimulates breathing by acting on the respiratory centre in the medulla, this finding is no surprise to us, but seems to be in line with the intentional clinical effect of this drug.

Moreover, this latter finding is even more clear in the results of the ratio between these Poincaré variability parameters. As these variabilities resemble each other increasingly after caffeine is administered, the ratio increases. Nonetheless, this finding can have two logical causes or a combination of both. Since the short-term variability declines, eventually the long-term variability must become smaller as well. However, the fact that $SD2$ declines more than $SD1$ causes this not to be very plausible. A more likely and clinically relevant cause is the variability limitation of biological systems. It may be that caffeine administration will result in a trend towards a biological minimum of variability. When both variabilities cannot decrease any further, the ratio automatically tend to a value of 1.

Detrended Fluctuation Analysis

The decline in multifractal dimension suggests that caffeine may reduce the fractal dimension of diaphragmatic electrical activity. Therefore, the collected data appears to be less complex after caffeine administration. This result can be explained by the stimulating effect of caffeine on the breathing regulation as well. As the respiration becomes more regular, which was also noticed in the Poincaré outcomes, the complexity automatically decreases.

Entropy

Although caffeine increases the spatial regularity (IBIs) and diaphragm activity ([64]), it apparently does not influence the regularity or pattern of the dEMG amplitudes since the entropy outcomes seem not to be affected after caffeine administration.

Extubation Response

In this section, the results of the extubation response on the dEMG variability analyses are discussed.

Poincaré

It was assumed that the individuals of the failure group would show a more increased respiratory effort MV was stopped in comparison to the success group as they were not ready for extubation yet. The flattened variation (SEM) in IBI outcomes of the failure group emphasizes a compression to necessary activity only and stresses their need for respiratory support.

While a fluctuation in $SD\ ratio$ can be observed at the successfully extubated population, the failed population shows a constant increase of this parameter over time (*Figure 12.b*). As the respiratory system for the failure group was suggested to be compressed to basic activity, this latter result was as expected. Moreover, this finding is in agreement with the results of the study in which this dataset was acquired ([18]).

Detrended Fluctuation Analysis

Successfully extubated children show more complex dEMG data in comparison to those who failed. It has been hypothesised that they are able to have more variance in diaphragm activity as they are not compressed to breathe in a survival state. This latter assumption can also be made after observation of the difference in outcomes between groups at the width of the multifractal spectrum.

Entropy

Both the Shannon and Approximate entropy analyses do not seem useful to distinguish between patients who fail and succeed extubation, based on the utilized dEMG data. However, there is a difference between groups in Sample entropy (*Figure 12.d*). This result suggests that for those who fail extubation, less information (input data) is needed to predict the data yet to come. Moreover, as the distinctness between these groups appears to be greatest at start, it might be interesting to investigate this variable as extubation readiness parameter in future studies. However, there were no noticeable changes over time in this parameter per group after extubation. Therefore, this parameter is suggested not to be beneficial for investigations that focus on a physiological development as in the main research of this thesis.

8. Exploratory Research - Conclusion

From these exploratory results, one can conclude that the Poincaré analysis outcomes seem to be most useful. Both the caffeine effect and extubation response on dEMG data were observable in the outcomes of this variability analysis. Moreover, these outcomes were in agreement with the results of the corresponding publications. Finally, as auxiliary pleasant bonus, the Poincaré analysis is far out the most simple and fast among the three proposed approaches.

DFA on dEMG did not show remarkable outcomes on both caffeine effect and extubation response. Similarly, Shannon and Approximate entropy analyses did not result in noteworthy outcomes either. However, Sample entropy seems to be interesting to investigate in future studies as possible extubation readiness parameter.

Although only the Poincaré analysis seems to be effective and is used in the next sections about the main research question, software has been coded into a graphical user interface (GUI) including all the previously described analyses. This allows researchers to easily apply these analyses in future explorative studies. A screenshot of this application including some explanation can be found in the *Appendix*, *Figure 19*.

9. Methods

To be able to answer the main research question, two scientific studies were proposed and are currently running. Both studies took again place at the NICU of the Emma Children's Hospital. The first study was set up to investigate the correlation between the optimal pressure setting at HFOV as determined with FOT and the optimal pressure as defined by clinicians. Therefore, this study should give answer to the first sub-question of the main research and will hereinafter referred to as '*FOT study*'. The second study served to further examine the correlation between the reactance of the respiratory system determined with FOT, the applied pressure settings, as well as dEMG and clinical parameters. This study is meant to give answer on the remaining sub-questions. Since this second study utilizes two non-invasive techniques, FOT and dEMG, of which it is hoped they can be like equal siblings to each other, this study is hereinafter referred to as '*Gemini study'*, like the constellation of twins.

FOT Study

This study has a single centre prospective observational design and is not subject to the Medical Research Involving Human Subjects Act ('Wet Medisch-wetenschappelijk Onderzoek met mensen' (WMO in Dutch)) since only FOT measurements are added to the standard care. Moreover, the FOT modality has been approved and is implemented in the neonatal ventilator. The study is ongoing, but the data described in this report has been collected between the start in August 2021 and May 2022. As the desired amount of fifteen participants had not been reached within this period, the study continues.

Study Population

Preterm- and term born infants admitted to the NICU who received HFOV-MV were eligible to participate in this study. Infants who received nitric oxide therapy were excluded from participation in this study. When palliative care was initiated, participation was also not allowed. Another criterium was that the infant should be ventilated by the Fabian HFOi neonatal ventilator (Vyaire Medical, Il, US), with an operational FOT modality. At last, study measurements were only executed when written parental (or legal guardian) consent for studies not subject to the WMO was confirmed. Based on previous FOT studies in intubated neonates, a convenience sample of fifteen participants was desired to be included.

Medical Equipment

The Fabian HFOi neonatal ventilator, as part of the standard care at the NICU, was used in this research. This ventilator provides both the pressure (CDP) and oxygen (FiO_2) to the patient, which can be observed on the ventilator. Furthermore, this ventilator allowed us to perform FOT measurements, providing X_{rs}. For these measurements, the ventilator uses a fixed FOT frequency of 10Hz.

Procedure

After intubation, respiratory support settings were determined by clinicians for the first time. As part of the standard procedure, the optimal pressure setting is titrated by clinicians (P_{CLIN}). Simultaneously, as part of this study, the optimal pressure setting will be determined by FOT measurements (P_{FOT}). Therefore, the respiratory system reactance is determined for each gradually increased and afterwards decreased pressure step as in usual respiratory support titration in infants receiving HFOV.

When the cycle of measurements is performed, the obtained reactance values are plotted against their corresponding CDP values, of which an example can be seen in *Figure 13*. In this way, the optimal pressure setting (P_{FOT}) is represented by the lowest pressure value corresponding to the highest reactance as indicated by the red circle in *Figure 13*.

As part of standard care, optimal pressure setting titration may be repeated by clinician during the MV period, therefore, one single period of MV can result in multiple *FOT study* measurements. Both P_{CLIN} and P_{FOT} as well as the difference between these pressures were noted at all these times.

Since the FOT measurements were new to clinical practice at the NICU of the Amsterdam UMC, the usefulness of the FOT modality and measurements was investigated as well. For this purpose, the endotracheal tube size, the eventual extra time needed for the FOT measurements, as well as the success rate of these measurements and any other important outcomes was noted.

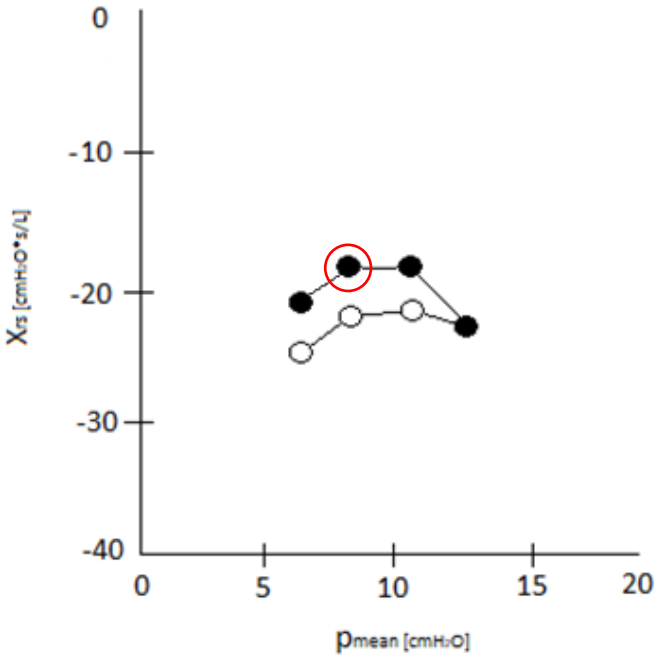

***Figure 13**: Theoretical example of FOT data as plotted by the Fabian ventilator. White circles represent measurements with increasing pressure whilst black circles represent those with gradually decreased pressure. The red circle indicates the optimal pressure setting as determined with FOT (P_{FOT}).*

Data Storage

The obtained P_{CLIN} and P_{FOT} and FOT measurement outcomes were noted. These data were processed into an Excel sheet (Microsoft, WA, US), saved and stored on a dedicated research computer.

Analysis

Data analysis was done utilizing Excel and analytical MATLAB software (MathWorks, Natick, MA, US). Moreover, for statistical purposes SPSS-26 (IBM, Armonk, NY, US) and GraphPad Prism version 9.3.1 (GraphPad Software, Inc., San Diego, CA, US) were utilized. The correlation between the obtained P_{CLIN} and P_{FOT} data was determined. In addition, the usefulness of the FOT measurements was examined.

Gemini Study

The *Gemini Study* was designed as a WMO obliged single centre prospective observational study. The study is ongoing and started in October 2021. The abstract of the study proposal to the Medical Ethics Review Committee (MERC; METC in Dutch) and both the subject information sheet and the informed consent form of this study can be found in the *Appendix*.

Study Population

Preterm- and term born infants admitted to the NICU who were mechanically ventilated >24 hours were eligible to participate in this study. Exclusion criteria were: 1. A GA <26 weeks, because of the frailty of the premature skin, making transcutaneous dEMG measurements undesirable. 2. Congenital malformations not compatible with dEMG measurements. 3. Nitric oxide therapy or therapeutical hypothermia. At last, when palliative care was initiated, participation for this study was also not allowed. The infant should again be ventilated by the Fabian HFOi neonatal ventilator (Vyaire Medical, Il, US), with an operational FOT modality in order to be included.

Study measurements were only performed after written parental (or legal guardian) informed consent was confirmed. Nonetheless, to increase the inclusion rate of this study, it was chosen to utilize two previously initiated studies which are not subject to the WMO. Due to the combination of both of these studies, we were able to perform all *Gemini Study* related measurements in a legitimate way as well when consent for studies not WMO obliged studies was confirmed.

Based on previous FOT studies in intubated neonates, a convenience sample of at least 30 participants was desired to be included.

Medical Equipment

During this study, the same medical equipment was used as in the *FOT study* to obtain the applied pressure and FiO_2 settings, as well as the reactance values.

Peripheral oxygen saturation (SpO_2) was measured and monitored as in standard care by a non-invasive flexible wrap sensor (Nonin Medical, Inc., MN, US/ Nellcor, Covidien, Mansfield, MA, US) around the foot of the patient and a was displayed by a Philips monitor. Concentration of carbon dioxide in peripheral blood was measured by transcutaneous sensors (SenTec Inc., Fenton, MO, US).

