POLITECNICO DI MILANO PH.D. PROGRAM IN BIOENGINEERING

NEW METHODS FOR MONITORING LUNG FUNCTION IN PRETERM NEWBORNS TO OPTIMIZE VENTILATORY SUPPORT

DOCTORAL DISSERTATION OF:

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Life is an opportunity, benefit from it. Life is beauty, admire it. Life is bliss, taste it. Life is a dream, realize it. Life is a challenge, meet it. Life is a duty, complete it. Life is a game, play it. Life is a promise, fulfil it. Life is a promise, fulfil it. Life is a song, sing it. Life is a struggle, accept it. Life is an adventure, dare it. Life is luck, make it. Life is too precious, do not destroy it. Life is life, fight for it.

Mother Teresa of Calcutta

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LIST OF ABBREVIATIONS

V: flow **BPD: Bronco-Pulmonary Dysplasia CDP: Continuous Distending Pressure** C_{dvn}: dynamic Compliance of the respiratory system CMV: Conventional Mechanical Ventilation Crs: Compliance of the respiratory system **CT:** Computerised Tomography EAI: Expiratory Asynchrony Index E_{dvn}: dynamic Elastance **EELV: End-Expiratory Lung Volume** E_{FOT}: lung Elastance estimate from Xrs EIT: Electrical Impedance Tomography **ELBW: Extremely Low Birth Weight** ELGAN: Extremely Low Gestational Age Newborn eWOB: elastic Work Of Breathing FiO₂: Fraction of Inspired Oxygen FOT: Forced Oscillation Technique FRC: Functional Residual Capacity GA: Gestational Age **HFOV: High Frequency Oscillation Technique** HHHFNC: Heated, Humidified High-Flow Nasal Cannula HIT: High Interrupter Technique IAI: Inspiratory Asynchrony Index **IPPV: Intermitted Positive Pressure Ventilation IRDS:** Infant Respiratory Distress Syndrome Irs: Inertance of the respiratory system IT: Interrupter Technique LBI: Labored Breathing Index LIP: Lower Inflection Point LUS: Lung Ultrasound MAP: Mean Airways Pressure MIT: Multilinear Interrupter Technique **MOT: Multiple Occlusion Technique MV: Mechanical Ventilation** NAVA: Neural Adjusted Ventilatory Assist nBiPAP: Nasal Bilevel Positive Airways Pressure nCPAP: Nasal Continuous Positive Airway Pressure

NICU: Neonatal Intensive Care Unit nIPPV: Nasal Intermitted Positive Pressure Ventilation NIV: Non Invasive Ventilation **OEP: Opto-Electronic Plethysmography** P_{ALV}: alveolar Pressure P_{AO}: Airways Opening Pressure P_{BS}: Body Surface Pressure pCO₂: arterial partial pressure of carbon dioxide **PCV: Pressure Controlled Ventilation PEEP: Positive End-Expiratory Pressure** Pes: Oesophageal Pressure **PIP: Positive inspiratory Pressure** P_L: Transpulmonary Pressure P_{MUS}: Pressure generated by the respiratory muscles pO₂: arterial partial pressure of oxygen P_{PL}: Pleural Pressure P_{rp}: Retropharyngeal Pressure **PSV: Pressure Support Ventilation** PtcCO₂:Transcutaneous partial pressure of carbon dioxide PtcO₂:Transcutaneous partial pressure of oxygen **PTP: Pressure Time Product** P-V curve: Pressure-Volume curve R: Resistance **RDS: Respiratory Distress Syndrome RIP: Respiratory Inductive Plethysmography RR:Respiratory rate** Rrs: Resistance of the respiratory system rWOB: Resistive Work Of Breathing SIPPV: Synchronised Intermitted positive pressure ventilation **SLI: Sustained Lung Inflation** SOT: Single Occlusion Technique SpO₂: Oxygen saturation Ti:Te: inspiratory-expiratory time ratio **UIP: Upper Inflection Ioint** VCV: Volume Controlled Ventilation VILI: Ventilation-Induced Lung Injury V_L: Lung Volume V_T: Tidal Volume VTV: Volume Targeted Ventilation VV: Variable Ventilation WOB : Work Of Breathing WOB_e: Expiratory Work Of Breathing WOB_i: Inspiratory Work Of Breathing WOB¹⁰: Work Of Breathing of the lower respiratory system WOB^{up}: Work Of Breathing of the upper respiratory system. X₅: reactance of the respiratory system at 5Hz Xrs: reactance of the respiratory system

SUMMARY

Preterm birth is a significant perinatal health problem across the globe, affecting approximately 9.6% of all births worldwide (12.9 million/year) (Beck et al. 2010). The preterm lung is structurally and biochemically immature and vulnerable to injury and this has a great impact in terms of associated mortality, short and long-term morbidity and financial implications for health-care systems (Petrou et al. 2011).

In particular, the infant respiratory distress syndrome (IRDS) is by far the most common cause of respiratory distress in premature infants and a major contributor to neonatal mortality worldwide (Kamath et al. 2011). It is characterized by surfactant deficiency and consequently by the tendency of the lung to collapse. To counteract this tendency and to guarantee ad adequate amount of oxygen to the tissue these infants are treated with oxygen therapy and ventilatory supports. Although these treatments are often indispensable for the survival of patients with respiratory failure they can exacerbate lung injury and play a role in the development bronchopulmonary dysplasia (BPD), the most common chronic lung disease in childhood and one of the most severe long term complications of prematurity (Baraldi et al. 2007; Fawke et al. 2010).

Several studies suggested that combining less invasive care strategies that avoid excessive oxygen and ventilation may decrease the incidence and severity of chronic pathologies (Jobe 2011). In particular there is evidence that the choice of the correct ventilation strategy is crucial from the first moment of life on (Björklund et al. 1997; Wada et al. 1997; Morris et al. 2006).

For this reason, in the last years an intense research activity, both experimental and clinical, has examined different aspects of the ventilation practice in order to improve the treatments and provide a ventilation that is not only supportive but also, as far as possible, protective of pulmonary function and structure. Significant progresses have been made both in the definition of new invasive ventilation modalities and in the technology employed in the ventilators. Efforts have been also made to improve non-invasive modalities of ventilation in order to avoid intubation to the majority of the infants. Moreover, nowadays new strategies, employing recruitment manoeuvres, for resuscitation at birth are under investigation for helping infants in reaching an adequate lung volume at birth so to start ventilation in a contest of a more recruited lung.

There is also an increasing awareness that, in order to implement a real protective ventilation strategy, the treatment has to be tailored and continuously adapted according to lung function of each infant. In fact the extreme heterogeneity of condition of the respiratory system in newborns, together with the continuous changes the lung undergoes especially during the first weeks of life, makes it impossible to adopt a common strategy for all patients.

Optimisation of ventilator parameters is currently obtained by monitoring blood oxygenation. However, even if it can be used to indirectly monitor lung recruitment or airways collapse, gas exchange is the result of many processes (ventilation, diffusion, perfusion, circulation) and, therefore, it lacks in specificity for the detection of pressure-induced over-distension and tidal recruitment (Richard et al. 2004). Therefore, an optimization based on parameters that can specifically identify the best trade-off between minimal lung tissue distention and maximal lung volume recruitment could result in a more protective treatment and improvement in clinical outcomes. This optimisation procedure requires the availability of technologies for measuring and monitoring appropriate indices of lung function at the bedside suitable to be allied in clinical practice.

Even if several techniques have been developed for lung function measurement, their use in infants in clinical practice is still a challenge. Adequate tools to guide the physician to identify optimal ventilatory settings during resuscitation at birth as well as during invasive and non-invasive ventilation are still missing. The aim of this work is to develop methods and technologies to monitor lung function at bedside during the different approaches of the ventilatory support in preterm newborns to provide tools that improve the tailoring of the ventilatory strategy in order to minimise the negative outcomes that permanently compromise the quality of the future life of these infants.

CHAPTER 1: LUNG FUNCTION AND VENTILATORY SUPPORTS IN PRETERM NEWBORNS

Choosing how to support ventilation in a preterm newborn is a difficult task as his lung is highly vulnerable to injury, there is little knowledge about optimal gas target levels and about the threshold for harmful stress and strain to lung tissue necessary to implement protective ventilation strategies and, moreover, there are no measurements available at bedside that can help the clinician to personalize the treatment.

In this chapter it is provided a description of 1) the characteristic of the respiratory system in newborns, with the main acute and chronic pathologies; 2) the current progress in invasive and non-invasive ventilation and in the strategies for resuscitation at birth; 3) the available technologies to measure lung function and 4) an introduction to the concept of protective ventilation.

Birth represents a critical event associated with dramatic changes in the lung function: the foetal lung fluid needs to be absorbed, the lungs have to be filled with air, adequate gasexchanging surface area has to be established, the pulmonary blood flow has to greatly increase and the surfactant system has to ensure the lungs remain expanded by decreasing the alveolar surface tension. Moreover preterm newborns are compounded by an incomplete structural and biochemical development, a lower capacity to reabsorb foetal lung liquid, the lack of surfactant and increased thickness of the alveolar-capillary membrane that interferes with gas exchange. All these features make infants and especially the preterm ones very exposed to respiratory system injury (Hammer et al. 2005).

There is a diffuse awareness of the importance of a protective ventilation strategy for newborns that guarantee adequate amount of oxygen to the tissues and removal of carbon dioxide without injuring the lung. However, how to implement it in the clinical practice remains controversial. A lot of progresses have been made in the last years both in the definition of invasive ventilation modalities and in the technology employed in the ventilators. Together with the older more diffused forms of ventilation, different new promising modalities for treating newborns are now being studied. In parallel, different modalities of non-invasive ventilation (NIV) have been proposed for weaning but also as an alternative to invasive ventilation in the attempt to reduce ventilatory induced lung injury (VILI). Also new strategies to help preterm infants to establish adequate functional residual capacity (FRC) right at birth have been evaluated in order to start ventilation of a more recruited lung. However, there is still a need of studies investigating the mechanisms of functioning of these new forms of supports together with means to optimize ventilation settings of both invasive and non-invasive supports as well as to guide resuscitation at birth. Moreover because of the great inter-patient heterogeneity and the continuous changes due to growth and pathology development of the respiratory system functionality in newborns, a strategy that permits the customization of the treatment would realise a more protective ventilation. In order to achieve this, parameters that could track changes in the respiratory function during ventilator setting titration and that can be measured non-invasively in the clinical practice should be identified.

Assessment of lung function and mechanics in infants in clinical practice is still a challenge as equipment, methods, measurements' condition and data elaboration used in adults and older children must be adapted to meet the special requirements of this group age. Different techniques have been proposed and applied for research purpose but the technical demands, the need for specialized equipment and operators together with test duration, maximal repetition frequency, the requirement for sedation and the lack of appropriate reference values or proved clinical important impact have limited their used for clinical purpose. However the recent development of new technologies, the standardization of procedures and the appearance of commercial equipment have led to a renewed interest in this field.

One promising technique is the forced oscillation technique (FOT) as it is non-invasive and, with the development of appropriate set up, can measure the input impedance (Zin) of the respiratory system in clinical practice. Moreover, the recently proposed intra-breath analysis of Zin and the attention on its relative changes rather than on the absolute values as function of the stimulation frequency can lead to new insides in pathology and clinical management of the infants. Besides FOT, other promising techniques (such as the study of electrical activity of the diaphragm and the variability analysis of different signal related to the respiratory system) are under development and evaluated as new tools to study of the respiratory system. These techniques could provide important information especially during NIV, when all measurements of lung function are difficult or impossible and the spontaneous respiratory activity of the infant plays a crucial role.

CHAPTER 2: INVASIVE MECHANICAL VENTILATION

Although nowadays the tendency is to avoid as much as possible the recourse to invasive ventilation, it is indispensable for the survival of patients with the most complex clinical condition and lungs difficult to ventilate. However, excessive tidal volumes and inadequate lung recruitment may contribute to mortality and development of BPD by causing ventilator-induced lung injury. There is a diffuse awareness of the importance of a customized protective ventilation strategy for newborns as well as that the key for it is recruiting the lung and keep it open. However, how to implement it in the clinical practice remains controversial as evaluating oxygenation is not enough and the proposed strategy based on lung mechanics are of difficult application.

Recently, a new approach based on Zin measurement by FOT has been suggested. Dellacà et al. proposed the use of a single forcing frequency (at 5Hz) overimposed on conventional ventilation that allows high temporal resolution and to follow intra-tidal changes of the respiratory system reactance (Xrs). Therefore they can study the changes of Xrs with PEEP (positive end-expiratory pressure) at end expiration avoiding the influence of intra-tidal recruitment/derecruitment. They showed that Xrs can be used to monitor recruitment/derecruitment during ventilation in two different porcine models of lung collapse (Dellaca et al. 2009) and allows the definition of an optimal PEEP in agreement with CT data (Dellacà et al. 2011).