In order to perform a dEMG measurement, three transcutaneous Ag/AgCl hydrogel electrodes (Kendall H59P Cloth Electrodes, Covidien, Mansfield, MA, US) and the Porti-7 signal amplifier (TMSi, Oldenzaal, the Netherlands) were used. Electrodes were positioned as described earlier, of which a schematic representation can be observed in *Figure 6*.

Procedure

After (parental) consent was given, the first study measurements were performed as soon as possible. These first measurements include both FOT and dEMG. FOT measurements were performed on the current clinical applied CDP to 2cmH_2O above this pressure and back to the former clinical applied CDP in steps of 1cmH_2O. An example of these FOT measurements on increasing and decreasing pressures can be observed in *Figure 13*. These FOT measurements take place during the dEMG measurement, which is a recording diaphragm activity for one hour.

Each consecutive day of MV, the FOT measurements will be repeated to investigate the temporal behaviour of the reactance. On the last day of the intubation period, the last study measurements take place. These measurements are in fact a repetition of those performed at the start of the study. However, these last measurements preferably take place within six hours before extubation. An overview of all these research related interventions during an infants' period of MV can be observed in the *Appendix*, *Figure 20* at the end of this document. At all the aforementioned FOT and dEMG measurements the values of the clinical parameters, as well as the pressure level and day of intubation were noted.

Prior to the start, the medical staff was informed about this study and its protocol. Although it was thought not to induce any harm to the patients, the medical staff needed to be aware of the eventual cause of discomfort during study measurements. Logically, measurements were interrupted if any patient discomfort was noticed. Measurements were repeated if the cause of discomfort is abated and a stable situation was restored.

Data Storage

FiO_2, SpO_2 and $tcPCO_2$ as well as FOT measurement outcomes and pressure settings were noted. Next, these data were processed into an Excel sheet (Microsoft, WA, US), saved and stored on a dedicated research computer. Raw dEMG data were collected and saved utilizing the Porti-7 system (TMSi, Oldenzaal, the Netherlands) and stored on the same research computer.

Analysis

In order to derive a dEMG respiratory waveform, raw dEMG data was processed. Cardiac interference and motion artefacts were removed, the signal was rectified and lastly the RMS was calculated. Data analysis on dEMG was done utilizing Excel and analytical MATLAB software (MathWorks, Natick, MA, US). Moreover, for statistical purposes SPSS-26 (IBM, Armonk, NY, US) and GraphPad Prism version 9.3.1 (GraphPad Software, Inc., San Diego, CA, US) were utilized.

To describe diaphragm activity, the earlier described usual dEMG parameters (peak activity ($dEMG_{peak}$), tonic activity ($dEMG_{tonic}$) and the area under the curve ($dEMG_{AUC}$)) as well as Poincaré parameters were determined. Besides, the reactance values (X_{rs}) were obtained from the FOT measurements. These latter results were correlated to the usual and Poincaré dEMG parameters. In addition, X_{rs} values were correlated to the collected clinical parameters (SpO_2, FiO_2 and $tcPCO_2$).

At last, the changes over time during the mechanical ventilation period of all described parameters was calculated for each participant. The correlations between the changes over time in X_{rs} and all other described parameters was investigated.

10. Results

In this section the results of both the *FOT Study* and *Gemini Study* are shown. For the *FOT Study*, tables containing the subject characteristics and all the collected and computed results (respectively *Table 1* and *2*) are given. Besides, a figure about the most relevant result (*Figure 14*) is provided. For the *Gemini Study*, a table about the subject characteristics is given as well (*Table 3*). In addition, *Figures 15* to *17* are provided in order to answer the corresponding sub-questions. Complete results of this latter study are provided in the *Appendix, Table 6*.

Only a limited number of patients were suited to participate in both studies. Therefore, measurements were performed on nine and six participants only for respectively the *FOT Study* and *Gemini Study* during the period described in this report.

FOT Study

***Table 1**: Demographic characteristics of the FOT study participants (n=9). Data presented as median [interquartile range] and number (percentage).*

Gestational age at birth (weeks)	32.2 [26.7, 39.6]
Gestational age at inclusion (weeks)	32.7 [26.9, 40.3]
Birthweight (gram)	1730 [680, 2565]
<1000	5 (55.6%)
>2500	3 (33.3%)
Weight at inclusion (gram)	1737 [680, 2565]
Male sex	5 (55.6%)
Apgar at minute 1	7 [5, 8]
Apgar at minute 5	9 [8, 9]
Vaginal delivery	(55.6%)
Antenatal corticosteroids	3 (33.3%)
Days of mechanical ventilation	4.6 [4, 6]
Reason of intubation	
Necrotizing enterocolitis	1 (11.1%)
Surgery	2 (22.2%)
Meconium aspiration syndrome	1 (11.1%)
Lack of respiratory drive due to:	
Apnoea of prematurity	2 (22.2%)
Infection	1 (11.1%)
Hypercapnia due to:	
Infant respiratory distress syndrome	2 (22.2%)

Although *FOT Study* measurements were performed ten times (on nine different participants), only eight of them resulted in FOT parameter outcomes. At the two missing outcomes, no X_{rs} values were obtained due to unstable measurements. Therefore, there are only eight data points in *Figure 14*. However, in *Table 2*, all measurement attempts are shown.

Moreover, the endotracheal tube (ETT) size (in mm) and applied pressures are provided in this table as well. P_{Start} indicates the pressure at which the cycle of FOT measurements was started, whilst P_{Peak} and $P_{StepSize}$ indicate the highest applied pressure in this cycle and the pressure step size to reach for this peak value, respectively. P_{Diff} refers to the differences between optimal pressure as determined by FOT measurements (P_{FOT}) and by clinicians (P_{CLIN}).

***Table 2**: Results of the FOT Study. In total, measurements were performed on nine subjects, of which one was measured twice. At two measurements no P_{FOT} value was obtained, both shown in red.*

Subject	Measurement	ETT Size	P_{Start}	P_{Peak}	$P_{StepSize}$	P_{CLIN}	P_{FOT}	P_{Diff}
1	1	3.0	10	14	2	12	12	0
2	1	3.5	10	14	2	10	10	0
3	1	2.0	8	16	2	X	12	-
4	1	3.0	10	16	2	10	10	0
5	1	3.0	10	14	2	10	10	0
6	1	3.5	8	14	2	10	10	0
7	1	2.5	8	12	1	11	10	1
7	2	2.5	18	23	1	X	20	-
8	1	3.5	10	19	3	16	13	3
9	1	3.0	8	12	2	10	10	0

As one can observe in *Table 2*, six out of eight successful FOT measurements resulted in a similar optimal pressure as determined by clinicians. Five of these six similar outcomes were all on a pressure of 10cmH_2O and therefore result in identical data point in *Figure 14*. Because of this reason, the latter data point has been increased in size.

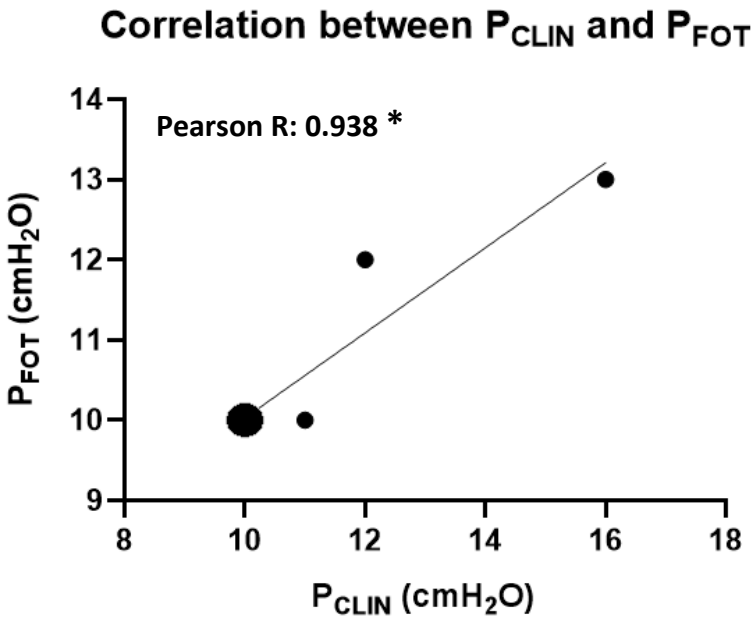

***Figure 14**: Pearson correlation analysis of 8 pairs of the optimal pressure setting as determined by the clinicians (P_{CLIN}) and the optimal pressure setting as determined with FOT (P_{FOT}). The larger data point covers the results of five identical pairs. *The two-tailed p-value of this correlation is <0.001.*

Gemini Study

For the *Gemini Study,* two participants were included. The study screening and enrolment flow chart can be observed in *Figure 15*. The subject characteristics of these participants are shown in *Table 3*.

Due to another previously initiated study at the NICU, which was not subject to the WMO, it was possible to increase the inclusion rate of the Gemini study. At this study, the already utilized ECG electrodes for standard monitoring at the NICU are relocated for the dEMG placement. This approach enables one to detect both the electrical activity of the hearth and the diaphragm. As the electrical activity of the hearth is dominant, the heart rate can still adequately be monitored. Moreover, the diaphragmatic electrical activity detected by this approach does not differ from the initial WMO approach if appropriate data modification is being applied. This allows one to combine data collected at both approaches. The described dEMG-ECG study is being conducted by a technical medicine PhD candidate and the corresponding manuscript is in preparation at the moment.

In addition, due to the *FOT Study*, the performance of FOT measurements was already possible without specific written parental consent for this study. When general consent for studies not subject to the WMO was received, we were legally allowed to perform all *Gemini study* related measurements. So, by this 'Non-WMO' approach, no extra written parental consent was required. This approach allowed us to collect and analyse data of four more participants, resulting in six participants in total. However, the desired number of participants for this study has still not been reached, so the measurements performed up to May 2022 are covered in this report.

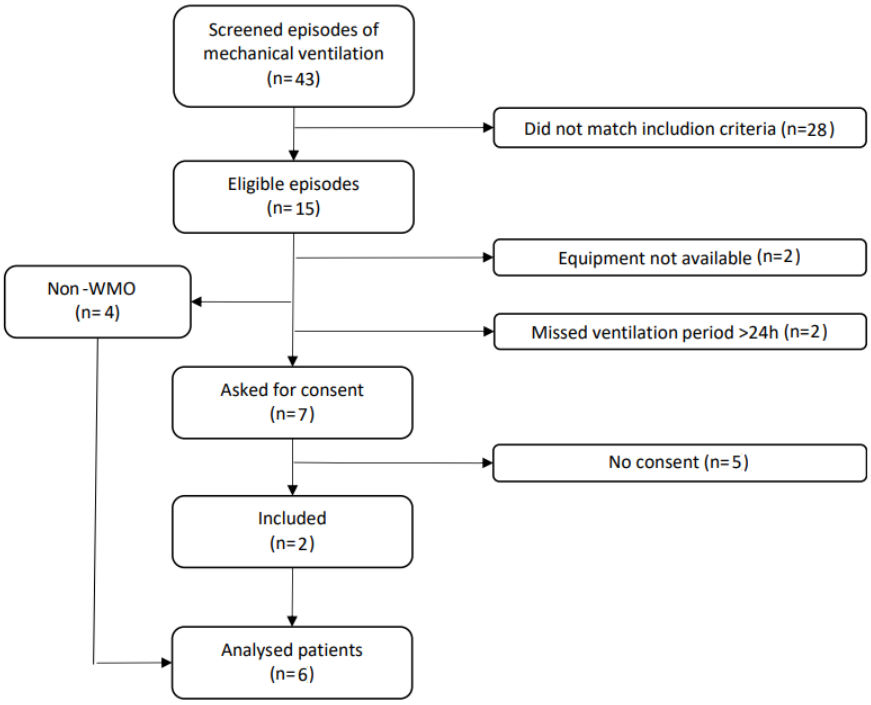

***Figure 15**: The screening and enrolment flow chart of the Gemini Study.*

Even though all NICU patients who received mechanical ventilation were eligible, most of the screened episodes of mechanical ventilation did not result into a study inclusion since the majority did not match all the inclusion criteria. Of those who did not meet the inclusion criteria (28), six received mechanical ventilation for a relatively short period (<24hours). Nonetheless, the majority were deemed in too poor condition to participate. The latter is also confirmed by the number of patients who received MV during this study (43), but eventually died (9), resulting in ±20%.

The technical equipment was insufficient for two possible inclusions. At one of these possible inclusions, there was no operational FOT modality in the used neonatal ventilator. At the other possible inclusion, no flow sensor was connected in the ventilator circuit, disabling FOT measurements. Although we kept a close watch to find eligible participants, two ventilation periods were missed. Moreover, five of the seven approached (pair of) parents did not give consent. Therefore, the number of patients included via the WMO approach was two. However, due to the Non-WMO approach, additional data of four patients was collected. Therefore, the total amount of analysed patients resulted to be six.

***Table 3**: Demographic characteristics of the Gemini inclusions (n=6). Data presented as median [interquartile range] and number (percentage).*

Gestational age at birth (weeks)	39.8 [33.3, 40.3]
Gestational age at inclusion (weeks)	40.4 [34.5, 41.3]
Birthweight (gram)	2702 [2201, 2945]
<1000	1 (17%)
>2500	4 (67%)
Weight at inclusion (gram)	2728 [2201, 2973]
Male sex	2 (33%)
Apgar at minute 1	8 [5, 9]
Apgar at minute 5	9 [9, 10]
Vaginal delivery	4 (67%)
Antenatal corticosteroids	2 (33%)
Days of mechanical ventilation	4 [3.25, 4.75]
Reason of intubation	
Necrotizing enterocolitis	2 (33%)
Surgery	2 (33%)
Meconium aspiration syndrome	1 (17%)
Lack of respiratory drive due to:	
Apnoea of prematurity	1 (17%)

Each reactance outcome was linked to the corresponding dEMG and clinical parameters determined at the time of the FOT reactance determination. In the following figures the Pearson correlation of all data points (both the pairs of linked outcomes on the first suitable moment after intubation and on the last suitable moment before extubation) is visualized. Outcomes of the first suitable moment after intubation are visualised by black circles, whilst white circles correspond to the results obtained on the last suitable moment before extubation. Only the most relevant results are provided in this section by *Figures 16* and *17*, the remaining outcomes can be observed in the *Appendix*, *Figures 21* to *23*.

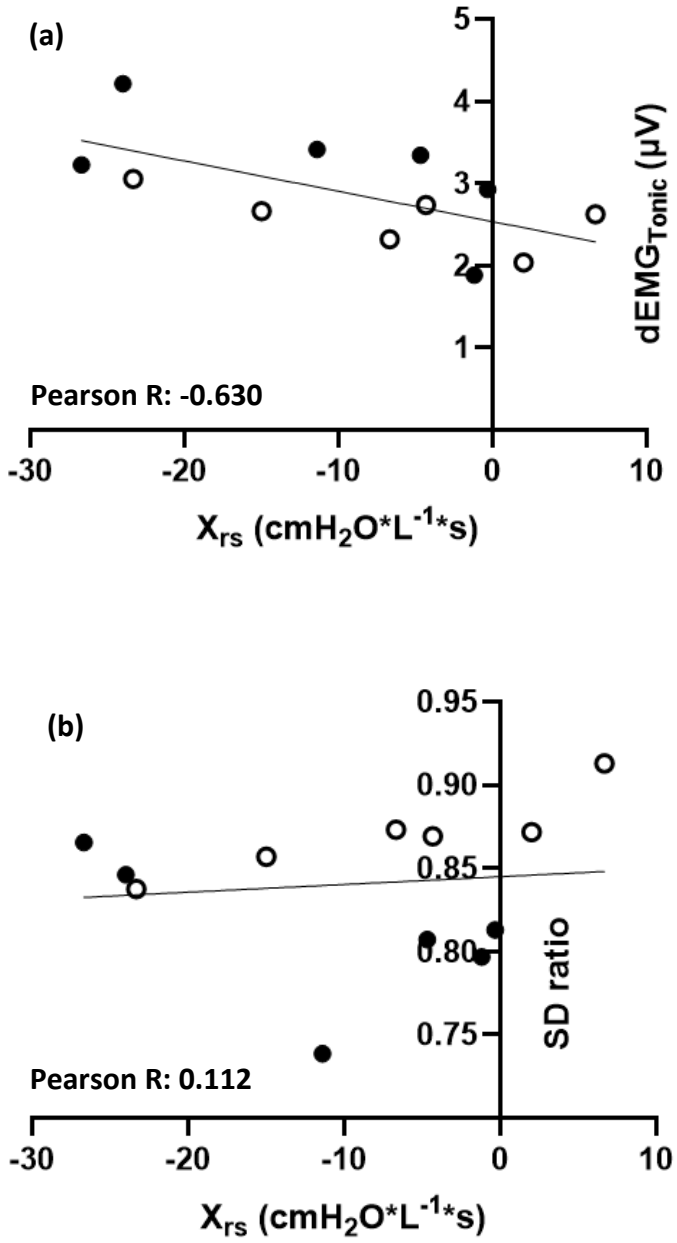

Figure 16: *Pearson correlation analyses of 12 pairs (6x2) of the tonic activity and SD ratio obtained from the dEMG measurements and the reactance as determined with FOT measurements (X_{rs}) during the dEMG recording. Black circles correspond to data collected on the first suitable moment after intubation, whilst white circles correspond to the data collected on the last suitable moment before extubation.* **(a)**: X_{rs} and *the mean tonic dEMG value, p = 0.028.* **(b)**: X_{rs} and *the Poincaré SD ratio outcome, p = 0.729.*

In *Figure 16.a* and *b*, respectively the correlations between X_{rs} and $dEMG_{Tonic}$ and X_{rs} and the $SD\ ratio$ can be observed. The correlations between X_{rs} and the $SD\ ratio$ is not significant, however, there is a remarkable difference between the outcomes of the first suitable moment after intubation (black circles) and those corresponding to the last suitable moment before extubation (white circles). The correlations between X_{rs} and the remaining dEMG parameters were assessed to be less relevant and are therefore visualized in the *Appendix, Figures 21* and *22.*

In the next figure, the correlations between X_{rs} and $tcPCO_2$ can be observed. The correlations between X_{rs} and the remaining clinical parameters were again assessed to be less relevant and are therefore visualized in the *Appendix* (*Figures 23.a* and *b*) as well.

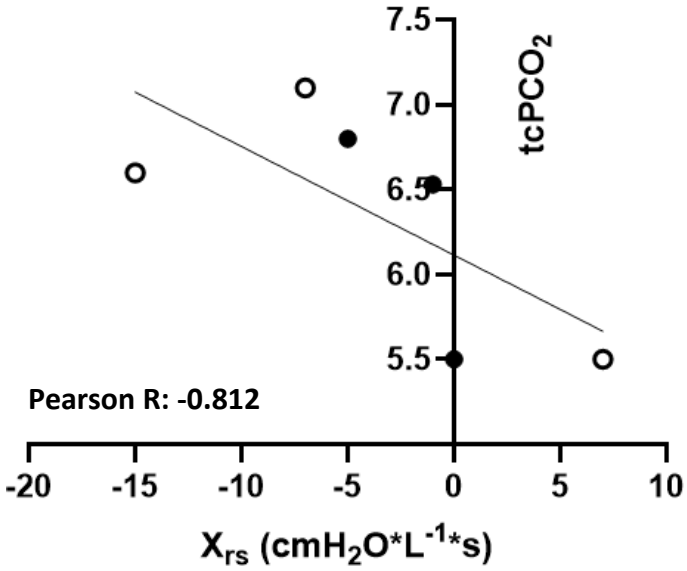

Figure 17: *Pearson correlation analysis of 6 pairs (3x2) of the collected $tcPCO_2$ and the reactance as determined with FOT measurements (X_{rs}) during the dEMG recording. Black circles correspond to data collected on the first suitable moment after intubation, whilst white circles correspond to the data collected on the last suitable moment before extubation. X_{rs} and $tcPCO_2$, p = 0.072.*

The change over time of each parameter was calculated for each participant as well. With this latter approach only one data point per patient was obtained, thus six in total. This latter approach did not result in any significant outcomes. The relation with the most strength in this approach was found between X_{rs} and the IBI with a Pearson R of -0.661 and corresponding p = 0.152. Nonetheless, the coefficient of this correlation is not in agreement with our hypothesis. The figures corresponding to the correlations between the changes over time in X_{rs} and all mentioned parameters can be observed in the *Appendix, Figures 24.a* to *c.*

11. Discussion

Both the *FOT Study* and *Gemini Study* provided interesting results, which will be further discussed in this section.

FOT Study

Overall, P_{FOT} and P_{CLIN} seem to result in similar values for the optimal pressure and therefore seem correlated, based on the available data. However, at two of the measurements (25%) an optimal pressure outcome difference was noticed. The optimal pressure as determined by FOT was lower than the optimal pressure as determined by clinicals at both cases.

Comparison

Zannin et al. concluded that X_{rs} has the potential as a bedside tool to optimize the applied airway pressure during HFOV [12]. However, the measurements in this research were only performed on lambs. We investigated reactance measurements as a bedside tool in clinical practice at the NICU and came to the same conclusion. Similar to our findings, Wallström et al. showed that FOT measurements resulted in lower optimal pressure settings. They found that the optimal PEEP during MV of extremely preterm infants, as evaluated by FOT in the first week of life, was lower than the clinically set PEEP [14]. Nonetheless, in our research, we only performed FOT measurements during lung volume recruitment manoeuvres in patients receiving HFOV. Moreover, we did not made distinctions in gestational nor chronological age. So, we included all infants admitted to the NICU receiving HFOV, independent of their GA nor the time elapsed after their birth.

Besides the optimal pressure investigation, we examined the usefulness of the FOT measurements in clinical practice in our research as well. Apart from the two cases in which the measurements were unsuccessful. The remaining measurements did not require any additional time and were successful at first attempt (*Table 1*). We found that the unsuccessful outcomes seem to be linked to the size of the ET. This finding is in line with the results of a previously conducted study at the NICU of the Emma Children's Hospital. They found deviated X_{rs} outcomes for small tube sizes in a bench study as well [66].

Strengths and Weaknesses

Although the study is ongoing since August 2021, all measurements were performed within half a year. Therefore, the same protocols, clinicians, researchers and medical equipment were involved at all measurements.

Although there was a small team of clinicians and researchers to perform this study, all measurements were performed and analysed by the same person. Therefore, no interpretive nor inter-observer bias should be suspected. Moreover, both the measurements and analyses were fulfilled in a standardised and structured way.

Both the inclusion and exclusion criteria for this research were composed beforehand to aim for a homogenous study population that suffer lung conditions. However, intubated patients with various underlying pathologies were suited for study participation. As these patients showed to have comparable outcomes, we can infer that our approach is applicable for patients that suffer other underlying pathologies that require intubation (and are not listed as exclusion criterium) as well.