These results, together with the theoretical possibility to implement FOT into a mechanical ventilator, could make it possible to provide a tool for tailoring mechanical ventilation and to help to manage patients in clinical practice. For these reason we decided to apply this methodology to the study of preterm infants.

To this aim, adequate set up and methods for data processing have to be developed to meet the specific requirements related to this group of patients. In fact, the very low flow and volume and the high breathing frequency add technical issues and constraints for the experimental set up and the data processing. In fact the traditional loudspeaker based FOT set up is not suitable for the study of ventilated preterm infants, as the additional compliance represented by the loudspeaker when connected to the ventilation circuit interfere with the ventilator trigger and the delivery of a proper ventilator waveform. Therefore we develop and validated a new set up for applying FOT in preterm newborns. Also, the algorithms used for Zin measurement in adults have been adapted to be applied in presence of lower signal to noise ratio and partial overlapping of the spontaneous breathing frequency components and the oscillatory forcing signal.

Once the set-up has been validated in vitro, in order to evaluate if FOT can provide a tool to optimize the treatment for these patients, we applied it to the studies of clinical interventions that could modify EELV and its distribution like PEEP and prone positioning. These studies were performed in collaboration with Ospedale Mangiagalli di Milano and Children Hospital of Uppsala.

As PEEP is the ventilatory parameter that mostly impacts on how many alveoli remain open at end-expiration, we first applied FOT to the study of the changes in lung mechanics during PEEP titration. Different group of patients (RDS, evolving BPD and healthy lung) have been considered to understand if their response to PEEP is different and whether and how Zin measurements could guide the tailoring of PEEP setting in these patients. RDS infants present a similar behaviour to animal models of RDS, suggesting that a PEEP optimized by measuring Zin could attenuate signs of VILI (Kostic et al. 2011). Moreover, the trend of Zin with PEEP in BPD patients suggests that the role of PEEP in this population could be different and related to maintaining airways patency. Even if these results are based on a small population and need further confirmation, they suggest that Zin could have a role also in optimizing the settings also for this group of patients.

Moreover, as during the first days of life the respiratory system undergoes dramatic changes, we also studied how the relationship between PEEP and impedance evolves during the first week of life in the same infant and how the optimal PEEP (from a mechanical point of view) changes during this period in comparison with the one clinically identified by oxygenation. Our results show differences in the relationship between Xrs and PEEP in the different days and suggest that on the 1st day of life particular care should be taken in setting PEEP as the lung is easily over-distended probably because of the not complete reabsorption of foetal fluid.

Finally, we also applied Zin measurements to the study of the effect of prone positioning as a potential tool for improving respiratory support and permitting adequate gas exchange with a less aggressive mechanical ventilation. Our results suggest that prone positioning does not offer significant advantages over supine positioning in the management of intubated and mechanically ventilated infants with RDS on short term basis. In patients with evolving BPD prone positioning is associated to lower Rrs values and could have a role in the clinical management of highly obstructed patient as it reduce the time constants of the respiratory system.

The data obtained from all these studies have provided important information also for understanding physiological mechanisms and for tuning algorithms and the approach to Zin measurement. Finally, the results of these studies emphasize the importance of customization and continuous adaptation of the treatment to the respiratory system condition. They provide a justification for the efforts required to integrate FOT into a mechanical ventilator in order to make Zin measurement possible in clinical practice. This will open the way to clinical studies evaluating the impact of this measure on clinical outcome of these small patients.

CHAPTER 3: NON-INVASIVE VENTILATION

Recently, there is an increasing interest in non-invasive ventilation not only for weaning from mechanical ventilation (MV) but as an alternative to it in the attempt to reduce VILI and the incidence of BPD. New modalities have been proposed in order to overcome the limits of the present technology and to prevent intubation. However there is still a need of studies investigating the mechanisms of functioning of these new forms of supports together with means to optimize non-invasive supports in general in order to avoid MV to the majority of the infants (Morley & Davis 2008).

Nasal continuous positive airways pressure (nCPAP) is the most diffused form of non-invasive support. It consists in applying a distending pressure to the respiratory system with the rationale of maintaining alveolar recruitment and airways patency. It is currently used as a primary form of support for preterm infants instead of intubation and MV. Efforts are being directed to improve its efficacy combining it with surfactant administration and evaluating different modalities of pressure generation. Also new interfaces with the infant have been designed to obtain less rigid and heavy nasal cannula that fit correctly the infant's nares without injuring them, minimising the dead space and therefore also the WOB required to the infant.

A new modality that is going further in the direction of a less invasive interface with the infant is called heated humidified high-flow nasal cannula (HHHFNC). It is emerging in a variety of clinical situations, thanks to its ease of use, better infants' tolerance, improved feeding and bonding. It consists in the delivery of a flow exceeding the patients' inspiratory flow (clinical range: 2-8 l/min) through not sealed nasal cannula. The working mechanisms of HHHFNC are not yet fully understood but the washout of the upper airways and the provision of a distending pressure are considered the most relevant ones (Locke et al. 2012; Spence et al. 2007; Dysart et al. 2009; Frizzola et al. 2011). However the pressure is generated in a different way in respect to nCPAP and it is not monitored. If it can play a role similar to nCPAP, requiring the same WOB to the infant is still to be verified. Therefore, in collaboration with Ospedale Mangiagalli di Milano, we compared the effect CPAP and HHHFNC on lung function in term of breathing pattern, resistance, dynamic compliance and WOB when the same level of distending pressure was generated. The results of the study support the use of this modality as, even if pressure is not the only mechanisms of functioning, it can be used also to generate a pressure with comparable effects to nCPAP.

The drawback of this tendency toward gentler interfaces with the infants is the increased difficulty in monitoring breathing signals. Therefore, even if in principle lung mechanics could play the same role in optimizing end expiratory pressure during these modalities than during invasive ventilation the not sealed connection with the infants makes it difficult to perform FOT measurements. Moreover during non-invasive modalities of respiratory support the activity of the infant plays a major role in the determination of EELV. In fact the absence of an ETT permits to the infant to actively elevate EELV also through the flow-breaking action of the larynx. Infant's efforts could compensate a low pressure at the cost of an increased WOB. A longer time of monitoring could be required to identify the optimal CPAP just on lung mechanics bases as short term changes could be masked by the increased infant's activity.

Variability analysis of breathing pattern has the advantage to be performed on different signals related to the respiratory system that can be measured non-invasively and it has been increasingly applied to the study of the respiratory system in infants. Information about control of breathing as well as mechanical properties of the thoraco-pulmonary system can be obtained.

Variability in the respiratory system has been found to be important for cell activity as it positively affects surfactant secretion (Arold et al. 2009; Arold et al. 2003); be related to the maturation of the breathing control in infants (U Frey et al. 1998; Engoren et al. 2009); be correlated with weaning success (Wysocki et al. 2006; Kaczmarek et al. 2013; Engoren 1998).

The application of pressure might modify EELV, lung mechanics, the input to the neural centres that arrives from the stress receptors, gas concentrations and consequently the variability of the breathing pattern. Goldman et al. showed that the application of a pressure at the airways opening modifies the multifractal properties of the intra breath interval in children (Goldman et al. 2008). Preterm babies could be more sensible to pressure levels as they have strong reflexes that play a large role in the control of breathing, immature control of breathing and they have to actively elevate EELV above the resting volume of the respiratory system. In newborns, variability of breathing pattern parameters has been found to be modified by assisted ventilation (Emeriaud et al. 2010). However, it is not known whether the nCPAP level influences breathing pattern variability in infants and if it can play a role in nCPAP titration. In collaboration with Ospedale San Gerardo di Monza and Boston University, to test this hypothesis, we applied variability analysis to the study of lung condition at different levels of nCPAP in preterm infants during their first day of life. In particular we looked at long term correlation present in EELV, V_{T} and RR by detrended fluctuation analysis (DFA). Between the different methods proposed to assess variability DFA has the advantages of non-requiring the stationarity of the data and distinguishing between intrinsic fluctuation generated by a complex system (exhibit long-range correlation) and those caused by external stimuli acting on the system (local effect, having characteristic time scales). For this first study we measured lung volume changes by OEP as, even if it is not suitable for clinical practice, it has the advantages not to be subject to drift, to provide different breathing pattern indices including EELV without assuming only two degree of freedom for the highly deformable infant chest wall or requiring a subject specific and difficult calibration. Moreover it has been shown that it permits accurate measurements in infants (Dellaca et al. 2010). We found that the application of a nCPAP that maximises oxygenation results in lower long term correlation in EELV, compared to the absence of nCPAP and to the highest nCPAP applied in this study. These preliminary results suggest that variability analysis of the breathing pattern could provide useful information for tailoring ventilatory support. Studying the variability on different time scale of different signals that can be easily obtained in clinical practice and quantified it by different metrics is a possibility worthy to explore.

CHAPTER 4: RESUSCITATION AT BIRTH

Crescent attention is focused on the identification of resuscitation strategies at birth that can optimise FRC establishment and realize a protective ventilation from the onset of ventilation.

In the transition from foetal life to air breathing, rapid clearance of foetal lung fluid and recruitment of alveoli are the critical steps for the survival of the newborn. Transpulmonary pressure plays a determinant role in this process (Hooper et al. 2007) and the application of a pressure to the airway opening helps preterm infants to remove the fluid and open closed alveoli. As once the alveoli are recruited less pressure is required to keep them open and to ventilate the lung, the application of recruitment manoeuvres at birth has the advantage to permit the onset of ventilation in the context of a more recruited lung, with a lower requirement of pressure, a more homogeneous distribution of lung volume and less stress applied to the tissue. On the other hand, at birth a surfactant-deficient lung can be injured even by few inflations with volumes that are probably harmless in other circumstances (Björklund et al. 1997; Wada et al. 1997). Therefore designing a protective strategy to recruit the lung without causing injury could have a great impact on the clinical outcome of the newborns.

Animal studies have shown that the application of a sustained lung inflation (SLI) followed by PEEP improve lung volume recruitment with respect to the conventional treatment consisting in intermittent application of pressure (te Pas, Siew, Wallace, Kitchen, Fouras, R. a Lewis, et al. 2009). The advantage of SLI could be linked to the application of pressure for a duration of time appropriate for the air/liquid interface to move into the distal airways. In fact, because of viscoelastance and other time-dependant phenomena, the tendency of previously collapsed lung units to open depends both on the applied transpulmonary pressure and duration of its application.

Despite these encouraging results on animal studies, efficacy and safety of such manoeuvres in infants are presently still under investigation as the optimal duration and level of initial inflation and subsequent level of PEEP remain unclear (Lista et al. 2012). The lack of knowledge about which is the best technique to recruit the lung (type of the manoeuvre, time and pressure settings) and its probable dependence on the specific characteristics of each lung has limited the diffusion of SLI in clinical practice.

The possibility of measuring lung condition during the manoeuvre and the availability of tools to customise it would lower the probability of injure the lung, favour the diffusion of recruitment manoeuvre at birth and permit the realization of a more protective ventilation strategy. It has been suggested that a lung volume targeted strategy could realise a more protective strategy than the application of fixed time/pressure SLI (te Pas, Siew, Wallace, Kitchen, Fouras, R. A. Lewis, et al. 2009; Tingay et al. 2013). However a strategy considering lung mechanics could have the additional advantage to be sensible to overdistantion. In particular, measurement of Zin by FOT has been proved to provide information on lung recruitment/distention (Dellaca et al. 2009) and has been successfully applied to the study of lung mechanics in ventilated preterm newborns (Dellacà, Veneroni, et al. 2013). In order to test the utility of FOT during resuscitation at birth, Zin measurements were tested in a preterm lamb at birth. Different strategy can be implemented on the bases of Zin and an intelligent device for the implementation and the comparison of these strategies in animal studies is currently under development.

However, even if the optimal recruitment strategy were known, it could not be applied in infants unless a FOT set up suitable for measurement during the critical moment of resuscitation at birth is available. Starting from a proper ventilator it would be possible to integrate a FOT set up into it without adding any hardware but only modifying the software. Therefore, in collaboration with Acutronic Medical System (Switzerland) we developed a set up that exploits FabianHFOV device for FOT measurements. In order to limit the changes of the ventilator software in this first phase we decided to connect a tablet to the ventilator and to implement part of the functionalities in an Android application. In particular: the ventilator generates the sinusoidal stimulus and measures flow and pressure, while the developed Android application handles Zin computation, data recording and the user interface for FOT measurements. This set up guarantees the safety of newborns during the manoeuvre and the possibility to support the respiration with mechanical breathes and requires only pressing a button to start Zin measurements. The accuracy of the measurements obtained with this set up was evaluated in vitro. After that, in order to test the feasibility of Zin measurements during recruitment manoeuvres, the impact of leakages and how to minimize them, the developed set up was used for a preliminary study on babies admitted to NICU for RDS in collaboration with Ospedale Mangiagalli di Milano. The main result of this study was that FOT allows monitoring lung mechanics changes during SLI in preterm babies. Leakages can be easily identified and the correspondent data can be discharged. In conclusion this set up represents a valuable tool to understand the role of SLI and recruitment manoeuvres in general in the establishment of FRC at birth. It could also provide useful information to guide the identification of a customised protective lung recruitment strategy.