Unfortunately, we were not able reach the desired number of participants for this study in the available time frame. As clinical care develops, we better understand how to treat certain patients. This also applies for new-born infants at the NICU. Therefore, the percentage of new-born infants who need MV is decreasing over the years. To be precise, since the start of these studies 322 patients have been admitted to the NICU of the Emma Children's Hospital, of which 43 received MV, resulting in ±13%. About 10 years ago, twice as much NICU admissions led to endotracheal intubations [67]. In addition, this 13% is also mechanically ventilated for a shorter period than a decade ago [68]. This decreases the pool suitable participants (e.g. MV longer than 24 hours) for these kind of studies, which underlines the difficulty of including study participants.

In addition, it was hard to time the exact moment of the measurements of this study. To cover all the time and have a researcher available at the moments of respiratory support titration in infants receiving HFOV, which were chosen by clinicians, to perform the (FOT measurements) turned out to be hard. Because of these reasons, we missed many possible *FOT Study* measurements. However, we were able to perform measurements on nine participants, resulting in noteworthy outcomes.

Interpretation

The optimal pressures as determined by FOT measurements seem to be quite correlated with the optimal pressures as assessed by clinicians. Therefore, one could argue that additional FOT measurements for optimal pressure titration at the NICU are superfluous since clinicians can do the same job. However, the measurements in which a difference in optimal pressure was found are actually the most interesting. The optimal pressure as determined by FOT in these patients was lower than the optimal pressure as determined by clinicals. As higher pressures are associated with VILI, one should apply the lowest possible pressure suited for the patient. From the in-between results of this study, FOT measurement might be able to result in lower pressures (CDP in this case), supported by objective respiratory mechanics measurements. Therefore, one can infer that additional reactance measurements are of added value for respiratory support titration. However, more research is needed to confirm this result.

As one can see in *Table 1*, two of the performed measurements did not result in a P_{FOT} value. At both times this was caused by unstable FOT measurements. Remarkably, at both times a relatively small tube size was involved as well. The relation between unstable measurements and small tube sizes can be explained as following. Due to the small diameter, a significant part of the opening of the tube can get overlaid with tissue or clogged with mucus quite quickly. As a result, the reflected oscillations can get hindered to find their way back to the ventilator in order to describe the mechanical properties. Besides, as one might know from fluid dynamics, the radius of a tube is an important factor in pressure drop, which can be seen in *Equation 25*.

Equation 25: $\Delta p = \frac{8\mu L Q}{\pi R^4}$

In this standard fluid-kinetics equation (for fully developed laminar flow in a straight circular tube, which roughly is the case in this application), Δp is the pressure difference, μ the dynamic viscosity, L the length of the tube, Q the flow rate and R the radius of the tube. Due to the power of four, a small radius tube leads to a notably different pressure in comparison to larger tube sizes, which may affect the computation of X_{rs} by the pressure sensor.

Another plausible reason for the unstable FOT measurements is the amount of air leakage. Air flow always seeks the path of least resistance. In case of a leak, less air flow will return to the flow sensor, which may affect the computation of X_{rs}. However, no leakages were noted during the performance of the FOT measurements.

To add to this lack, FOT measurements should especially be operable for small tube sizes. The smallest patients (usually have a low GA as well as a low birth weight) are most prone to respiratory failure, pointing out their need for MV and eventual advantage of repetitive respiratory system reactance determinations. Given the future in which probably even smaller patients will be admitted to the NICU (now GA>24 weeks, in future >23 or maybe even >22 weeks), this is an important point of discussion for improvement of FOT measurements in its used form.

Future Recommendation

First, it is important to find out the exact cause of the unstable FOT measurements and whether it can be prevented or corrected. Perhaps suctioning and aligning the patient (in direction of the tube) prior to the measurements may be helpful. This should be investigated at the upcoming participants of this study, or in a future study about reactance determinations utilizing FOT measurements.

In this observational study P_{FOT} is only measured, i.e. the computed P_{FOT} is not applied. In future, if the final outcomes of this study result in a similar correlation, a randomized control trial may be proposed in order to evaluate the difference in clinical outcomes between a population where support is titrated with respect to P_{FOT}, compared to the standard clinical care.

Gemini Study

As far one can conclude from the in-between analyses on the results of six participants, X_{rs} occurred to be significantly correlated with the tonic diaphragmatic activity utilizing this small amount of data. The peak diaphragmatic activity also seems to show a correlation with X_{rs}, but too little information has been collected so far to confirm. In addition, we found X_{rs} and $tcPCO_2$ outcomes to be correlated.

With respect to the results of the changes over time during the intubation period, there only seems to be a trend towards a significant correlation between X_{rs} and the mean IBI. However, the corresponding Pearson R-coefficient does not match our expectations.

Comparison, Strengths and Weaknesses

To our knowledge we are the first to investigate the correlation between respiratory system reactance and both diaphragmatic electromyography parameters and clinical parameters. In addition, we are also the first to investigate the added value of several variability analyses on dEMG data. These aspects underline the novelty of this research. However, this complicates one to compare our outcomes with published papers about this topic, as there are none available. Nonetheless, comparison with available literature about both subjects (FOT and dEMG) separately was possible. Similar outcome values of respiratory system reactance in neonates was found by both Zannin et al. [69] and Lavizzari et al. [70]. Studies of Leuteren et al. [71] and Williams et al. [72] resulted in comparable dEMG parameter outcomes for patients admitted to the NICU.

Roughly the same strengths as described in the discussion of the *FOT Study* apply to this study. However, all measurements took place in an even shorter period. Although the study is ongoing since October 2021, all measurements were performed within three months. Therefore, again the same protocols, clinicians, researchers and medical equipment were involved at all measurements.

Although the same small team of clinicians and researchers were involved at the initiation of this study, all measurements were performed and analysed by the same person. Therefore, again no interpretive nor inter-observer bias should be suspected. Furthermore, the measurements and analyses were again fulfilled in a beforehand set up standardised and structured way.

Both the inclusion and exclusion criteria for this research were also composed to aim for a homogenous study population that suffer lung conditions. However, again intubated patients with various underlying pathologies were suited for study participation. As these patients showed to have comparable outcomes, we can infer for this study again that our approach is applicable for patients that suffer other underlying pathologies that require intubation (and are not listed as exclusion criterium) as well.

In addition to the limited number of suitable participants for this kind of studies as described in the discussion of the *FOT Study*, this study initially started as subject to the WMO. Therefore, we had to ask for parental consent before the measurement can be performed, which was difficult to time. The periods of MV are as short as possible nowadays (to prevent any VILI). In order to perform measurements on two consecutive intubation days, action must be taken quickly after being informed of an intubation. On the other hand, one does not want to approach parents too soon. The closer to the moment of their child's intubation, the higher the emotional burden for parents and the less they are willing to think about study participation. Moreover, the parents should be given sufficient time to consider participation, which makes the time window in which the study can be performed even shorter.

Due to this difficulty, two potential participants were missed. For both these patients, on the same day we wanted to approach parents for consent, we were informed that the ETT would already be removed. This made these patients ineligible for participation as multiple days of measurements was no longer possible.

Furthermore, five of the seven eventually approached parents (±70%) did not give consent (*Figure 15*). Additional research interventions (even if it is non-invasive) is soon experienced as 'too much', which is understandable in this acute phase. Due to these reasons, only two patients were officially included in the *Gemini Study*. However, due to the Non-WMO approach, additional data of four patients was collected, resulting in a moderate amount of data to analyse.

Interpretation

Of the correlations among X_{rs} and dEMG parameters, $dEMG_{Tonic}$ showed to have the most strength (greatest $R^{(2)}$) and significance. This result can be explained by the relation between the FRC and tonic (end-expiratory) diaphragm activity. As preterm infants may lack a sufficient amount of surfactant, they are prone to develop atelectasis. In order to prevent alveoli closing, preterm infants may actively maintain adequate FRC by preserving inspiratory muscle (diaphragm) activation throughout expiration (tonic activity).
When the respiratory system improves, this tonic activity is expected to decrease. Moreover, higher values of X_{rs} (less negative) are associated with better lung conditions. Therefore, this relationship is as hypothesised.

As shown in the exploratory research, the $SD\ ratio$ is generally higher for better lung conditions (after caffeine effect and associated with extubation readiness). Therefore, less negative values of the reactance were suggested to correlate to relatively high $SD\ ratio$ outcomes as well. In *Figure 16.b*, a positive coefficient of this relation can be observed, but this result is far from significant.
A couple of deviated data points are present as well. Remarkably, all these deviating data points were collected on the first suitable moment after intubation. However, the white circles (last suitable moment before extubation) are in line with each other. The combination of both black and white circles seems to give a distorted picture. Apparently, there is a wide range of $SD\ ratios$ on the first suitable moment after intubation, which affects the overall correlation. This range can be explained by the fact that the duration before this first suitable moment after intubation differs considerably per patient, from six hours to three days. Within this period, the lung condition and physiology of the intubated infant can change considerably. This change might influence both the respiratory system reactance and the activity of the diaphragm and could therefore affect both X_{rs} and dEMG parameters. The correlation between X_{rs} and the $SD\ ratio$ outcomes of only the measurements performed on the last suitable moment after extubation has been computed. This resulted in a Pearson R coefficient of 0.627 with a significance of p = 0.037, which can be observed in *Figure 22.b* in the *Appendix*.

The least significant correlation among X_{rs} and clinical parameters was found at the applied fraction of inspired oxygen (FiO_2). This result can be explained by the fact that some patients do not need an extra amount of inspired oxygen during the period of MV, which skews the FiO_2 data to the left, thereby influencing the correlation as the outcome values may vary considerably. Therefore, no correlation was expected as well. If one would only plot the FiO_2 outcomes above 21% (average oxygen concentration in room air, thus the lower limit), a positive Pearson R-coefficient would appear, which is not in line with the expectations. Much more data is needed to investigate whether or not there is a link between reactance and FiO_2.

Simply thought, larger reactance increases over time during intubation should be related to SpO2 increases. However, this does not seem to be the case for the data collected so far. The saturation is generally maintained between 92 to 95%. For this reason, there is only limited variation over time in this parameter. In addition, the saturation of individuals of this study population may fluctuate each minute. The measured outcome is therefore more of a snapshot, which can fairly affect the correlation and emphasises the need to collect more data before any conclusions can be drawn.

Future Recommendation

Since all study related measurements can legitimately be performed without the need of parental consent, like in the Non-WMO approach, we should ask the METC to reconsider their decision to treat the *Gemini Study* as subject to the WMO. As the Non-WMO approach led to twice as many measurements (four in comparison to two), even in a shorter time (three months in comparison to eight months), it would be much more time efficient to continue the *Gemini Study* as a study which is not subject to the WMO.

Besides the increased inclusion rate, there would even be less risks for the subjects as less electrodes are used. First, the (extreme) premature skin can be frail, making them susceptible to adhesive-related skin injuries. Second, less electrodes means less wires to roam around the neonatal incubator, which may hinder the (clinical) work convenience. An amendment should be proposed to the METC in order to transform this study into a non-MWO study. This amendment should focus on the increased inclusion rate, the decreased intrusiveness to parents (i.e. increased rest), the reduced consumption of medical equipment (electrodes) and the decreased risks to the subjects, which often are in a critical condition.

At last, it is recommended to investigate the applied pressures in the obtained correlations as well. Therefore, if a sufficient amount of data is collected, three dimensional figures should be made with the applied pressure during the measurements on the z-axis. In this way, more detailed information is visualized, which may reveal more relevant information about the interaction between lung mechanics and respiratory muscle activity in developing infants.

12. Conclusion

Our study showed that FOT measurements may be beneficial for the titration of respiratory support pressure settings. The optimal pressure settings as determined by this medical technology seem to be lower than these pressure settings as determined by clinicians in the results of the *FOT Study*. However, the small amount of data prevents us to draw firm conclusions. When the desired amount of 15 patients has been reached, the performed analyses should be repeated in order to confirm the correlation. Moreover, additional reactance determinations so far did not extend the duration of the lung volume recruitment manoeuvres. Nonetheless, the relation between small ETT sizes and unstable FOT measurements should further be investigated.

The combination of both FOT and dEMG provides information about the condition of different aspects of the respiratory system. Some of the analysed parameters showed moderate correlation in the results of the *Gemini Study*. Unfortunately, little is confirmed by the limited amount of data collected so far. The study needs to continue to ascertain the results as described in this thesis. To increase the inclusion rate for the *Gemini Study*, it is suggested to propose an amendment to the METC in order to make this study not subject to the WMO. However, it is still believed that the respiratory system reactance as measured with FOT will show correlation with several diaphragmatic activity parameters when a sufficient amount of data is collected.

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15. Appendix

In this section the additional tables and figures as mentioned in this report are provided. Besides, the abstract of the METC proposal as well as the study information sheet and informed consent form of the *Gemini Study* are provided.

Additional Tables and Figures

***Table 4**: Results (mean (SEM)) (n=18) of the applied variability analyses on dEMG data before (Pre) and at fixed time points after caffeine administration (30, 60, 90 and 120 minutes).*

	Poincaré				
	Pre	**+ 30min**	**+ 60min**	**+ 90min**	**+ 120min**
SD1	0.31 (0.02)	0.29 (0.02)	0.26 (0.02)	0.29 (0.02)	0.27 (0.02)
SD2	0.38 (0.02)	0.35 (0.03)	0.30 (0.02)	0.33 (0.02)	0.30 (0.02)
SD ratio	0.80 (0.02)	0.83 (0.02)	0.86 (0.03)	0.88 (0.02)	0.89 (0.02)
Mean IBI	1.00 (0.02)	1.04 (0.02)	0.99 (0.03)	1.02 (0.02)	1.07 (0.02)
	DFA				
Alpha	1.56 (0.02)	1.55 (0.02)	1.54 (0.03)	1.55 (0.02)	1.54 (0.02)
MF width	4.32 (0.87)	4.07 (0.80)	4.01 (0.81)	3.08 (0.75)	3.21 (0.67)
	Entropy				
Shannon	-1.35E+08 (1.58E+07)	-1.25E+08 (1.57E+07)	-1.40E+08 (2.08E+07)	-1.62E+08 (2.57E+07)	-1.90E+08 (3.92E+07)
Approximate	0.46 (0.02)	0.45 (0.02)	0.42 (0.02)	0.42 (0.03)	0.44 (0.02)
Sample	4.16 (0.04)	4.10 (0.04)	4.19 (0.03)	4.22 (0.04)	4.18 (0.04)

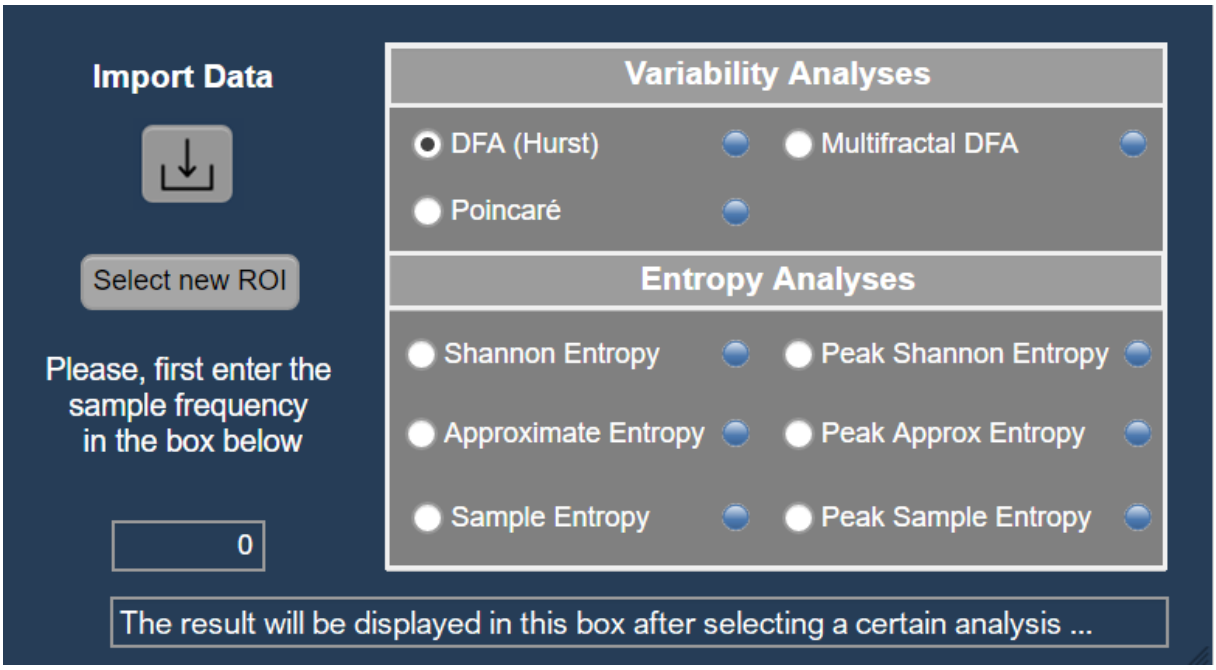

Figure 19: *The designed graphical user interface (GUI) including all the nonlinear variability analyses described in this report. With the 'Import Data' button, a file can be selected in order to be analysed. The sample frequency of the selected file can manually be set. Hereafter, the analysis of interest can be selected. When an analysis is performed, the obtained results are visualized into a plot and shown in the box at the bottom of the GUI. Moreover, after the performance of a certain analysis, a blue dot appears next to it to keep the user informed of the performance (useful if one have to perform the same analyses repeatedly on a bunch of data). If a new file or new region of interest (ROI) in selected, the dots disappear again.*

Table 5: *Results (mean (SEM)) of the applied nonlinear variability analyses on dEMG data before (Pre) and at fixed time points after the study subject was extubated (30, 60, 90 and 120 minutes). While the results of the study subjects who succeed extubation (n=32) are indicated by 'Success', the results of the study subjects who failed extubation (n=10) are indicated by 'Fail'.*

Poincare										
	Pre		+ 30min		+ 60min		+ 90min		+ 120min	
	Success	Fail	Success	Fail	Success	Fail	Success	Fail	Success	Fail
SD1	0.33 (0.01)	0.30 (0.02)	0.28 (0.01)	0.28 (0.02)	0.29 (0.02)	0.29 (0.02)	0.28 (0.01)	0.27 (0.01)	0.26 (0.02)	0.28 (0.02)
SD2	0.35 (0.02)	0.34 (0.02)	0.31 (0.01)	0.31 (0.03)	0.30 (0.01)	0.32 (0.02)	0.31 (0.02)	0.29 (0.02)	0.30 (0.02)	0.30 (0.02)
SD Ratio	0.94 (0.01)	0.89 (0.02)	0.91 (0.02)	0.92 (0.02)	0.94 (0.02)	0.93 (0.02)	0.92 (0.02)	0.94 (0.01)	0.90 (0.02)	0.95 (0.01)
Mean IBI	1.06 (0.02)	1.03 (0.02)	1.03 (0.02)	1.02 (0.04)	1.03 (0.02)	1.00 (0.03)	1.04 (0.02)	0.98 (0.01)	1.02 (0.02)	0.98 (0.01)
DFA										
Alpha	1.31 (0.03)	1.29 (0.04)	1.26 (0.02)	1.29 (0.04)	1.26 (0.03)	1.27 (0.03)	1.27 (0.03)	1.24 (0.03)	1.28 (0.02)	1.21 (0.02)
MF width	1.10 (0.16)	1.00 (0.14)	0.98 (0.05)	0.89 (0.10)	0.95 (0.04)	0.87 (0.07)	0.98 (0.06)	0.74 (0.10)	0.94 (0.05)	0.78 (0.07)
Entropy										
Shannon	-3.13E+04 (1.05E+04)	-4.37E+04 (2.03E+04)	-4.62E+04 (1.51E+04)	-3.57E+04 (2.30E+04)	-4.62E+04 (2.03E+04)	-3.30E+04 (2.10E+04)	-2.13E+04 (8.20E+03)	-3.43E+04 (2.13E+04)	-4.34E+08 (2.33E+04)	-4.11E+04 (2.43E+04)
Approximate	0.94 (0.04)	0.89 (0.08)	1.00 (0.03)	0.94 (0.06)	0.95 (0.04)	0.98 (0.05)	0.99 (0.03)	0.97 (0.05)	0.94 (0.04)	0.86 (0.07)
Sample	3.98 (0.08)	3.54 (0.08)	3.87 (0.09)	3.73 (0.13)	3.83 (0.09)	3.53 (0.14)	3.73 (0.09)	3.42 (0.18)	3.76 (0.09)	3.14 (0.09)

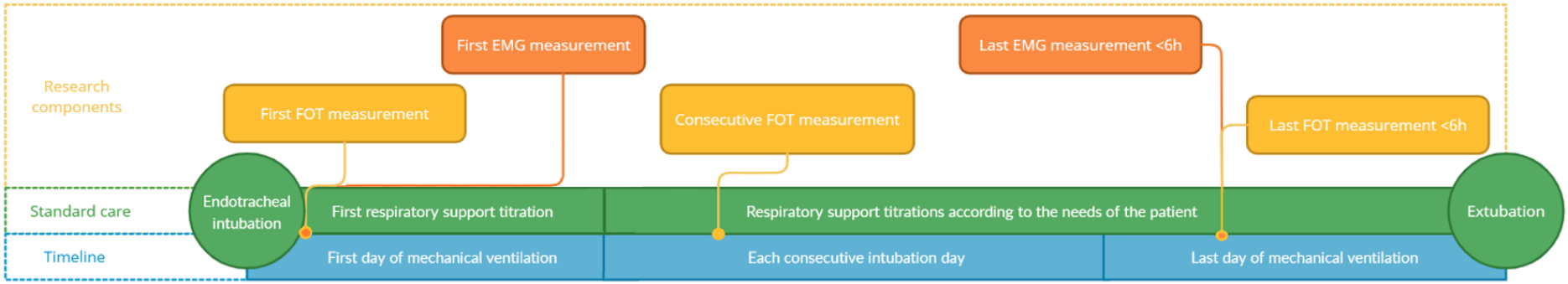

Figure 20: *An overview of the study procedure. It has to be noted that standard care will not be interrupted. The first FOT measurement takes place during the first dEMG measurement. These first measurements ideally take place within the first day of MV, however, we may only start after parental consent is given. The FOT measurements are repeated each consecutive day of MV. Within 6 hours before extubation, both last FOT and dEMG measurements are performed.*

Table 6: *An overview of the up to now collected and computed FOT, dEMG and clinical parameters of the Gemini Study.*