Conclusions

Choosing how to support ventilation in a preterm newborn is a difficult task as his lung is highly vulnerable to injury and there is little knowledge about protective ventilation strategies. Customization and continuous optimization of the treatment from the first moment of life play a key part to avoid secondary injure. In particular, it is important to: i) recruit the lung at birth without overdistending it, ii) keep it open by applying the lowest pressure that prevents derecruitment during invasive or non-invasive supports and iii) adjust the settings according to the lung function changes related to lung growth and to the course of the pathology. However in order to achieve this, measurement tools available at bedside that can monitor lung function and provide useful information for optimizing the treatment are needed.

Therefore different methods and technologies have been developed to be applied during the various procedures and types of ventilatory support. In particular, both the assessment of lung mechanics by FOT and variability analysis of the breathing pattern by DFA have been proposed in the attempt to account for: i) the different measurement constraints entailed by the different modalities of treatment and ii) the complexity of the respiratory system that includes not only the mechanical system (lung, chest wall, respiratory muscles) but also the control of breathing and shows lag, cumulative, memory and disproportionate effects in response to treatments and stimuli.

In vivo studies have shown that the developed methods permit monitoring of lung function in infants and provide information useful to improve the tailoring of the ventilatory strategy in order to minimise the negative outcomes that permanently compromise the quality of the future life of premature infants.

INTRODUCTION

Preterm birth is a significant perinatal health problem across the globe, affecting approximately 9.6% of all births worldwide (12.9 million/year) (Beck et al. 2010). The preterm lung is structurally and biochemically immature and vulnerable to injury and this has a great impact in terms of associated mortality, short and long-term morbidity and financial implications for health-care systems (Petrou et al. 2011). The infant respiratory distress syndrome (IRDS) is by far the most common cause of respiratory distress in premature infants and a major contributor to neonatal mortality worldwide (Kamath et al. 2011). It is characterized by surfactant deficiency and consequently by the tendency of the lung to collapse. To counteract this tendency and to guarantee ad adequate amount of oxygen to the tissues these infants are treated with oxygen therapy and ventilatory supports. Although these treatments are often indispensable for the survival of patients with respiratory failure they can exacerbate lung injury and play a role in the development bronchopulmonary dysplasia (BPD), the most common chronic lung disease in childhood and one of the most severe long term complications of prematurity. In fact, BDP is not only a paediatric issue as substantial obstructive lung disease persists into adolescence and young adulthood (Baraldi et al. 2007; Fawke et al. 2010). From the histological point of view, BPD is characterised by alveolar simplification and enlargement, and by highly dysmorphic capillary configuration.

Several studies have suggested that combining less invasive care strategies that avoid excessive oxygen and ventilation may decrease the incidence and severity of chronic pathologies (Jobe 2011). There is evidence that the choice of the correct ventilation strategy is crucial from the first moment of life on. In fact it has been shown that even few inflations with volumes that are probably harmless in other circumstances can injure a surfactant-deficient lung at birth (Björklund et al. 1997; Wada et al. 1997). In addition, it has been pointed out that early ventilation strategies, during the apparently stable first days after birth, have significant implications on long term morbidity in patients with RDS (Morris et al. 2006).

For this reason, in the last years an intense research activity, both experimental and clinical, has examined different aspects of the ventilation practice in order to improve the treatments and provide a ventilation that is not only supportive but also, as far as possible, protective of pulmonary function and structure. Significant progresses have been made both in the definition of invasive ventilation modalities and in the technology employed in the ventilators. Together with the older more diffused forms of ventilation, different new promising modalities for treating newborns are now being studied. In the same time, new forms of non-invasive support are being developed and not only considered as complementary to invasive ventilation but they are increasingly seen as an alternative to intubation. Moreover, also new strategies for resuscitation at birth, employing recruitment manoeuvres are under investigation for helping newborns in reaching an adequate lung volume at birth in order to start ventilation in a contest of a more recruited lung.

However there is still a need of studies investigating the mechanisms of functioning of these new forms of supports together with means to optimize ventilation settings of both invasive and non-invasive supports as well as to guide resuscitation at birth. Some attempt have been made to identify the best range of pressure for supporting infants with specific pathologies and characteristics (Essouri et al. 2011; Khirani et al. 2013; Mathe et al. 1987) and to identify important features of recruitment strategy at birth (te Pas, Siew, Wallace, Kitchen, Fouras, R. A. Lewis, et al. 2009; te Pas, Siew, Wallace, Kitchen, Fouras, R. a Lewis, et al. 2009) according to the obtained lung volume, mechanics or fatigue indices. These studies, employing techniques that are not suitable for clinical practice, aimed to identify protective settings and procedures that could be applied for the treatment of infants.

However, the extreme heterogeneity of conditions of the respiratory system in newborns, together with the continuous changes the lung undergoes especially during the first weeks of life, makes it impossible to adopt a common strategy for all patients. To identify the general characteristic of a protective ventilation is just a first step for improving ventilatory outcomes. In order to implement a real protective ventilation strategy, the treatment has to be tailored and continuously adapted according to lung function of each infant.

Ventilatory settings are currently optimised on gas exchanges bases. However even if this permits to indirectly monitor lung recruitment, gas concentrations are the result of many processes (ventilation, diffusion, perfusion, circulation) and, therefore, they lack in specificity for detecting pressure-induced over-distension and tidal recruitment (Richard et al. 2004). Therefore, an optimization based on parameters that can specifically identify the best trade-off between minimal lung tissue distention and maximal lung volume recruitment could result in a more protective treatment. However it requires the availability of technologies for measuring and monitoring lung function at the bedside in clinical practice. Different techniques have been developed for lung function measurement but their assessment in infants in clinical practice is still a challenge. Adequate tools to guide the physician to identify optimal ventilatory settings during resuscitation at birth, invasive and non-invasive ventilation are still missing.

The aim of this work is to develop methods and technologies to monitor lung function at bedside during the different approaches of ventilatory support in preterm newborns to provide tools that improve the tailoring of the ventilatory strategy in order to minimise the negative outcomes that permanently compromise the quality of the future life of these infants.

1 LUNG FUNCTION AND VENTILATORY SUPPORTS IN PRETERM NEWBORNS

Choosing how to support ventilation in a preterm newborn is a difficult task as his lung is highly vulnerable to injury, there is little knowledge about optimal gas target levels and about the threshold for harmful stress and strain to lung tissue necessary to implement protective ventilation strategies and, moreover, there are no measurements available at bedside that can help the clinician to personalize the treatment.

Birth represents a critical event associated with dramatic changes in the lung function: the fetal lung fluid needs to be absorbed, the lungs have to be filled with air, adequate gasexchanging surface area has to be established, the pulmonary blood flow has to greatly increase and the surfactant system has to ensure the lungs remain expanded by decreasing the alveolar surface tension. Moreover preterm newborns are compounded by an incomplete structural and biochemical development, a lower capacity to reabsorb foetal lung liquid, lack of surfactant and increased thickness of the alveolar-capillary membrane that interferes with gas exchange. All these features make infants and especially the preterm ones very exposed to respiratory system injury (Hammer et al. 2005).

The goal of the ventilator support is to guarantee adequate amount of oxygen to the tissues and removal of carbon dioxide without injury the lung. Several studies have suggested that combining less invasive care strategies that avoid excessive oxygen and ventilation may decrease the incidence and severity of chronic pathologies (Jobe 2011). For this reason, an intense research activity, both experimental and clinical, has examined different aspects of the ventilation practice in order to improve the treatments and provide a ventilation that is not only supportive but also, as far as possible, protective of pulmonary function and structure. Target concentrations of O_2 and CO_2 in tissue have been reconsidered as a lower O_2 target and permissive hypercapnia permit to decrease the FiO_2 and the pressure applied to the respiratory system (Saugstad et al. 2011; Woodgate et al. 2001). Other aspects of the ventilatory treatment are under evaluation (Sweet et al. 2013). Together with the older more diffused forms of ventilation, different new promising modalities for treating newborns are now being studied. In the same time, new forms of non-invasive support are being developed and not only considered as complementary to invasive ventilation but they are increasingly seen as an alternative to intubation. Moreover, also new strategies for resuscitation at birth, employing recruitment manoeuvres are under investigation for helping newborns in reaching an adequate lung volume at birth in order to start ventilation in a contest of a more recruited lung.

However, even in a homogeneous group of infants for pathology and GA, the heterogeneity of lung conditions and clinical situations is very high. Moreover in the first weeks of life the respiratory system undergoes several fast and dramatic changes because of lung growth and the development of pathology. Therefore continuously adapting the treatment, optimizing it according to lung mechanics and condition of each infant, could permit a better implementation

of the concept of protective strategy. This approach has the potential to reduce ventilatory induced lung injury (VILI) and permit the achievement of the O_2 target with lower FiO₂. However it requires the availability of technologies for measuring lung function at the bedside in clinical practice. Different techniques have been developed for lung function measurement but their assessment in infants in clinical practice is still a challenge. However, the recent developments in technology together with new proposed methodologies and approaches open the way toward the availability at bedside of parameters that reflect lung function, the exploration of their usefulness and the identification of the ones that could guide the choice of the ventilatory treatment.

1.1 THE INFANT RESPIRATORY SYSTEM

Structural anatomical and physiological reasons make infants less able to cope with a given stress to the respiratory system than adults and older children. Moreover preterm birth leaves insufficient time to the lung to fully develop, predisposing these infants to the development of acute and chronic pathologies.

In particular infant respiratory distress syndrome (IRDS) is the most severe form of acute lung injury and is responsible for high rates of neonatal morbidity and mortality. It is characterized by surfactant deficiency and consequently by the tendency of the lung to collapse, which is counteracted in clinical practise by the application of positive pressures at the airways opening through mechanical ventilation. Although the ventilatory support is indispensable for the survival of these patients, it can contribute significantly to establish lung injury. Ventilator-induced lung injury (VILI) is recognised as one of the main causes of the development of a chronic lung pathology called bronchopulmonary dysplasia (BPD) which is characterized as an arrest of lung development at the gestational age of birth with significant pulmonary sequel during childhood and adolescence.

1.1.1 BASIS OF RESPIRATORY MECHANICS AND LUNG VOLUMES

Lung ventilation, the movement of air to and from the alveoli, represents the first step for respiration and has the function to convey enough oxygen to the gas exchange area and remove CO₂. The comprehension of the interaction between the gas flow, going into and out of the respiratory system, the lung volume and the pressure acting on lung and chest wall (i.e. of the mechanical behaviour of the respiratory system) is fundamental to understand the impact of ventilatory support on the respiratory system.

A simple mechanical model of the respiratory system includes four elements: airways, lung, chest wall and respiratory muscle (Figure 1 1). The properties of each of these elements and of the whole respiratory system and can be described by a lumped parameter electrical analogues model. The simpler model results from a resistance, a capacitance and an inductance in parallel representing respectively the resistance, compliance and inertance of the considered element or of the whole respiratory system. The values of these parameters can be obtained by measuring the volume and the pressure across the considered element. For example lung properties can be estimated measuring lung volume and the transpulmonary pressure (P_L).



Figure 1-1 Left: Schematic representation of the forces acting on the respiratory system. *PAO*: airways opening pressure, *PALV*: alveolar pressure, *PPL*: pleural pressure, *PBS*: body surface pressure, *PMUS*: pressure generated by the respiratory muscles, *PL*: transpulmonary pressure, \dot{V} : flow, *VL*: lung volume, AW: airways, T: tissue, L: lung, CW: chest wall, MUS: muscles. Right: Electrical model. It can be applied to a single element or to the whole respiratory system. *R*: resistance, *C*: capacitance and *L*: inductance representing respectively the resistance, compliance and inertance of the considered system.

THE STATIC PRESSURE-VOLUME (P-V) CURVE

As lung and chest wall are distensible structures their volume depends on their elastic properties and their distending pressures. There are different reasons for the elastic behaviour of the lung: elastic tissue constituted by fibres of elastin and collagen; compressibility of the gas and the surface tension at the alveolar air-liquid interface which is reduced by the presence of surfactant (a tension-active lipoprotein formed by type II alveolar cells).

The elastic behaviour of the whole respiratory system is described by its static pressurevolume (P-V) curve that results from the sum of the P-V curves for the chest wall and the lung (Figure 1-2 A).The volume resulting from the static passive balance between the outward recoil of the chest wall and the inward recoil of the lung is the resting volume of the respiratory system or functional residual capacity (FRC). The slope of the P-V curve is known as static compliance and describes the distensibility of the respiratory system: the steeper the slope, the grater the distensibility.