Measurements on the first suitable moment after intubation										
		Usual dEMG parameters			*Poincaré parameters*			*Clinical parameters*		
Subj.	**Xrs ($cmH_2O*L^{-1}*s$)**	**$dEMG_{Peak}$ (µV)**	**$dEMG_{Tonic}$ (µV)**	**$dEMG_{AUC}$**	**SD ratio**	**Mean IBI (s)**	**Pressure (cmH_2O)**	**$tcPCO_2$**	**FiO_2 (%)**	**SpO_2 (%)**
1	-11,4	6,943	3,417	0,166	0,739	1,248	6	-	21	93
2	-1,2	3,508	1,887	0,057	0,797	1,307	5	6,53	21	96
3	-26,67	5,239	3,228	0,075	0,866	1,194	9	-	32	94
4	-24	9,595	4,213	0,201	0,846	1,363	12	-	21	92
5	-0,33	7,356	2,926	0,342	0,713	1,286	7	5,5	21	96
6	-4,67	7,520	3,346	0,591	0,807	1,318	10	6,8	80	92
Measurements on the last suitable moment before extubation										
1	2	7,296	2,039	0,142	0,872	1,460	6	-	21	96
2	-15	4,234	2,663	0,061	0,857	1,725	5	6,6	21	100
3	-4,33	6,167	2,741	0,058	0,869	1,109	11	-	28	96
4	-23,33	8,312	3,057	0,187	0,838	1,415	11	-	21	95
5	6,67	6,5761	2,6256	0,1437	0,7130	1,719	5	5,5	21	96
6	-6,67	5,6479	2,3216	0,3020	0,8732	1,315	7	7,1	55	94

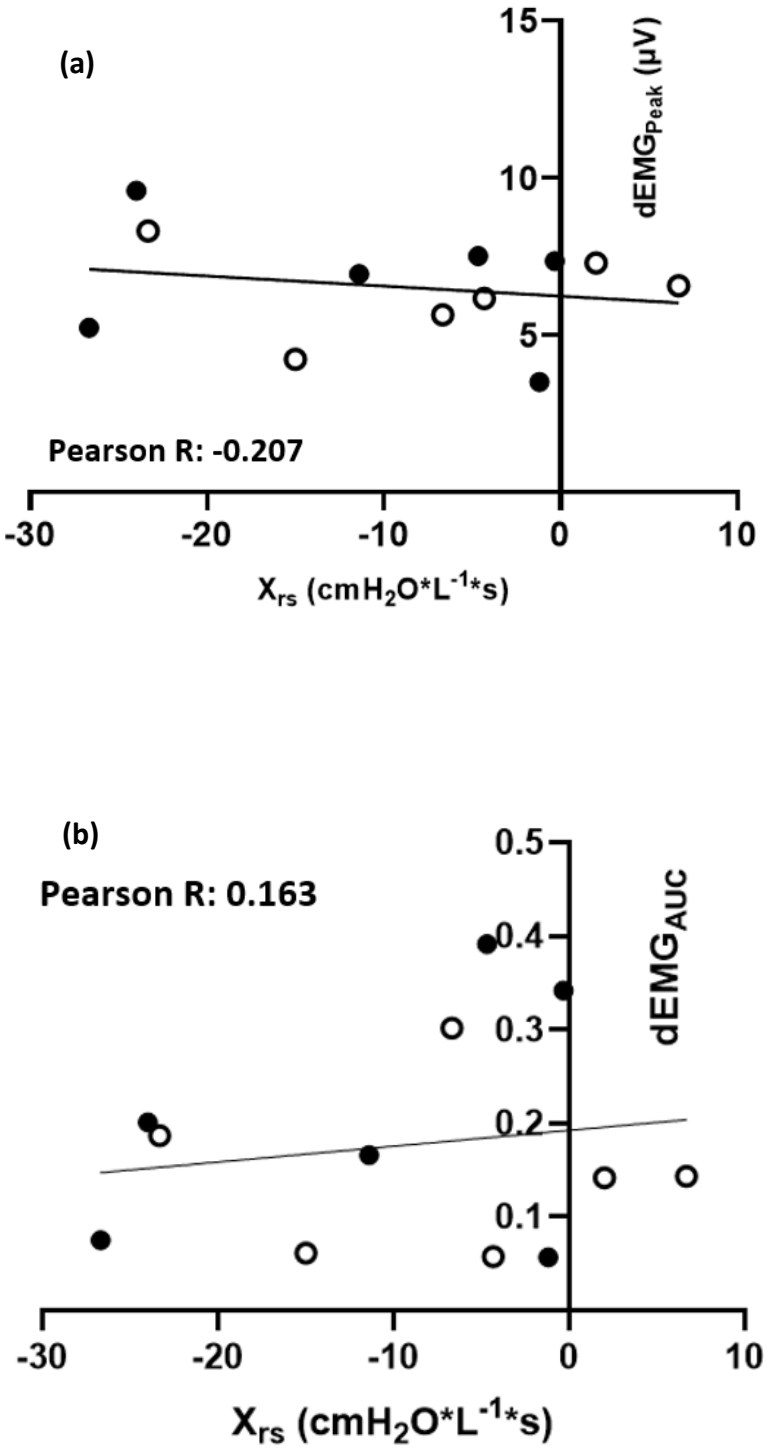

Figure 21: *Pearson correlation analyses of 12 pairs (6x2) of the parameters obtained from the dEMG measurements and the reactance as determined with FOT measurements (X_{rs}) during the dEMG recording. Black circles correspond to data collected on the first suitable moment after intubation, whilst white circles correspond to the data collected on the last suitable moment before extubation.* **(a)**: X_{rs} and *the mean peak dEMG activity, p = 0.520.* **(b)**: X_{rs} and *the mean dEMG area under the curve, p = 0.613.*

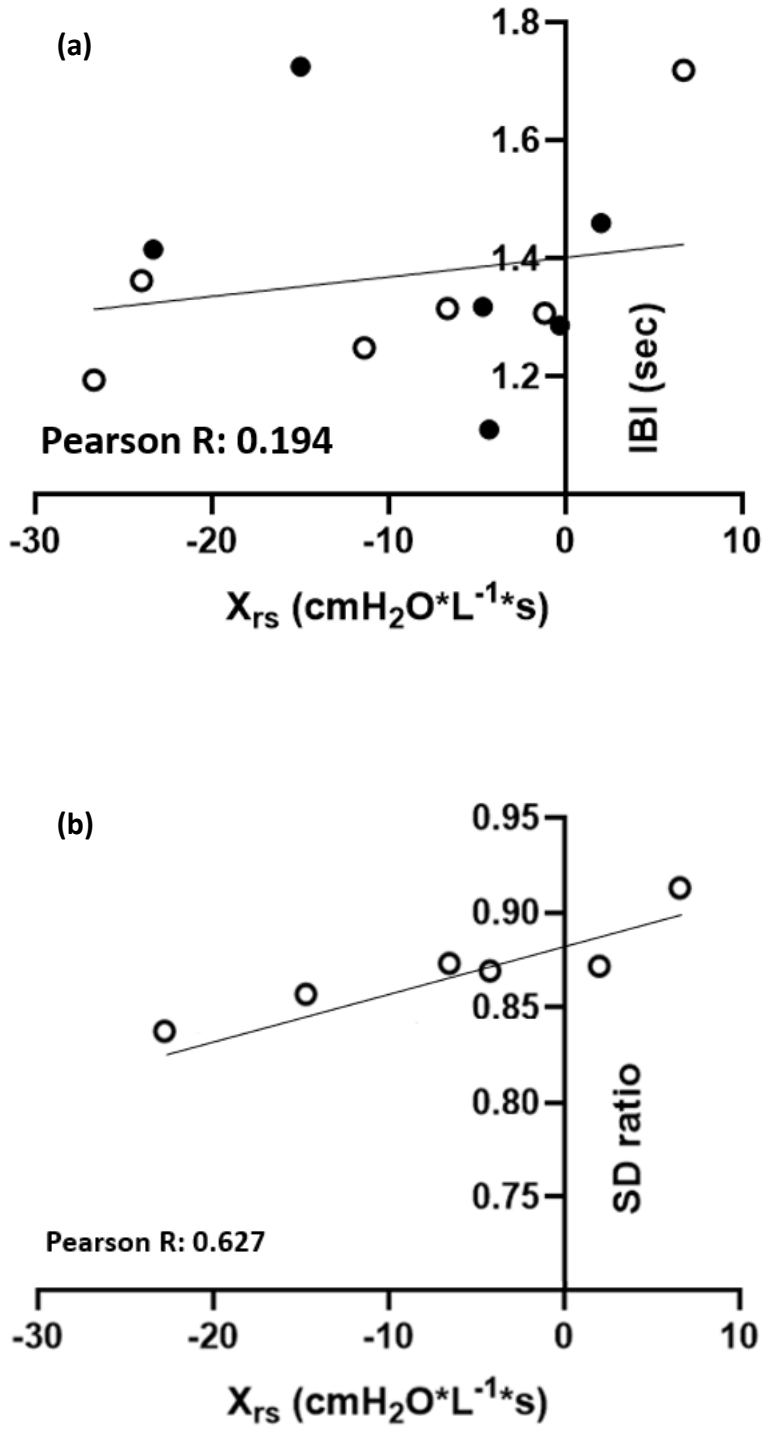

Figure 22: *Pearson correlation analyses of 12 pairs (6x2) of the parameters obtained from the Poincaré analyses on the dEMG measurements and the reactance as determined with FOT measurements (X_{rs}) during these dEMG recording. Black circles correspond to data collected on the first suitable moment after intubation, whilst white circles correspond to the data collected on the last suitable moment before extubation* **(a)**: X_{rs} and *the mean IBI, p = 0.547.* **(b)**: X_{rs} and *the SD ratio outcomes of only the last suitable moment before extubation, p = 0.037.*

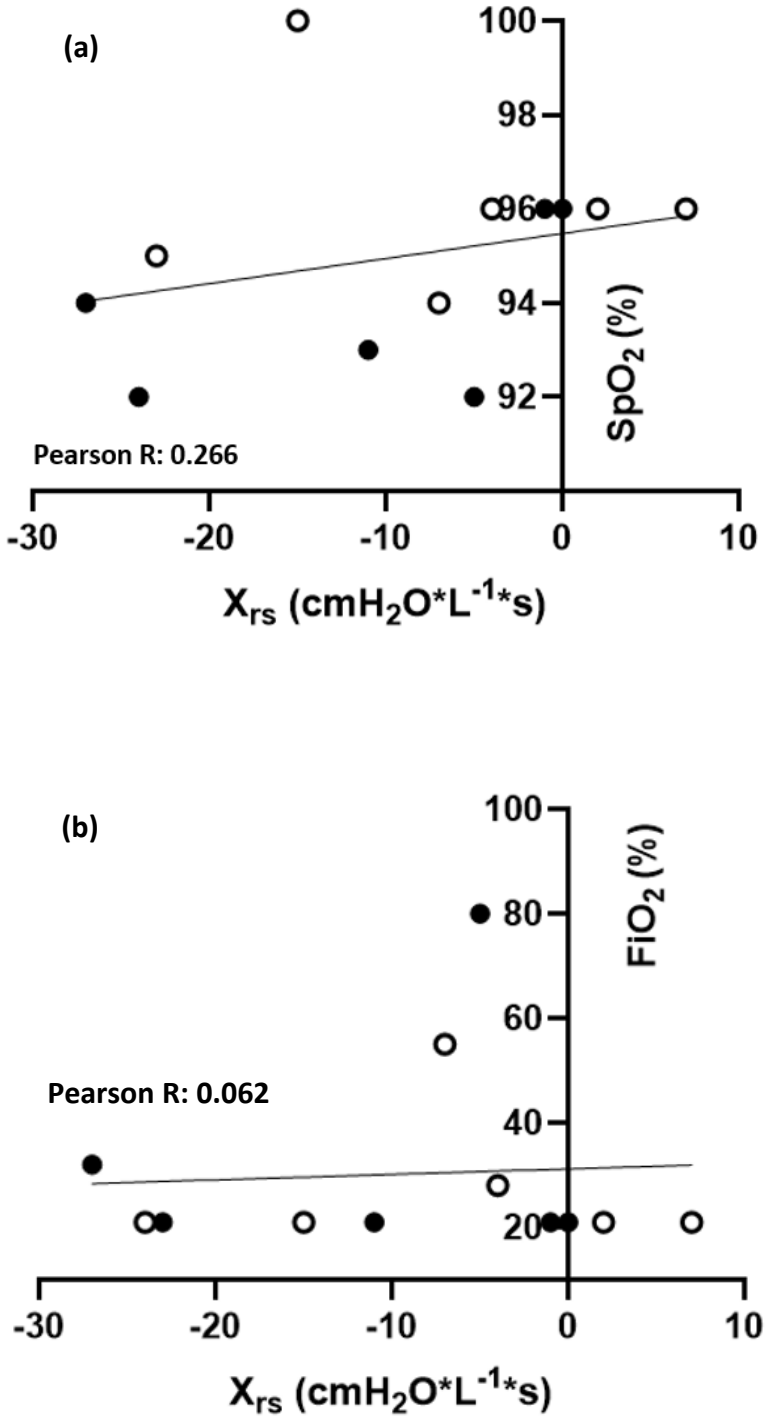

Figure 23: *Pearson correlation analyses of 12 pairs (6x2) of the collected clinical parameters and the reactance as determined with FOT measurements (X_{rs}) during the dEMG recording. Black circles correspond to data collected on the first suitable moment after intubation, whilst white circles correspond to the data collected on the last suitable moment before extubation.* **(a)**: X_{rs} and SpO_2, *p = 0.402.* **(b)**: X_{rs} and FiO_2, *p = 0.847.*

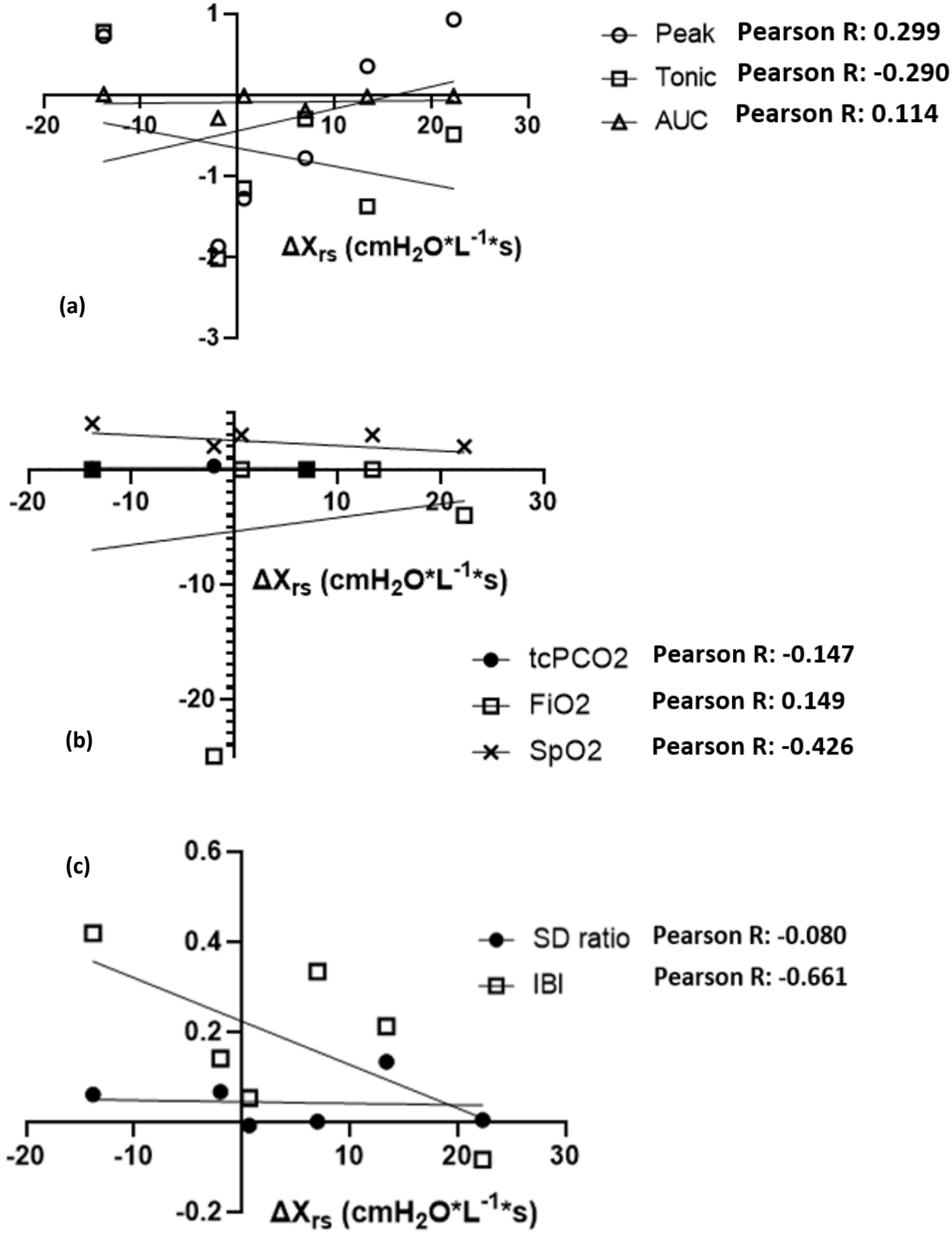

Figure 24: *Pearson correlation analyses of the reactance as determined with FOT measurements (X_{rs}) and both all the dEMG and clinical parameters. In these figures, the correlation between the difference of these parameters between the first and last measurement is analysed. While on the x-axis the change over time in X_{rs} is shown, the changes over time in the other parameters are shown on the y-axis.* **(a)**: X_{rs} and *usual dEMG parameters. The two-tailed p-values of these correlations are 0.565, 0.578 and 0.830 for respectively dEMG peak, tonic and AUC.* **(b)**: *X_{rs} and clinical parameters. The p-values are 0.906, 0.778 and 0.399 for respectively $tcPCO_2$, FiO_2 and SpO_2.* **(c)**: X_{rs} and *Poincaré dEMG parameters. The p-values are 0.881 and 0.152 for respectively the SD ratio and mean IBI.*

Gemini Study METC Proposal Abstract

Rationale: At the Neonatal Intensive Care Unit (NICU) newborn infants may need mechanical ventilation (MV). The appropriate ventilator settings to achieve optimal oxygenation and ventilation should be adapted to the infant's lung function, which changes due to developing (patho)physiology. In clinical practice, titrating the level of respiratory support is difficult, as there is no standard used technique to monitor the infant's lung function. Therefore, peripheral oxygen saturation (SpO_2), oxygen demand (FiO_2), and transcutaneous carbon dioxide (tCO_2) are used as secondary measures for adequate gas exchange. However, these clinical parameters are not only dependent on current lung function, but on the circulatory condition as well. If a technique was available which could solely examine the lung function this would provide important new insights which could aid in clinical practice to stabilize and improve the lung condition during MV.

Studies in infants show that the Forced Oscillation Technique (FOT) is a feasible method to acquire information on lung mechanics (i.e. resistance (R) and reactance (X)), without impact of the cardiovascular condition. Recently, FOT has been implemented in a neonatal ventilator, which makes it easily accessible for lung function evaluation on a daily basis.

Next to FOT, the level of diaphragm activity (measured with transcutaneous electromyography (dEMG)) could also describe the current lung function based on increased/decreased respiratory effort. Using both FOT and dEMG in clinical practice could have a clear impact on clinical practice as it might provide a clear image of the infant's lung condition, combining lung mechanics (FOT) and respiratory effort (dEMG). However, to our knowledge, never before has this unique and non-invasive combination of methods been investigated in (mechanically ventilated) infants.

Objective: This study's first objective is to assess reactance values measured with FOT to describe mechanical properties of the respiratory system during a MV episode in clinical practice. The second objective is to examine diaphragm activity measured with dEMG to assess the respiratory effort during the MV period. Lastly, exploratory analysis will be performed to determine to what extent the dEMG and FOT data are related, in order to speculate if the combination could aid the examination of lung function.

Study design: Prospective, observational cohort study.

Study population: Infants with a post conceptual age >25 weeks, who receive invasive MV from the Fabian HFOi neonatal ventilator at the NICU department of Emma's Children's Hospital in Amsterdam for more than 24 hours.

Main study parameter/endpoint: The reactance measured with FOT and diaphragm activity measured with dEMG to examine lung function over time.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: This is a non-invasive observational, prospective study that only uses certified techniques. No changes are made to the caregiving routine. The study population will not benefit from participating in this study. This study will expand our physiological knowledge on non-invasive ways to monitor lung function, which will benefit future neonatal invasive ventilatory care

Study subject information for parents/guardians for participation in medical research

Neonatal lung function measurements

Official title: Non-invasive lung function measurements in children using the Forced Oscillation Technique and diaphragm electromyography

Introduction

Dear Sir/Madam,

With this letter, we would like to ask you for your child's participation in a medical study. Participation is voluntary. You have received this letter because your child is admitted to the Neonatology Intensive Care Unit (NICU) and is being mechanically ventilated through a tube. You can read about the medical study in this information sheet, what it means for your child, and what the pros and cons are. It is a lot of information. Can you please read the information and decide if you want your child can take part? If you want your child to take part, complete the form in Appendix C.

Ask your questions

You can take your decision based on the information in this information sheet. We also suggest that you do this:

- Put your questions to the investigator who gave you this information.
- Talk to your partner, family or friends about this study.
- Ask questions to the independent expert, dr. R.A. Bem.
- Read the information on www.rijksoverheid.nl/mensenonderzoek.

1. General information

The Neonatology of the Amsterdam UMC, location AMC, has set up this study. Below, we always call the Amsterdam UMC, location AMC the 'sponsor'. Investigators, these can be doctors, technical clinicians and nurses, conduct the study. For this study, 30 test subjects are needed who are collected at location AMC. The Medical Ethics Review Committee (MERC) of the Amsterdam UMC, location AMC has approved this study.

2. What is the purpose of the study?

In this study, we look at whether we can use the combination of two measuring techniques to measure the lung function in children who are ventilated through a tube. By means of this research, we hope to be able to tailor respiratory support settings more adequately in children in future.

3. What is the background of the study?

Your son or daughter requires mechanical ventilation because of preterm birth or illness. Clinicians always do their utmost best to tailor the settings of the respiratory support to the child's need. To optimally tailor the support, monitoring of the child's lung function is very important, as your child grows and his or her lung condition may change.

In order to determine respiratory support settings, clinicians monitor oxygen saturation and the amount of carbon dioxide in the blood of the infant as well as any additional administered oxygen. These factors provide information about gas exchange, but as these factors are not solely dependent on the lung function, it can be difficult to determine the optimal respiratory support settings. Therefore, we are looking for new non-impacting measuring techniques to determine the lung function of infants who receive mechanical ventilation.

Therefore, two measuring techniques are investigated in this study that can provide information about the lung function and breathing of the infant. The first one is called the Forced Oscillation Technique (FOT). The FOT modality is provided by the ventilator that supports your infant. By this modality, small, imperceptible vibrations (the so-called 'forced oscillations') are send into the lungs. The damping of these vibrations in the respiratory system provides information about the aeration of the lungs. This technique has been tested and is safe for newborn infants.