This curve has a sigmoid shape: at low lung volumes compliance is low, at the centre of the curve compliance increases, and finally at high lung volumes, above the upper inflation point (UIP), the lung gets stiffer opposing further volume increases. In the pressure-volume curve of the lung the inflation and deflation limbs are different: the lung volume at any given pressure during deflation is larger than during inflation (Figure 1-2 B). This behaviour is known as hysteresis and it is due to the viscoelasticity of lung tissues.

Changes of the elastic properties of lung or chest wall modify this curve. In particular three abnormalities are typical of pathologies characterised by surfactant deficiency: the appearance of a lower inflection point (LIP), which corresponds to the opening pressure of the collapsed alveolar units; reduction of the slope of the ascending limb, which indicates the loss of lung aeration; and lowering of the volume that corresponds to the upper inflection point, which increases the risk over-distension induces by mechanical ventilation (Fisher et al. 1980; Gerhardt et al. 1987).



Figure 1-2 A) P-V curves for the chest wall and the lung (continuous line) and their sum that represents the P_V curve of the whole respiratory system (dashed line). FRC is the resting volume of the respiratory system. B) Static P-V curve of the respiratory system in a healthy lung (grey dashed line) and in a lung with lack of surfactant (black solid line). LIP and UIP represent the Lower Inflation Point and the Upper Inflation Point respectively. Adapted from (Moloney 2004).

THE EQUATION OF MOTION OF THE RESPIRATORY SYSTEM

The balance of forces acting on the respiratory system during dynamic conditions as during breathing is regulated by the equation of motion of the respiratory system:

$$P_{AO} + P_{MUS} = \frac{V}{C_{rs}} + R_{rs} \cdot \dot{V} + \dot{I}_{rs} \cdot \ddot{V}$$
(1.1)

 P_{AO} and P_{MUS} are the pressures applied to the open airways and generated by the respiratory muscles, while V, \dot{V} and \ddot{V} are the volume of the lung above end-expiratory volume and its time derivatives, flow and acceleration, respectively. Pressure and volume (with its time derivatives) represent the state variables of the respiratory system.

The equation reported above describes the behaviour of respiratory system as a simple linear system with the dynamic compliance (C_{rs}), resistance (R_{rs}) and inertance (I_{rs}) being the parameters of the equation and describing the mechanical characteristics of the respiratory system.

Dynamic compliance describes the combined distensibility of the lung and chest wall. In a more general model, dynamic compliance is dependent on tidal volume amplitude and frequency and it is lower than the static compliance.

Resistance represents the opposition to flow due to frictional forces. It is the sum of the tissues' and chest wall's viscous resistance and the airways resistance due to the relative movement between the gas molecules and the airways walls.

As the resistance of the respiratory system is rarely linear the term $R_{rs}\dot{V}$ in Eq. 1.1 can be replaced by term of Rohrer's equation:

$$K_1 \cdot \dot{V} + K_2 \cdot \dot{V}^2 \tag{1.2}$$

Where K_1 and K_2 are called the Rohrer constants.

 K_1 is directly proportional to the viscosity of the gas and the length of the conducting airways and inversely correlated to the fourth power of their internal diameter.

 K_2 is proportional to the density and viscosity of the gas, to the length of the conduction airways and inversely proportional to the fifth power of their internal diameter.

The first term of the Rohrer equation describes the relationship between pressure and flow in laminar regime while the second accounts for turbulent flow. The presence of laminar or turbulent flow is related to the Reynolds number; in general, when it is above 4000 a flow is considered completely turbulent while when it is below 1500 completely laminar.

Reynolds number is directly proportional to the fluid velocity, the gas density and the diameter of the conducting airways while inversely correlated to the viscosity of the gas.

As the sum of the internal diameter of the airways increases dramatically as they extend towards the periphery, K_1 and K_2 decreases markedly and total respiratory system resistance results manly associated to the resistance of proximal airways.

Inertance is a measurement of the tendency of the respiratory system to resist changes in flow. In the range of frequencies reached during spontaneous breathing and conventional mechanical ventilation inertance can be neglected.

LUNG VOLUMES

Different lung volumes can be defined (Figure 1-3) and from the PV curve of a subject is possible to know the pressure required to reach a specific volume. In pathological lungs higher pressure are usually required.



Figure 1-3 Left: Volume trace over time during normal breathes and a maximal expiration and inspiration with reported the different lung volumes. Right: P-V curve of the respiratory system for healthy and pathological lung with overimposed the volume changes during tidal volume.

The tidal volume (V_T) is the gas volume change during a breath. The inspiratory reserve volume (IRV) is the maximum volume that can be inspired from an end-tidal inspiratory level while the expiratory reserve volume (ERV) is the maximum volume that can be expired from the end-expiratory level. The inspiratory capacity (IC) is the maximum volume that can be inspired from the end-expiratory level and the vital capacity (VC) is the volume of the deepest breath.

The residual volume (RV) and the total lung capacity (TLC) are the volume of gas in the respiratory system after as much gas as possible has been exhaled or inhaled respectively. The functional residual capacity (FRC) is the volume of gas in the lungs and airways either at the end of spontaneous expiration or at the resting volume of the respiratory system.

1.1.2 PRETERM BIRTH AND EFFECTS ON THE RESPIRATORY SYSTEM

The development of the lung covers a period that begins with the appearance of lung bud in embryo and ends in the first infancy.

Birth is not the end of development of the respiratory system and does not correspond to a definite transition from one developmental stage to another but represents a critical event associated with dramatic changes in the lung function.

During fetal life, the lung is filled with liquid and receives only a small fraction of cardiac output. When the organ of gas exchange, changes from the placenta to the lung, the fetal lung fluid needs to be absorbed, the lungs has to be filled with air, there has to be an adequate gasexchanging surface area, the pulmonary blood flow has to greatly increase and the surfactant system has to ensure the lungs remain expanded by decreasing the alveolar surface tension.

The birth that occurs before the 37th complete week of gestation is defined "preterm". It therefore comprises a very heterogeneous population, with a wide range of gestational age, associated with very different outcomes.

The preterm lung is characterised by an incomplete structural development, a lower capacity of liquid reabsorption, functional immaturity due to surfactant deficiency and increased thickness of the alveolar-capillary membrane that interferes with gas exchange.

With extreme preterm birth (GA<27wk) the dense vascularization of the mesenchyme is just at the beginning and there is still little differentiation of alveolar epithelium cells of type I and II. This results in a minimal pulmonary functionality. Also if the birth occurs between 27th and 37th week of GA, the lung is not yet completely developed and this results in the presence of an airblood barrier that has just begun to dwindle, a vascularization still incomplete and immature type II cells. The birth at this level of development results in the presence of simplified distal pulmonary acini with reduced number of alveoli, bronchial smooth muscle hyperplasia, abnormal capillary morphology and variable interstitial cellularity and fibroproliferative level (Joshi et al. 2007).

Apart from the immediate effects of birth, there is a considerable remodelling of each of the structural lung components during the first periods of life. Airways, alveoli and blood vessels increase in number as well as size with different patterns of growth. Also the structure of the rib cage and the respiratory muscle undergo modification to reach their complete development (Merkus et al. 1996).

<u>Central control of breathing</u>. The infants' breathing pattern is irregular with considerable breath-to-breath variability and periodic breathing at times. The central control of breathing has to adapt to air breathing as the fetal qualitative and quantitative responses to hypercapnia or hypoxia are different from the adult ones. Moreover complete postnatal adaptation of the peripheral chemoreceptors to arterial oxygen tensions does not happen instantaneously after birth and requires time.

A considerable amount of maturation of control of breathing occurs in the last few weeks of gestation, which explains the high prevalence of apnoea in infants born prematurely.

<u>Upper and lower airways</u>. Airways resistance is higher than in adults because of the smaller size and the higher collapsibility of the airways. In fact the larynx, trachea and bronchi are considerably more compliant and therefore highly susceptible to distending and compressive forces while the activity of the muscles responsible for maintaining upper airway patency is decreased as the elastic recoil provided by the alveoli. Thus, during forced respiratory efforts, in presence of any obstruction of the upper airway, significant dynamic inspiratory collapse of upper airway structures can occur, while, in presence of lower airway obstruction dynamic expiratory lower airway collapse can occur further limiting expiratory flow (Hammer et al. 2005).

The airways of a child are relatively large in comparison with those of an adult but in absolute terms they are small, and minor changes in the airway radius create a much larger increase in resistance to airflow, since the resistance increases by the fourth power of any reduction in

radius. In the adult lung, peripheral airways provide a large cross-sectional area and thus contribute less than 20% to total airway resistance. Conversely, small peripheral airways contribute about 50% to the total airway resistance in the infant lung. Therefore, diseases that affect the small airways and cause large changes in peripheral resistance may be clinically silent in an adult, but can cause significant problems in infants (e.g., bronchiolitis).

<u>Lung parenchyma</u>. Alveolar development starts relatively late in the 7th month of intrauterine life. The alveoli, as they exist in adults, do not begin to appear until about 8 weeks of age and then the increase in alveolar number and the maturation of the elastic fibres require years to be completed. Moreover the lack of collateral ventilation prevents alveoli beyond obstructed airways to be ventilated and predisposes the infant to the development of atelectasis. In addition, in preterm newborns because of the lack of surfactant: tissue stiffness is increased, alveolar stability reduced and alveolar collapse and absorption of fluid into the alveolar space cannot be prevented. This leads to low specific lung volume and low lung compliance with a thickened alveolar-capillary membrane that impairs gases exchange.

<u>Chest wall</u>. The chest wall is highly compliant (at least 5 times higher than the lung) because of non-ossified ribs and of low muscular tone. It has reduced outward elastic recoil, therefore the relaxation volume of the respiratory system (see paragraph 1.1.1) is low making the infant susceptible to alveolar instability, airways collapse and atelectasis.

Therefore infants have to constantly re-establishing lung volumes with crying and movement and actively elevate the end-expiratory lung volume above the elastic equilibrium volume. This is achieved mainly by three energy-consuming mechanisms: high respiratory rate with insufficient time to exhale to the elastic equilibrium volume; laryngeal adduction during exhalation to increase the resistance to airflow, and post-inspiratory diaphragmatic muscle activity.

Moreover the respiratory pump is poorly effective because of two main reasons: the ribs are aligned horizontally allowing for less anteroposterior movement during respiration; the high compliance of the chest wall and the more horizontal angle of insertion of the dome-shaped diaphragm at the lower ribs produces an inward distortion of the ribs rather than shucking air in when the respiratory muscles contract.

<u>Respiratory muscle</u>. At birth, the respiratory muscles appear to be histochemically poorly equipped to sustain high workloads, and the majority of the muscles mass are type II 'fast twitch' fibres. Maturational changes in the respiratory musculature occur, with increased mass and a progressive increase in the fatigue-resistant type I muscle fibres. In addition, premature infants have to undergo a considerable amount of maturational changes in respiratory muscle coordination of the upper airway muscles and the diaphragm which make them susceptible to obstructive sleep apnoea.

The mentioned physiological and anatomical differences between infants and adults, together with the newborns' larger surface area to body weight ratio, higher basal metabolic rate and energy requirements for growth, make newborns less able to cope with a given stress to the respiratory system (Hammer et al. 2005). All these differences are particularly evident and marked for preterm newborns, who thus are more prone to respiratory failure.

1.1.3 INFANT RESPIRATORY DISTRESS SYNDROME

The infant respiratory distress syndrome (IRDS), also called hyaline membrane disease, is by far the most common cause of respiratory distress in premature infants and a major contributor to neonatal mortality worldwide (Kamath et al. 2011).

The pathophysiology is highly complex. Immature type II alveolar cells produce less surfactant, causing an increase in alveolar surface tension and a decrease in compliance. The resultant atelectasis causes pulmonary vascular constriction, increases ventilation perfusion mismatch and

hypoperfusion. In fact the alveoli containing surfactant tend to hyperexpand when the surrounding surfactant-free alveoli collapse, creating a nonhomogeneous distribution of gas in the lung. The hyperexpanded areas tend to be poorly perfused due to the flattening of the pulmonary capillaries while hypoventilated areas are, in contrast, hyperperfused.

The severity of surfactant deficiency also influences the development of pulmonary oedema.

This situation results in lung tissue injury which is mediated by a marked pulmonary inflammatory response. The expression of the acute injury is the production of hyaline membranes form through the combination of sloughed epithelium, protein, and oedema which further hinder gas exchange (Avery et al. 1959).

In order to prevent collapse and the development of atelectasis, positive end-expiratory pressure (PEEP), continuous positive airways pressure (CPAP) or continuous distending pressure (CDP) may be applied and relatively high ventilator pressures may be required (Sweet et al. 2007).

1.1.4 BRONCO-PULMONARY DYSPLASIA

The bronco-pulmonary dysplasia (BPD) is the most common chronic lung disease in childhood and one of the most severe long term complications of prematurity. Its incidence depends on different factors (medical centre, years considered, diagnostic criteria applied). To have an idea of its prevalence, it was found to affect the 68% of the infants in a study involving 9575 infants extremely low GA (22–28 weeks) newborns between 2003 and 2007 (Stoll et al. 2010).

Infants with BPD have significant pulmonary morbidity during childhood and adolescence. These children have more chest deformities, more asthma, and more respiratory symptoms than the other children (Fakhoury et al. 2010; Balinotti et al. 2010; Filippone et al. 2012).

Definition and classification of this pathology are difficult tasks. Nowadays BPD diagnosis is generally made on the base of oxygen dependency at specific postnatal ages (Jobe et al. 2001). This definition has some limitations: even if the oxygen level is influenced also by the medical centre and by pharmacological treatment, it is involved in both pathogenesis and treatment of BPD and it does not reflect necessary a chronic stage.

Lung characteristics of infants with BPD have been changing with progress in neonatal care such as extensive introduction of antenatal glucocorticoids, surfactant treatment and gentler ventilation strategies, so that it is possible to distinguish between an "old" and a "new" form of BPD. The "new" BPD is characterised from the histological point of view by alveolar simplification and enlargement and by highly dysmorphic capillary configuration, whereas interstitial cellularity and fibroproliferation are variable and generally less represented than in the "old" form of BPD.

BPD is a multifactorial pathology and presents a complex pathogenesis, resulting from dynamic processes involving inflammation, injury, repair and maturation. The abnormal lung growth is consequent to various injuries to the foetal and preterm lung but the associations of a single causal factor with BPD is confounded by the complex relationships of other interdependent factors that have played a role in the single infants' lung development.

Inflammatory processes are known to play a key role in the development of BPD and the main risk factors include, apart from prematurity and subsequently incomplete lung development, prenatal and postnatal infections, supplementary oxygen therapy and mechanical ventilation (Speer 2006; Kallapur et al. 2006; Desai et al. 2007; Saugstad 1997).

In particular strong correlations between BPD and days of mechanical ventilation and oxygen therapy have been reported (Mailaparambil et al. 2010; Thoresen et al. 1988; Sampath et al. 2009; Saugstad 2010; Cerny et al. 2008).

Genetic factors have also a role in BPD development. Up to now genomic candidates have been identified but no particular gene or gene pathway are jet inferred to be contributing substantially to the underlying etiology of BPD (Shaw et al. 2013).

Stem cell therapy are under investigation for injury repairmen (van Haaften et al. 2009; Aslam et al. 2009) but to decrease the incidence and the severity of the pathology preventive care strategies that could reduce lung injury are fundamental (Jobe 2011). These include non-invasive ventilation, advanced invasive ventilator management, avoiding prolonged oxygen exposure and treatment of the associated inflammation.

1.1.5 FACTORS INFLUENCING LUNG GROWTH

The development of the lung starts in the third week of embryonic life and continues into postnatal life until after adolescence (Joshi et al. 2007; Merkus et al. 1996).

A comprehensive understanding of the genetic and environmental factors influencing lung growth and lung function is challenged by the need to separate the interdependent effects of genetic predisposition, premature birth, neonatal respiratory disorders, treatment strategies on the growing lung and the environment.

Figure 1-4 shows some factors that could affects lung growth before and after birth.



Figure 1-4. Factors influencing lung growth. Dashed circles represent possible medical treatments and their possible drawbacks. Mechanical ventilation and oxygen therapy are lifesaving for preterm infants but they can lead to VILI and oxidative stress (Speer 2006). Prenatal and postnatal steroids are administered to stimulate the maturation of the respiratory system but can partially modify lung growth (Vyas et al. 1997).

1.2 VENTILATORY STRATEGIES

There is a diffuse awareness of the importance of a protective ventilation strategy for newborns, however how to implement it in the clinical practice remains controversial. Several progresses have been made in the last years both in the definition of invasive ventilation modalities and in the technology employed in the ventilators. Together with the older more diffused forms of ventilation, different new promising modalities for treating newborns are now being studied. In parallel, different modalities of non-invasive ventilation have been proposed for weaning but also as an alternative to invasive ventilation in the attempt to reduce VILI. Also new strategies to help preterm infants to establish adequate FRC right at birth have been evaluated in order to start ventilation of a more recruited lung.

However there is still a need of studies investigating the mechanisms of functioning of these new forms of supports together with means to optimize ventilation settings of both invasive and non-invasive supports as well as to guide resuscitation at birth. Moreover because of the great inter-patient heterogeneity and the continuous changes due to growth and pathology development of the respiratory system functionality in newborns, a strategy that permits the customization of the treatment would realise a more protective ventilation. In order to achieve this, parameters that could track changes in the respiratory function during ventilator setting titration and that can be measured non-invasively in the clinical practice should be identified.

1.2.1 MECHANICAL VENTILATION

When, for any reasons, the respiratory muscles cannot provide the necessary pressure for respiratory flow, lung ventilation must be guarantee by artificial ventilation.

Negative pressure ventilation is carried out by the application of a direct force to the chest. During inspiration the chest wall is exposed to sub-atmospheric pressure which lowers intrapleural pressure and allows air to enter the lungs while the elastic coil of the chest and lungs leads to passive exhalation when the pressure around the chest wall returns to atmospheric levels. This technique has the advantages that it can be used without tracheotomies or tracheal tubes and it is the most physiological one, but requires keeping the patient's chest in a cabinet therefore today the most used form of mechanical ventilation is positive pressure ventilation.

<u>Conventional mechanical ventilation (CMV)</u> refers to the application of positive pressure waveform at the airways opening to provide or support the patient's minute ventilation.

The principles of the CMV are governed by the equation of motion of the respiratory system (Eq. 1.1), which states that to deliver adequate tidal volumes and inspiratory flow rates a pressure is required to overcome elastic, resistive and inertial forces. Since flow and volume are function of each other, the mathematical form of just one of the three state variables (pressure, volume and flow) can be predetermined, making it the independent or control variable and making the others the dependent variables.

Conventionally the ventilatory cycle is divided into four phases (the change from expiration to inspiration, inspiration, the change from inspiration to expiration, and expiration) that allow examining how a ventilator starts, sustains and ends an inspiration and what it does between consecutive inspirations. According to this convention, phase variables can be divided into trigger variables, limit variables, cycle variables and baseline variables. The trigger variable controls the onset of inspiration; the limit variable sets an upper bound for pressure, volume or flow; the cycle variable is the one that, once it has reached a pre-set value, makes expiration start; and baseline variable is the one that is controlled during expiration. In some modalities there are also conditional variables: before each breath the ventilator controls if the value of

those variables is above a pre-set threshold and according to that selects one breath pattern or another.

It is possible to differentiate between *controlled ventilation*, in which the ventilator does all the work of breathing, and *assisted ventilation* in which the ventilator contribute in various extents to the work of breathing.

Spontaneous breaths are those initiated and terminated by the patient. On the contrary, if the ventilator determines either the start or end of inspiration, than the breath is considered mandatory. In the same ventilator modality different types of breaths can occur (R.L.Chutburn 2006).

A diffused alternative to CMV is represented by the <u>high frequency oscillation ventilation</u> (<u>HFOV</u>). A constant distending airways pressure is applied over which small tidal volumes are superimposed at a super-physiological rate. Even though tidal volumes are usually below anatomical dead space, other gas transport mechanisms in addition to direct ventilation and bulk convection ensure adequate oxygenation and CO_2 removal with higher end-expiratory and lower peak inspiratory alveolar pressures than conventional mechanical ventilation. These mechanisms include pendelluft, which is the gas exchange between adjacent alveolar units with different time constants; convective change due to asymmetrical velocity profiles; Taylor dispersion, the mixing that occurs along the front of a high-velocity flow profile; and molecular diffusion that occurs within the individual alveolar units (Pillow 2005). It is considered a lung protective ventilation modality because of its low tidal volume but up to now there is no evidence of significant improvements in comparison with CMV (Cools et al. 2009; Cools et al. 2010).

CONVENTIONAL VENTILATIONS' MODALITIES

Different ventilation modalities with different advantages and drawbacks are defined depending on the combination of the chosen control, phase and conditional variables.

For treating neonates pressure control ventilation (PCV) has been usually preferred over volume control ventilation (VCV) as during VCV high inspiratory peak pressure may be reached and volume control may be complicated by the compressible volume of the ventilator circuit and requires correction for the air leaks due to the uncuffed endotracheal tube (Young et al. 1990). However, during PCV the delivered volume depends on lung compliance and may result insufficient to guarantee the correct exchange of gas or excessive and may damage the lung.

Several progresses have been made in the last years both in the definition of ventilation modalities and in the technology employed in the ventilators. Together with the older more diffused forms of ventilation, different new promising modalities for treating newborns are now being studied.

Commercial ventilators offer a range of ventilation modalities slightly different among the ventilator manufacturers and sometimes the same ventilation modality has different names generating confusion. Up to now, it has not been proved that a ventilatory modality is better than another and there are still more questions than answers about the most protective ventilation strategy. In clinical practice, the choice of one mode over the others depends on the infants' characteristic and on the clinician's experience and familiarity with a specific mode.

One of the most diffused PCV modality is the intermitted positive pressure ventilation (IPPV), during which inspiration is induced by raising the airways pressure intermittently above the PEEP. Intermittent mandatory ventilation (IMV) is similar to IPPV but the patient is allowed to breathe spontaneously between the ventilator strokes. These two modalities are especially used in their synchronised form. Synchronised modes of ventilation (S-IPPV, also known as assist/control A/C; S-IMV) permit to achieve adequate gas exchange at lower inflating pressure, reduce risk of pneumothorax and intra-ventricular haemorrhage and prevent rapid respiratory muscle atrophy (Bernstein et al. 1996; Anzueto et al. 1997). Not only the initiation but also the

termination of ventilator inflations is determined by the infant's efforts during pressure support (PS) which can have some advantage as weaning mode.

Recently, especially thanks to the technological improvement of ventilators that permit to accurately measure tidal volume, volume target ventilation (VTV) is widespreading. Depending on the ventilator, one or more of the peak inspiratory pressure, inflation time and inspiratory flow are adjusted to obtain the set volume. Pressure regulated volume control (PRVC) and volume Guarantee (VG), eventually used in combination with other modalities as SIPPV (SIPPV+VG), are some of the VTV modalities that aims to combine the advantages of PCV and VCV. They try to provide the newborn infant with, on average, a more stable assisted tidal ventilation from breath to breath, free from the perturbations in tidal volume that PCV produces while limiting the risk of developing high pressures of VCV. It has been shown that VTV reduce death/BPD, duration of ventilation, pneumothoraces, hypocarbia and periventricular leukomalacia/severe intraventricular haemorrhage (Wheeler et al. 2011; Klingenberg et al. 2011; Wheeler et al. 2010). However at present, data are lacking on the adequate V_T to be targeted by the clinician during the different phases of lung disease in preterm infants.

With the growing awareness of the importance to adapt the support continuously to the variable and instable respiratory condition of the infants, automatic modes of respiratory support have been developed to adjust some ventilatory parameters as FiO_2 (Claure, D'Ugard, et al. 2009; Claure et al. 2001) and respiratory frequency (Claure et al. 1997; Claure, Suguihara, et al. 2009). However other studies are needed before the introduction of them in the clinical practice (Laney et al. 1997; Claure et al. 2013)(Bancalari et al. 2013).

Although during natural breathing respiratory rate and tidal volume vary appreciably; during mechanical ventilation they are fixed. Variable ventilation (VV) is a ventilation modality which provides variable tidal volume or peak pressure adding noise to the set pressure or tidal volume settings in order to mimic the natural variability of the respiratory variables. Its theoretical bases are linked to the noise-enhanced amplification of a useful signal in a non-linear system by stochastic resonance. In a stochastic system, increasing of a small amount the standard deviation of the noise permits the amplification of a weak input improving the signal to noise ratio, in a similar way including appropriately designed noise in mechanical ventilators improves gas exchange (Suki et al. 1998). Animal studies have shown that VV improves ventilation of lung injury (Berry et al. 2012; Pillow et al. 2011).

Another field of resource is related to proportional ventilation. In these modalities the pressure generated by the ventilator is proportional to the effort of the patient. In this way the load to the respiratory muscles is reduced while the spontaneous breathing pattern can be reproduced. Not only the breathing frequency but also each breath morphology is controlled by the infant, realizing the ventilation chosen by its center of breathing control. The effort of the patient can be estimated indirectly by measuring flow and volume (PAV: proportional assist ventilation) or directly by measuring the electrical activity of the diaphragm (Edi; NAVA: neural adjusted ventilatory assist). NAVA permits better synchronization and trigger also breathes with under threshold volume for PAV. This modality is more diffused as non-invasive ventilation modality when accurate mouth flow measurements are very complicated (see the following paragraph). However, the role of these types of ventilation is still to be defined for preterm infants with an immature control of breathing.