Another technique of which we believe can help us to determine the optimal amount of respiratory support, is the measurement of the activity of most important respiratory muscle, the diaphragm. This technique is called diaphragm electromyography (dEMG), and measures muscle activity with several adhesive electrodes on the skin. The dEMG provides information about the amount of effort the child uses to breathe. By combining the FOT and the dEMG measurement, we believe we can track the lung function development more accurately. In addition, with this information we might be able to tailor respiratory support settings more adequately in future. After all, it is important that the lungs are well aerated (to be measured with FOT), but your child does not become exhausted to achieve this (measured muscle activity with dEMG).

4. What happens during the study?

How long will the study take?

Is your child taking part in the study? Then it will last during the period in which your child receives respiratory support through a tube.

Step 1: is your child eligible to take part?

Your child can participate in the study if she/he needs respiratory support through a tube for more than 24 hours and you give consent for participation. Only if it turns out that the measurements cannot be performed for whatever reason, your child will not be eligible to take part in the study.

Step 2: study and measurements

If you decide to include your child in the study, only extra non-impacting measurements are performed. One dEMG measurement will be performed on the next suitable moment after consent is received and one on the last day of the ventilation period. For the dEMG measurement three small adhesive electrodes will be applied to the skin during nursing care. These adhesive electrodes will be removed during the next moment of nursing care. In this way, your child will not be disturbed more than necessary. The dEMG measurement takes one hour.

In addition, a daily FOT measurement is performed during the ventilation period. One FOT measurement takes about 10 seconds and are executed on the applied pressure and on one small pressure step above. This small change in pressure is comparable with the pressure fluctuations that accompany kangaroo care or clinical handling. After this FOT measurement, the pressure is reset to the pressure applied prior to the measurement.

These measurements will be conducted at the ward your child is admitted and only take place during the respiratory support period. Your child will not notice either of the measurements (FOT and dEMG) and you are allowed to attend these measurements. We do ask you not to touch your child during the FOT measurements. The moments of the application of the adhesive electrodes and measurements will always be coordinated with the nurses and/or physicians so that you and your child are disturbed as little as possible. An overview of the study measurements can be observed in appendix B.

What is the difference with standard care?

We would like to emphasize that this study is not very different from standard care. We will not change your child's monitoring nor treatment within this study. Your child will receive the standard treatment as prescribed by the treating physician. We only perform the before mentioned extra non-impacting measurements.

5. What are the pros and cons if your child takes part in the study?

There are no benefits for your child from participating in this study. As we currently only execute measurements, no adjustments in care will be made. However, participation may contribute to improved possibilities to tailor ventilation settings in newborns in the future. There is already plenty experience with EMG measurements in newborn infants and there are no disadvantages to expect when participating in this study. Occasionally there was a slight redness of the skin after the dEMG measurement, but this disappears quickly and it not harmful. The FOT measurements are non-impacting and have no disadvantages.

6. Resistance of your child

It is possible that your child resists (does not cooperate) at some point during the study. The investigator must immediately stop the study if this happens. It is difficult to describe exactly what resistance means. If the doctor, nurse and/or researcher has the feeling that your child is suffering during a measurement, the measurement will be discontinued. However, there is no reason in advance to assume that your child will resist. The investigator will follow the rules of the Code of Conduct on the Resistance of Minors.

7. When does the study end?

The investigator will let you know if there is any new information about the study that is important to you. The investigator will then ask you if your child can continue to take part.

Your child's participation in the study will stop if:

- Your child does not need mechanical ventilation anymore.
- All measurements according to the schedule are finished. See appendix B.
- You choose to stop your child's participation to the study. You can stop at any time. Report this to the investigator immediately. You do not have to explain why you want to stop your child's participation.
- The investigator thinks it is better for your child to stop.
- One of the following authorities decides that the study should stop:
    - The Amsterdam UMC, location AMc
    - The government, or
    - The Medical Ethics Review Committee assessing the study.

What happens if you stop your child's participation in the study?

The investigators use the data that have been collected up to the moment that you decide to stop your child's participation in the study.

8. What happens after the study has ended?

Will you get the results of the study?

After your child took part in the study, the investigator can inform you about the most important results of the study. Do you prefer to know? Please tell the investigator.

9. What will be done with your child's data?

Is your child taking part in the study? Then you also give consent to collect, use and store your child's data.

What data do we store?

We store these data:

- your child's name
- the date of birth of your child
- the gestational age

- your child's weight at birth
- your child's gender
- information about your child's health
- (medical) information that we collect during the study

Why do we collect, use and store your child's data?

We collect, use and store your child's data to answer the questions of this study. And to be able to publish the results.

How do we protect your child's privacy?

To protect your privacy, we give a code to your child's data. We only put this code on your child's data. We keep the key to the code in a safe place in the Amsterdam UMC, location AMC. When we process your child's data, we always use only that code. Even in reports and publications about the study, nobody will be able to see that it was about your child.

Who can see your child's data?

Some people can see your child's name and other personal information without a code. These are people checking whether the investigators are carrying out the study properly and reliably. These persons can access your child's data:

- Members of the committee that keeps an eye on the safety of the study.
- An auditor who is hired by the Amsterdam UMC, location AMC.
- National supervisory authorities. For example, the Healthcare and Youth Inspectorate.

These people will keep your child's information confidential. We ask you to give permission for this access.

In addition, there is a collaboration with Vyaire Medical (a medical company which develops equipment for respiratory support) and the Politecnico di Milano University in Milan during this study. These two parties conduct studies in the field of lung function measurements in newborns as well. Both parties will receive encrypted data from the study, and not any data from which the identity of your child could be retrieved. At all times you have the right to decide to refrain from sharing the data with these parties. The data will then be destroyed by these parties. The shared data will periodically be examined on usability. Data that is no longer usable will be destroyed.

For how long do we store your child's data?

We store your child's data in the Amsterdam UMC, location AMC for 15 years.

Can we use your child's data for other research?

Your child's data may also be important after this study for other medical research in the field of lung function measurements in newborns. For this purpose, your child's data will be stored in the Amsterdam UMC, location AMC for 15 years. Please indicate in the consent form whether you agree with this. Do you not want to give consent for this purpose? Then your child can still take part in this study. Your child will get the same healthcare.

Can you take back your consent for the use of your child's data?

You can take back your consent for the use of your child's data at any time. This applies both to the use in this study and to the use in other (future) medical research. But please note: if you take back your consent, and the investigators have already collected data for research, they are still allowed to use this information.

Do you want to know more about the privacy of your child?

Do you want to know more about your child's rights when processing personal data? Visit www.autoriteitpersoonsgegevens.nl.

Do you have questions about your child's rights? Or do you have a complaint about the processing of your child's personal data? Please contact the person who is responsible for processing your child's personal data. For the present, this is: The researchers involved from the Amsterdam UMC, location AMC. See Appendix A for contact details.

If you have any complaints about the processing of your child's personal data, we recommend that you first discuss them with the research team. You can also contact the Data Protection Officer of the Amsterdam UMC, location AMC. Or you can submit a complaint to the Dutch Data Protection Authority. See appendix A for contact details.

10. Is your child insured during the study?

Your child is not additionally insured for this study. Because taking part in the study has no additional risks. That is why the sponsor of the MERC of the Amsterdam UMC, location AMC does not have to take out additional insurance.

11. Do you have any questions?

You can ask questions about the study to the research team. Would you like to get advice from someone who is independent from the study? Then contact dr. R.A. Bem. He knows a lot about the study, but is not a part of this study.

Do you have a complaint? Discuss it with the investigator or the doctor who is treating your child. If you prefer not to do so, please visit the complaints committee of the hospital. Appendix A tells you where to find this.

12. How do you give consent for the study?

You can first think carefully about this study. Then you tell the investigator if you understand the information and if you want to take part or not. If you want to take part, we ask you to fill in the consent form that you can find with this information sheet. You and the investigator will both get a signed version of this consent form.

Thank you for your attention.

13. Appendix

A. Contact details Amsterdam UMC, location AMC
B. Overview of study measurements
C. Informed consent form

Appendix A: Contact details Amsterdam UMC, location AMC

Main investigator Amsterdam UMC
Dr. G.J. Hutten, pediatrician - neonatologist
Tel. +31 20-5663668

Executive investigators Amsterdam UMC
Dr. R.W. van Leuteren, technical physician
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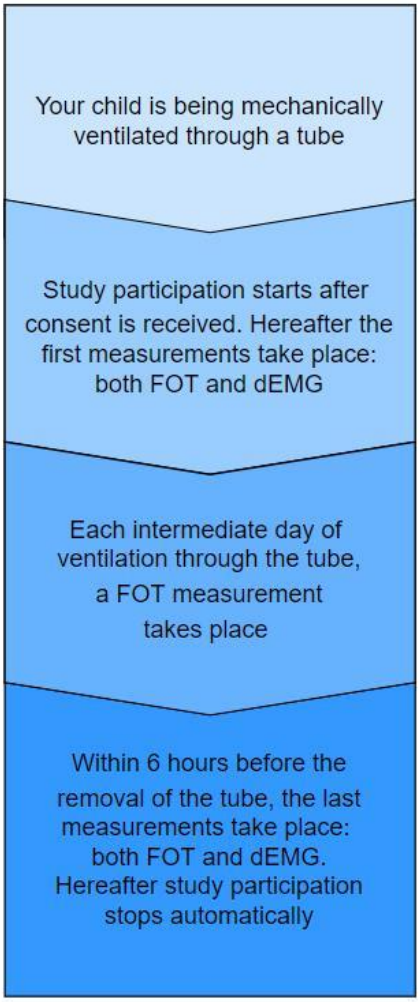
Tel. +31 20-5663675

Independent expert Amsterdam UMC
Dr. R.A. Bem, pediatrician – pediatric intensivist
Tel. +31 20-5665769

Data protection officer Amsterdam UMC
privacy@amsterdamumc.nl

Complaints committee Amsterdam UMC
Tel. +31 20-5663355
patientenvoorlichting@amc.nl

Appendix B: Overview of study measurements



Appendix C: Informed consent form

Belonging to:

Neonatal lung function measurements

I have been asked to give consent for my child to participate in this medical study:

Name of subject (child): …….. Date of birth: __/__/__

I have read the information sheet for parents/guardians. I was able to ask questions. My questions have been answered well enough. I had enough time to decide if I want my child to take part.

I know that taking part is voluntary. I also know that I can decide at any time that my child will not take part after all. I do not have to explain why.

I give consent to collect and use my child's data. The investigators only do this to answer the question of this study.

I know that some people will be able to see all of my child's data to review the study. These people are mentioned in this information sheet. I give consent to let these people see my child's data for this review.

Please tick yes or no in the table below.

I give consent to have my child's data stored for use in other research, as stated in the information sheet.	Yes ☐	No☐
I give consent that my child's encrypted data may be shared and used by Vyaire Medical, the Politecnico di Milano University and possibly for the benefit of its partners, as stated in the information sheet.	Yes ☐	No☐

I agree that my child takes part in this study.

Parent/guardian name: ……

Signature: …… Date: __/__/__

Other parent/guardian name: ……

Signature: ……..… Date: __/__/__

I declare that I have fully informed the person(s) mentioned above about the said study.

If any information becomes known during the study that could influence the parent/guardian's consent, I will let them know in good time.

Investigator name (or their representative): ……

Signature: …… Date: __/__/__