Together with the choice of the ventilation modality, the difficult task of setting ventilation parameters is to be faced.

SETTING OF VENTILATORY PARAMETERS

During CMV, depending on the ventilatory modality, a parameter can be set and imposed for each breath or result from the combination of other settings.

All the ventilation parameters are interdependent and the choice of the best parameters' combination for the individual infant is a difficult challenge as it must result in an adequate gas exchange without injuring the lung. Moreover, as the newborn lung condition is likely to be instable, the ventilatory parameters are to be continuously adjusted accordingly, in a dynamic process which lasts until the end of artificial ventilation.

Presently, parameters are set on base of gas exchange, chest radiography and subjective evaluation of the infants' conditions as no other information are available at bedside. However lung volumes or, and especially, mechanics targeted strategies may be more protective and efficient, as they could follow changes in the properties of the lung, finding continuously the best compromise between the pressure needed for lung recruitment and ventilation and the stress applied to the tissues.

If the compliance of the respiratory system increases (or decreases) the equilibrium tidal volume and the time constant increase (or decrease) proportionally. Changes in resistance have effect on the time constant and affect the equilibrium tidal volume, not directly, but through changes in the flow rate. These effects apply not only to the whole lung but also to regions which may have different compliances, resistances and time constants. Therefore, any therapy, as the surfactant one, that rapidly changes lung mechanics may also results in lung damage if there are not changes in the ventilatory management to reflect the altered characteristics.

The main parameters for conventional mechanical ventilation are the following:

<u>Fraction of inspired oxygen (FiO₂)</u>. Inhalation of any gas with a fraction of inspired oxygen higher the FiO₂ of room air can potentially be toxic. To minimize the risk of toxicity, the lowest FiO₂ that achieves adequate oxygenation should be employed (Gitto et al. 2009).

<u>Positive end expiratory pressure (PEEP)</u>. PEEP is applied to prevent the collapse of alveoli and terminal airways during expiration as it changes FRC in accordance with the P-V curve of the respiratory system (Figure 1-3) and reduces airways resistance in accordance to the inverse relationship between lung volume and airways resistance and improves gas exchange.

It was also shown that the application of PEEP in preterm neonates produces a more regular breathing pattern thanks to chest wall stabilization and reduction of thoracic distortion (Michna et al. 1999; Herman et al. 1973; Argiras et al. 1987). However, too high levels of PEEP may injury the lung overdistending the alveoli and increasing pCO_2 as the dead volume to tidal volume ratio increases. Moreover, although low levels of PEEP decrease pulmonary oedema and right-to-left cardiac shunting, high levels reduce venous return and hence reduce cardiac output and pulmonary perfusion and enhance ventilation-perfusion mismatching, resulting in a lower pO_2 (Ricard et al. 2003). The lack and the importance of a method to guide selection of appropriate PEEP levels for infants with RDS or BPD was the conclusion of recent review about PEEP setting (Bamat et al. 2012).

<u>Tidal volume ($V_{\underline{T}}$), positive inspiratory pressure (PIP), respiratory rate, inspiratory-expiratory</u> <u>time ratio (Ti:Te)</u>. All these variables are deeply connected to each other and only a subgroup of them can be set at a time.

Low tidal volumes may lead to decreased ventilation and an elevated arterial partial pressure of carbon dioxide (pCO₂). On the contrary high tidal volumes can cause increased endothelial and epithelial permeability, altered lung fluids balance and significant tissue damage. In pathological lungs, because of lung heterogeneity, areas of normal tissue are interspersed with collapsed or edematosus zone. The ventilated part of the lung is thus reduced respect to a normal lung and is more prone to overdistension at traditional tidal volumes (Gattinoni et al. 1987). PIP requirements are determined by the desired tidal volume, the set PEEP, the inspiratory time and the compliance of the respiratory system: the stiffer the lung or the lower the inspiration time, the greater PIP required.

For controlled ventilation, the respiratory rate equals the total number of ventilatory breaths the patient will receive while for synchronized ventilation, the rate represents the minimal number of breaths; depending on the inspiratory sensitivity set, the patient may initiate more than the minimal amount.

Prolonging the inflation time will increase the MAP and is an alternative to other manoeuvres such as increasing PIP. A prolonged inflation time, however, will predispose to lung overdistension and gas trapping, as it is likely to reduce the expiratory time unless the rate is altered to account for the change. For these reasons the expiratory time usually exceeds the inspiratory one regardless of the rate strategy used in order to prevent inadvertent alveolar over-distension (Connors et al. 1981).

<u>Mean airway pressure (MAP).</u> MAP is the mean pressure applied to the respiratory system and it results from PEEP, PIP and Ti:Te settings.

<u>Pressure waveform.</u> Most infant ventilators provide means to modify the inspiratory pressure pattern (rectangular, exponential, ramp and sinusoidal waveforms) for some ventilation modalities. Changing the waveform without changing PEEP, PIP, respiratory rate and Ti:Te impacts on MAP, the required flow and the delivered tidal volume.

<u>Sensitivity</u>. If the trigger sensitivity is set too high the detection of patient effort can fail while with a low trigger sensitivity, the ventilator may deliver breaths too frequently (commonly referred to as auto-cycling) and produce severe respiratory alkalosis (Gurevitch et al. 1989).

<u>Gain control.</u> During proportional ventilation PEEP and gain control are the only parameters to be defined. It represents the proportional factor between patient's efforts and pressure delivered by the ventilator. It is decreased progressively for weaning.

Different parameters are to be set during HFOV:

<u>Oscillatory frequency</u>. The oscillatory frequency influences the delivered tidal volume and the attenuation of pressure swings down the endotracheal tube (ETT). Higher frequencies result in lower tidal volumes and in lower magnitude of the alveolar pressure swings. Frequency selection should minimize pressure swings while not reducing V_T at the extent that CO_2 removal is compromised. Oscillatory frequencies between 8 and 15 Hz are most often used in newborns (Pillow 2005).

<u>Peak-to-peak pressure</u>. Changes in pressure amplitude are used to control ventilation and in particular the pCO_2 value. Increasing pressure amplitude, and thus tidal volume, improves carbon dioxide elimination. To avoid hypocapnia, the peak-to-peak pressure should be adjusted to the lowest value consistent with normocapnia that produces visible chest vibrations (Pillow 2005).

<u>Inspiratory-expiratory time ratio (Ti:Te) and pressure waveform</u>. Traditionally clinicians used asymmetric ratios to avoid gas trapping and the associated dangers of hyperinflation, air leak, and cardiovascular compromise during HFOV. However, the use of Ti = 33% results in a drop in the mean intrapulmonary pressure. Ventilation is also influenced by the pressure waveform delivered at the airways opening as it can have one (sinusoidal shape) or more frequency components. When asymmetric Ti:Te ratios are used, the fundamental frequency will be different during inspiration and expiration (Pillow 2005).

<u>Continuous distending pressure (CDP).</u> CDP is the primary variable affecting lung volume and oxygenation. Because distal airway pressure changes are minimal during HFOV, CDP may be considered similar to the PEEP level in conventional ventilation and high/low CDP levels can injure the lung as low/high PEEP levels do.

VENTILATION INDUCED LUNG INJURY (VILI)

Mechanical ventilation, independently of the ventilatory mode, results in forces applied to the respiratory system. These forces can injure the lung depending on the stress and strain magnitude applied to the tissue and the duration of ventilatory support. The additional damage cause by mechanical ventilation is known as ventilation-induced lung injury (VILI). During ventilation of an infant lung, VILI is even more dangerous as it contributes to the development of chronic diseases and alteration of lung development (Zoban et al. 2003).

It is well known that VILI magnify the severity of lung injury by causing mechanical damages and triggering inflammatory signalling cascades (Ricard et al. 2003) but the pathophysiological, biomechanical, and biochemical mechanisms responsible for it are still not completely understood.

Mechanical damage can occur even with low pressure set on the ventilator. In mechanical ventilation, pressure is applied at the mouth, to all the respiratory system but in an inhomogeneous lung with collapse of small pulmonary airways and alveoli, or fluid occlusion of non-collapsed airways/alveoli together with healthier part, it results in uneven local force distribution. This leads to additional stress and strain of the yet non-injured region (Dreyfuss et al. 1992).

In addition to this, during the respiratory cycle shear stress injury occurs in presence of cyclic alveolar recruitment/derecruitment when the end expiratory pressure is not enough to maintain open all the alveoli (Ghadiali et al. 2011).

Cells are sensitive to both internal and external mechanical forces and respond to stress and strain by the activation of inflammation and repair mechanisms via a process known as mechanotransduction (Halbertsma et al. 2005). Inflammation can, if excessive, injure the parenchymal tissue. Moreover cytokines are released from the lung into the systemic circulation impacting not only the lung, but also other organs (Murphy et al. 2000).

Disruption of the alveolar-capillary barrier leading to increased filtration and oedema, changes in gene expression alterations in cell rheology are some of the reported consequences of these processes (Halbertsma et al. 2005; Santos et al. 2000).

1.2.2 NON-INVASIVE VENTILATORY SUPPORTS

Non-invasive ventilatory supports have a complementary and alternative role to MV and are gaining more importance in the effort to avoid MV and reduce the incidence of BPD.

In non-invasive ventilation the endotracheal tube is replace by nasal cannula or less commonly by nasal mask or nasopharyngeal prongs. However the optimal interface design has not yet been determined as they are often too rigid or oversized or heavy for newborns. They are less invasive and associated with better clinical outcome than MV but because of airleaks and difficulties to maintain the nasal cannula in place, it is not sure that the set pressure really reach the respiratory system (Peter G Davis et al. 2009).

Complications of non-invasive supports include gastric distension (Jaile et al. 1992; Yamada et al. 2001), gastrointestinal perforations (Garland et al. 1985), nasal trauma (Yong et al. 2005) (Fischer et al. 2010a) and pneumothorax (Hall et al. 1975).

Recently, as there is an increasing interest in non-invasive ventilation, new modalities have been proposed in order to overcome the limits of the present technology and to prevent reintubation. However there is still a need of studies investigating the mechanisms of functioning of these new forms of supports together with means to optimize them and non-invasive supports in general in order to avoid MV to the majority of the infants (Morley & Davis 2008).

<u>nCPAP</u>. Nasal continues positive airways pressure (nCPAP) is the most diffused form of noninvasive support. It consists in applying a distending pressure to the respiratory system with the rationale of maintaining alveolar recruitment and airways patency. Studies have shown that the effects of nCPAP include improving oxygenation, maintaining lung volume, lowering upper airway and reducing obstructive apnoea and work of breathing (De Paoli et al. 2003).

It is established as an effective method for weaning from ventilator and preventing extubation failure and it is used in the management of apnoea of prematurity. Presently in the attempt to reduce VILI there are studies that aim to verify if it can replace MV for the treatment of the majority of infants with RDS (Diblasi 2009; Morley, Davis, et al. 2008; Morley & Davis 2008; Peter G Davis et al. 2009). Recent randomised studies have compare intubation at birth with early nCPAP (Morley et al., 2008; N. N. Finer et al., 2011; Dunn et al., 2011). They did not found differences in rate of death or BPD but infants treated with nCPAP had more incidences of pneumothorax, lower FiO₂ at 28 days, fewer days of ventilation and reduced rate of postnatal corticosteroid use. Therefore, they suggest that nCPAP could be considered an effective alternative to routine intubation and surfactant administration though the endotracheal tube.

However there are still several questions that are under evaluation including the effects of prophylactic vs rescue nCPAP (Sandri et al. 2004) and the combination of nCPAP with prophylactic or rescue surfactant treatment (Dunn et al. 2011; Stevens et al. 2010).

As it is well know that surfactant treatment improves important outcomes for intubated infants and can reduce pneumothorax incidence (Soll 2000b; Soll 2000a), ways of combine surfactant administration with non-invasive support have been studied. Aerosolised surfactant (Jorch et al. 1997; Berggren et al. 2000) and administration of surfactant via a thin catheter passed under direct vision into the trachea (Kribs et al. 2007; Kribs et al. 2008) have been proposed but the only used in clinical is the INSURE (INtubate, SURfactant, Extubate) approach.

Different strategies and devices are available to deliver CPAP, however it is unclear whether they play a role in improving outcomes in infants. Beside the traditional nCPAP, that employ the ventilator end expiratory pressure valve to control the nCPAP delivered, variable flow nCPAP devices (including infant-flow driver system) and bubble nCPAP are the more diffused. Variable flow nCPAP devices use a fluidic flip system to generate the pressure at the nasal interface in according to the set flow passing through the nasal device. Several studies have compared this mode of CPAP generation with the traditional one with not univocal results (Stefanescu et al. 2003; Boumecid et al. 2007). Bubble nCPAP, is accomplished by submerging the expiratory limb of the respiratory circuit within a fluid column. The generated pressure is determined by the depth of submersion and is independent of flow rate. It has been suggest that the noisy pressure waveform arising from bubble continuous positive airway pressure may promote airway opening events as a result of stochastic resonance. Even if it has been shown that it increases peripheral airway patency and decreases the lung clearance index in preterm lambs (J Jane Pillow et al. 2005; Pillow et al. 2007; Narendran et al. n.d.), a short-term cross-over study of human neonates comparing fast bubbling with minimum bubbling did not find any difference in blood gases (Morley et al. 2005).

<u>nBiPAP</u>. An alternative to CPAP is the nasal Bilevel positive airways pressure (nBiPAP) that provides two alternating levels of CPAP. Its theoretical benefits are the recruitment or prevention of collapse of unstable alveoli thanks to the periodical increasing in pressure together with the generation of a tidal volume by the delta pressure between two levels of CPAP that decrease the respiratory work. It has been shown that nBiPAP, as compared to nCPAP, improves gas exchange in the same cohort of preterm infants with repeated cycles of the two supports (Migliori et al. 2005) and is associated with better respiratory outcomes versus nCPAP, and allowed earlier discharge, in a population of newborns with moderate RDS (Lista et al. 2010).

<u>NIV.</u> Efforts to reduce intubation at birth and nCPAP failure rates prompted the use of noninvasive ventilation (NIV) that combines nCPAP with superimposed ventilator breaths. Its mechanism of action of remains uncertain as it is unclear whether mechanical inflations are transmitted to the lungs (Peter G Davis et al. 2009) enhancing tidal volume, whether it could act
as a stimulus reducing apnoea or the higher MAP and clearance of the exhaled gas plays a role (Bancalari et al. 2008).

It has been shown that NIV is more effective than nCPAP in improving gas exchange (Migliori et al. 2005) in decreasing WOB in premature infants (Aghai et al. 2006; Ali et al. 2007) in preventing reintubation (Moretti et al. 2008; P G Davis et al. 2009) and BPD (Kugelman et al. 2007; Bhandari et al. 2007), even if this data requires confirmation.

The different types of invasive mechanical ventilation have been used as NIV modalities (nIMV; nSIMV; nA/C or nSIPPV; nPSV and nHFV), however their relative advantages and disadvantages have not been clarified. Moreover there are no data on what the optimal settings are. Synchronization is thought to lead to a more effective support even if definitive data are still lacking (Bancalari et al. 2008). Detection of the onset and ending of the spontaneous inspiration with sufficient accuracy is difficult and different strategies, involving measurements of pressure swings on the abdomen (Graseby pressure capsule) or spontaneous inspiratory flow, have been proposed (Bancalari et al. 2013).

A different synchronization strategy is employed during NIV-NAVA (non-invasive ventilationneural adjusted ventilatory assist) that leads to a better synchronization with infant's inspiratory efforts. In this modality the electrical activity of the diaphragm (Edi) is used to determine the timing and magnitude of the ventilator pressure as the pressure generated by the ventilator is proportional to the effort of the patient. This modality reduces the load to the respiratory muscles while reproducing the spontaneous breathing pattern, realizing the ventilation chosen by the infant's center of breathing control. However, the role of these types of ventilation is still to be defined for preterm infants with an immature control of breathing.

<u>HHHFNC.</u> Heated, humidified high-flow nasal cannula (HHHFNC) consists in the delivery of flow exceeding patients' inspiratory flow trough not sealed nasal cannula.

Proposed mechanism of functioning includes: washout of nasopharyngeal dead space, attenuation of inspiratory resistance of nasopharynx, improved conductance and pulmonary compliance secondary to improved gas warming and humidification, reduction in energy expenditure for gas conditioning and provision of a distending pressure for lung recruitment (Dysart et al. 2009).

Several studies have underlined the importance of deliver adequately warm and humidified air to avoid nasal mucosal injury and bleeding, resulting in pain and creating a portal of entry for infectious agents (Kopelman et al. 2003; Woodhead et al. 2006a; Waugh et al. 2004).

It has emerged in a variety of clinical situations, thanks to its ease of use, better infants' tolerance, improved feeding and bonding (Dani et al. 2009a; Manley, Dold, et al. 2012a; Lee et al. 2012a; Manley, Owen, et al. 2012a; Spentzas et al. 2009a). In particular, it has been applied for apnoea of prematurity (Sreenan et al. 2001a), prevention of reintubation (Woodhead et al. 2006b; Holleman-Duray et al. 2007a; Miller et al. 2010a), weaning and in case of contraindications to nCPAP (Miller et al. 2010a). More recently a randomised study have shown that it appears to have similar efficacy and safety to nCPAP when applied immediately post-extubation or early as initial non-invasive support for respiratory dysfunction (Yoder et al. 2013a).

The amount of pressure generated is related to the flow rate, the size of the leak around the nasal cannula, and the degree of mouth opening. Concerns about the generation of potentially dangerous pressure have been raised.

Despite an increasing spread out of HHHFNC, most of its mechanisms of functioning are still unclear and studies aiming at evaluating the effects of HHHFNC on gas exchange, breathing pattern, lung mechanics and work of breathing (WOB) are still lacking.

1.2.3 RESUSCITATION AT BIRTH

Rapid clearance of foetal lung fluid plays a key role in the transition from foetal life to air breathing. A great part of lung fluid is removed from the interstitium before birth during labour, due to several factors as reverse activation of epithelium sodium channels and secretion of catecholamine and hormones (Bland 1990). When infants are delivered preterm, especially by caesarean section before the onset of spontaneous labour, the foetus is often deprived of the hormonal changes, making the neonatal transition and the establishment of FRC more difficult (Jain et al. 2006).

Repeated manual inflations with a self-inflating bag and mask followed by nasal continuous positive airway pressure is a diffuse procedure at birth to help preterm infants to establish an adequate FRC and efficient gas exchange. However various questions have been raise about the effects of such manoeuvres on the surfactant-deficient lung.

Multiple variables such as inspiratory pressure, tidal volume, positive end expiratory pressure, and the oxygen concentration could injure the preterm lung at birth and their relative contribution and interaction is still not fully understood.

Some studies investigated the effects of oxygen concentration in the inspired gas. They demonstrated than high FiO_2 values may affect lung function inducing atelectasis and changes in control of breathing while low values of FiO_2 leads to less oxidative stress, inflammation and risk of BPD (Vento et al. 2009; Finer et al. 2010; Tan et al. 2005; Kapadia et al. 2013).

Other studied have considered the impact of different ventilation strategies to recruit the lung evaluating the role and the possible dangerous consequences of pressure values or of tidal volumes delivered.

Hooper et al. using an imaging technique demonstrated the role of transpulmonary pressure in airway liquid clearance at birth as only during the inspiratory activity the air/liquid interface moved toward the distal airways (Hooper et al. 2007). Even if high pressures can be needed to remove the liquid from the lung and fill it with air, few breaths with high tidal volume can injure the lung. Animal studies have pointed out that at birth a surfactant-deficient lung can be injured even by few inflations with volumes that are probably harmless in other circumstances (Björklund et al. 1997; Wada et al. 1997).

The role of positive end-expiratory pressure (PEEP; see paragraph 1.2.1) has been evaluated and recruitment manoeuvre such as SLI (sustained lung inflation; see paragraph 1.4) has been proposed to recruit the lung before the beginning of ventilation in the attempt to find a lungprotective resuscitation strategy. Animal studies have shown that the application of PEEP at birth improve lung volume recruitment (Siew et al. 2009; Probyn et al. 2004). Te Pas and coworkers investigated the effect of the time duration of a sustained inflation at birth and of its combination with PEEP using phase-contrast radiography to image the lung of newborn rabbit pups. They found that a SLI of 20-sec resulted in better aeration than either a 1 or 5-sec SLI. (te Pas, Siew, Wallace, Kitchen, Fouras, R. A. Lewis, et al. 2009) and that combining a SLI and PEEP improved FRC formation and uniformity of lung aeration. PEEP prevented the re-closing of alveoli after SLI and had a greater influence than SLI on FRC (te Pas, Siew, Wallace, Kitchen, Fouras, R. a Lewis, et al. 2009).

In preterm infants, a randomized controlled trial stated that the application of SLI followed by nCPAP (nasal continuous positive airways pressure; see paragraph 1.2.2) lead to a decrease need for intubation in the first 72 hours, shorter duration of ventilator support, and BPD in comparison to face mask ventilation (te Pas et al. 2007) while another on failed to show a benefit from the same treatment (Lindner et al. 2005).

Presently efficacy and safety of such manoeuvres are still under investigation while the optimal duration and level of initial inflation and subsequent level of PEEP remains unclear (Lista et al. 2012).

Moreover SLI is not the only possible recruitment manoeuvre (see paragraph 1.4) and different strategies can be employed and compared in the attempt to reduce the stress applied to the lung tissue. For example recruitment manoeuvres with steps of increasing PEEP might have some advantages as they reduce the initial peak of pressure gradient. However they have not been tested at birth in infants and optimal step duration, step amplitude and the range of PEEP to cover are unknown and probably depend on the time constant and mechanical properties of each lung.

In fact, the different characteristics of each lung suggest that there are not univocal values of pressure, inflation duration or tidal volume beneficial for all the newborns but there is a need of choosing pressure/tidal volume according to the infant's characteristics.

However personalizing the treatment is a difficult task especially at birth as imply finding an adequate variable to monitor the respiratory system condition and the ability to routinely measure it in this difficult moment.

1.3 Assessing lung function in preterm newborns

Assessment of lung function and mechanics in infants in clinical practice is still a challenge. Particular care is needed when studying the newborn population (Gaultier et al. 1995). Equipment, methods, measurements' condition and data elaboration used in adults and older children must be adapted to meet the special requirements of this group age.

Different techniques have been proposed and applied for research purpose but the technical demands, the need for specialized equipment and operators together with test duration, maximal repetition frequency, the requirement for sedation and the lack of appropriate reference values or proved clinical important impact have limited their used for clinical purpose. However the recent development of new technologies, the standardization of procedure and the appearance of commercial equipment have led to a renewed interest in this field. Moreover there is an increasing awareness of the importance of quantitative assessment of lung function for improving the clinical treatment of infants. In fact it can improve the treatment through different ways: 1) increasing our understanding of the lung development and the impact of diseases; 2) permitting monitoring and evaluation of disease severity; 3) detecting small changes in clinical conditions otherwise undetectable; 4) objectively quantifying the effects of treatments and assist recognition of the most favourable ones; 5) optimising and permitting the continuous tailoring of the treatment at the individual patient level.

Between the current techniques, forced oscillation technique (FOT) should be taken into consideration as it is non-invasive and with the development of appropriate set up can be easily applied in clinical practice. Moreover the recently proposed intra-breath analysis and the attention on relative changes in lung impedance rather than on its absolute values as function of the stimulation frequency can lead to new insides in pathology and clinical management of the infants. Besides FOT, other promising techniques (such as the study of electrical activity of the diaphragm and the variability analysis of different signal related to the respiratory system) are been developed and applied to the study of the respiratory system. These techniques could provide important information especially during NIV, when FOT measurements are made difficult for long periods by the presence of leakage and the spontaneous respiratory activity of the infant plays a more important role.

1.3.1 CURRENT TECHNIQUES

Aeration pattern, lung volumes, respiratory mechanics, work of breathing, muscles activity and their variability over time are of interest when assessing lung function. Different techniques have been developed for clinical or resource purpose that permit to measure different parameters. Advantages and disadvantages are related both to the chosen technic and its target parameter. Different measurements can be combined in order to have a better understanding of the respiratory system condition.

IMAGING

Imaging plays an acknowledged role in the management of newborns requiring intensive care.

<u>Plain chest radiograph</u> could be routinely performed because it is associated to a low radiation load but it does not provide quantifiable information because of anatomical superimposition of the structures.

<u>Computerised tomography (CT)</u> provides detailed information about air distribution, lung collapse and hyperinflation however because of high radiation load it is not used to assess lung function in newborns.

<u>Electrical impedance tomography (EIT)</u> is a new non-invasive, method available at bedside for determining regional changes in lung aeration as it was demonstrated in a comparative study with CT (Frerichs 2002). It is based on the fact that changes in regional air content modify the electrical properties of lung tissue (Nopp 1993). However since electrical impedance is influenced by both air and fluid content, care should be taken in interpreting observed changes. Even if the low spatial resolution and the transformation of an irregular body shape to a circular image prevent the extraction of precise morphologic information, EIT is considered one of the most promising techniques for continuous monitoring of lung function in infants (Pillow et al. 2006).

<u>Lung ultrasound (LUS)</u> imaging is a non-invasive technique performable at the bedside and has been recently proposed to evaluate the pulmonary water balance in neonate with respiratory disease (Carlo & Bancalari, 2007; Copetti, Cattarossi, Macagno, Violino, & Furlan, 2008). Pulmonary fluid balance and clearance is crucial for postnatal adaptation to respiration and strictly related to the changes in interstitial pressure and to the integrity of interstitial structure. Moreover in patients with adult respiratory distress syndrome, a significant correlation was found between alveolar recruitment and the ultrasound re-aeration score even if PEEP-induced lung hyperinflation cannot be assess (Bouhemad et al. 2011).

LUNG VOLUMES AND BREATHING PATTERN

Monitoring of tidal volume is of fundamental importance especially during mechanical ventilation as excessive tidal volumes can cause lung injury. <u>Flowmeters</u> can measure the mouth flow and lung volume changes are obtained by mathematical integration. Breathing frequency, tidal volume, minute ventilation, inspiratory/expiratory time and other parameters can be obtained by this measure. However for non-intubated infants, the need of a face mask makes this measure affected by air leak and unsuitable for long-time monitoring.

Breath by breath changes in end-expiratory lung volume (EELV) can be used to monitor important phenomena as recruitment, derecruitment or development of oedema. The <u>respiratory inductive plethysmography (RIP)</u> and the <u>opto-electronic plethysmography (OEP)</u> have been used for this purpose.

RIP uses two compliant belts displaced around the rib cage and the abdomen to measure the local cross-sectional area. The weighted sum of the modification of these two sections yields an estimation of the volume change of the thoracic cavity.

OEP is based on the high accuracy and high temporal resolution 3D localization of retroreflective passive markers positioned on the infants' thoraco-abdominal wall by stereophotogrammetry. From their position the chest wall surface is reconstructed and by means of the Gauss's theorem absolute chest wall volume is computed (Cala et al. 1996).

These two techniques can also measure the degree of thoraco-abdominal asynchrony providing a quantitative assessment of paradoxical motion with OEP being more accurate and permitting a more detailed compartmentalisation of the thoraco-abdominal cavity and a quantification of asymmetry in ventilation. This is useful in evaluating maturation and efficacy of ventilation.

However drift problem and critical calibration of RIP and OEP cumbersome equipment prevents their diffusion for clinical practice.

Assessing of absolute lung volume is also of great importance as the correct lung ventilation and gas exchanges relays on the establishment of an adequate lung volume and its maintaining during all the respiratory cycle (see paragraph 1.4).

The *whole body plethysmography* and the *gas dilution techniques* can provide FRC measures.

The first technique requires the infant to be enclosed within a rigid, close container in supine position. During an end expiratory occlusion at the airways opening the infants' respiratory efforts results in alveolar pressure and volume changes. Changes in alveolar pressure are reflected and therefore measured at the airway opening while changes in alveolar volume are estimated from the measure of the box pressure. The total occluded volume is calculated by relating these changes in pressure and volume. However this technique is not suitable for continuous lung function monitoring at newborns' bedside because of its bulky and complex equipment.

The gas dilution technique preformed in newborns is the multiple breath washout and it is performed during tidal breathing while the wash-in or washout of a specific gas into/out of the lung is recorded. FRC can be calculated if the volume of the indicator gas washed in/out of the lung and its initial and final concentrations are known. Differently from the FRC measured by whole body plethysmography, the FRC measured by this technique does not include the volume of any gas trapped behind obstructed airways.

Knowing only lung volumes is not enough especially in a heterogeneous lung. In a homogeneous lung, every fibre shares an equal proportion of the total force applied and develops an equal tension and equal strain. If part of parenchyma remains collapsed during inflation the interwoven fibres in the diseased region bear the force load. When the stress and strain are not homogeneously distributed, greater tension and distortion develop in some regions and the fibre tension may lead to at the mechanical rupture. How air is distributed in the lung can be only seen by imaging but it is possible to obtain information on the efficiency of gas mixing and a number of ventilation heterogeneity (VI) indices of by multiple gas washout from plots of normalized tracer concentration vs. lung turnover number.

Recently improvements have been made in the set up and in the reproducibility of the results, however there are still limitations to be overcome including the influence of leakages (Sinhal et al. 2010; Pillow et al. 2006).

Forced expiratory manoeuvres have been used extensively in adults and children for assessing airways functionality. As newborns can't be instructed to perform such manoeuvres, either a pressure is externally applied to the chest wall and the abdomen (*rapid thoraco-abdominal compression technique*) or a negative pressure is imposed at the airway opening of intubated infants (*forced deflation technique*). Because of how these manoeuvres are performed they can't be used in clinical practice on very small or sick infants.

PULMONARY MECHANICS

The respiratory system can be described by a lump parameter model that describes its mechanical properties of resistance; compliance and inertance (see paragraph 1.1.1). The structural modification due to pathologies in turn results in changes in the mechanical properties of the respiratory system. Therefore, monitoring the mechanics of the respiratory system it is

possible to identify phenomena as airways' occlusions, expiratory flow limitation and lung recruitment or tissue distention, providing further pathological insights and guiding in choice of therapy.

Different techniques have been developed to estimate Rrs and Crs. However there is not a single value of Rrs and Crs for the respiratory system as they depend on the flow rate and volume at which they are measured.

The following techniques attempt to respond to the need of lung mechanics properties measurement.

<u>Multilinear regression analysis and Esophageal manometry</u>. The mechanical properties of the lung can be estimated from simultaneous measurements of lung volume and transpulmonary pressure (P_L) (1.1.1). Unless the patient is paralysed the pleural pressure (P_{PL}) is needed to compute P_L and it is estimated by the esophageal pressure measured by esophageal manometry. The data can be analysed using a variety of techniques, including the Mead-Whittenberger and multiple linear regression (MLR) techniques. With MLR, changes in flow, volume, and P_L are analysed throughout the entire respiratory cycle as a linear model, and depending on the chosen model, some mechanical parameter are calculated (Gappa et al. 2006). Crs obtained with these techniques is usually called C_{dyn} to emphasize that it is measured under dynamic condition. Correct placement of the esophageal catheter is mandatory to achieve valid results. Moreover this parameter is affected by non-linearity and tidal recruitment.

<u>Whole body plethysmography</u>. The same manoeuvre required for FRC (see paragraph before) permits also the computation of Rrs and the specific Rrs (Rsr*FRC). The use of this technique is limited by the complexity of the set up.

<u>Single occlusion technique (SOT)</u>. The airway opening is occluded at the end of a tidal inspiration while measuring flow and pressure. Resistance and compliance are calculated from the expiratory time constant of the respiratory system which is estimated as the slope of the descending portion of the observed volume-flow loop.

<u>Multiple occlusion technique (MOT)</u>. Occlusions are performed several times at different points of expiration. The volume above functional residual capacity remaining in the lung at the time of the occlusion is plotted against the corresponding pressure. Crs is calculated as the slope of curve while the intercept on the volume axis is the extent to which EELV is being dynamically elevated. Both MOT and SOT are based on a hypothesis difficult to be verified in infants: the presence of a stable EELV.

<u>Interrupter technique (IT)</u>. Rrs is obtained by dividing the rapid change in tracheal pressure immediately following the flow interruption by the flow measured just prior to interruption. It can be done every 3-4 breathes but requires rapid equilibration of pressure in the lung. This is not easily obtained in an inhomogeneous lung.

<u>Multilinear interrupter technique (MIT).</u> Multiple interruptions of a single passive expiration result in several pairs of V-P data that have common EELV permitting the estimation of Rrs and of Crs as for the IT and for the MOT respectively.

<u>High interrupter technique (HIT)</u>. A rapid interruption of the flow at the airway opening causes a step change in flow providing in this way a broad spectrum signal that excites the respiratory system and permits the computation of its input impedance. This is a promising technique available at bedside. However it is greatly affected by non-linearity as the stimulus applied to the lung contains different frequencies interfering with each other and has high amplitude.

<u>Forced oscillation technique (FOT)</u>. A pressure stimulus is applied at the airway opening or at the chest wall and the response of the system in terms of flow at the airway opening or of chest wall motion is measured (see paragraph 1.3.2). This technique has the potential to overcame the limits of the previous ones.

WORK OF BREATHING (WOB)

WOB can increase during pathological condition and can be too much for the newborns to sustain. Monitoring WOB can be useful in choosing the ventilatory treatment.

It can be calculated from \dot{V} and P_{AO} measurements and estimation of P_{PL} changes by oesophageal pressure measured with an oesophageal balloon.

Correct computation of WOB require the measurements of the relaxation curve of the chest wall and the lung elastic recoil curves that can't be assessed in spontaneously breathing in infants. Therefore WOB is usually calculated hypothesizing: 1) a constant compliance during over the breathing cycle; 2) that changes in total respiratory system pressure-volume curve around the operating lung volumes are mainly determined by the lung; 3) that the end-expiratory lung volume does not change significantly during the recordings.

MUSCLES ACTIVITY

Muscles activity can provide information about the effort required to the infant to breath. It can be monitored with superficial EMG however it not practical in the clinical practice because of the required set up and the poor quality of the measurements in infants. However the diaphragmatic electrical activity (Edi) can be measured trough miniaturized electrodes mounted on a conventional nasogastric feeding tube. This makes possible to study the Edi easily and to monitor its changes during the breathing cycle evaluating the effort required for inspiration and the residual end expiratory activity required to maintain adequate EELV (Emeriaud et al. 2006; Beck, Tucci, et al. 2004).

VARIABILITY ANALYSIS

Respiratory system includes many non-linear feedback mechanisms and a network of subsystems that are interacting with each other (vagally mediated peripheral stretch and chemoreceptor feedback control loops, central chemoreceptors, non-respiratory systems influences) and influence the respiratory generator within different time scales and gain. The controlled physiological parameters fluctuate within acceptable limits under non-equilibrium conditions (Frey et al. 2011). Variability analysis of these parameters provides an evaluation of the overall properties of such a complex system and is affected both by maturation and disease (Baldwin et al. 2006). Both lower and excessive variability has been associated with pathological conditions that results respectively in reduced adaptability of the system or loss of control (Frey et al. 2011).

The study of variability can play a role in the clinical management of patient and in particular could be an useful tool for monitoring the temporal behaviour of the disease, risk prediction, determining the severity of disease, phenotyping, and evaluating the impact of external treatments (Frey et al. 2011). Moreover variability in the respiratory system has been found to be important for cell activity as it positively affects surfactant secretion (Arold et al. 2009; Arold et al. 2003); be related to the maturation of the breathing control in infants (U Frey et al. 1998; Engoren et al. 2009); be correlated with weaning success (Wysocki et al. 2006; Kaczmarek et al. 2013; Engoren 1998) and be modified by RDS and assisted ventilation in newborns (Emeriaud et al. 2010). For the management of preterm babies, measure of variability can be also exploit to evaluate if the natural variability is maintained during mechanical ventilation and modified by ventilation setting and might be useful to identify a ventilation strategy that can preserve the natural variability or help the correct development of the control of breathing.

For variability analysis, time series obtained from different respiratory signals can be considered. The variability of parameters obtained from Edi signal it is more specific for the study of the control of breathing as it represents the output of the neural centres. The tidal-flow waveform contains information about the control of breathing as well as mechanical properties of the thoraco-pulmonary system. Separate the effect of control of breathing and mechanical properties of the respiratory system requires careful interpretation of the data. Another possibility is to study variability in lung mechanics. Whichever is the chosen signal, care should be taken when measuring it as the set up can modify the measure. For example the use of mask implies an additional resistance and dead space that can influence the breathing pattern. Also the influence of the measurements condition as posture and sleeping stage should be taken into account.

Not only different parameters but also different time scale can be considered. Time series can be obtained with a inter sample time interval of seconds, minutes, hours, days or months depending on the phenomena of interest. If the chosen parameter can be continuous monitoring also breath by breath variability or even intra-breath variability can be studied.

Moreover the study of the correlation between variations in different parameters could provide additional information.

A recent review reports more than 70 variability techniques that have been proposed to describe the time series under study in the attempt to untangle the information hidden within biological signal variability (Bravi et al. 2011). However up now just some of them have been applied to the study of the breathing pattern of newborns.

<u>Statistical domain</u>. These techniques describe the stochastic properties of the data and include standard statistical features, form factor, some symbolic dynamics features, turns count.

<u>Geometrical domain</u>. These techniques describe the properties that are related to the shape of the dataset in a certain space. Grid counting, poincaré plot features and recurrence plot features are included in this group.

<u>Energetic domain</u>. These techniques describe features related to the energy or the power of the data and include frequency and time-frequency features analysis.

<u>Informational domain</u>. These techniques describe the degree of irregularity/complexity inherent to the order of the elements in a time-series. The different metrics developed to measure entropy, entropy rate and differential entropy rate are included in this group.

In preterm infants entropy measurements have been applied to the study of changes in complexity of V_T and respiratory rate with age and size and between patients who failed or not extubation (Engoren et al. 2009; Engoren 1998).

<u>Invariant domain</u>. These techniques describe the properties of a system that demonstrates fractality or other attributes that do not change over either time or space. The presence of a similar pattern at different scales suggests that there is some form of long term memory or correlation among events within the system. Fractal properties in respiration have been reported in several studies starting from foetal breathing movements (Akay et al. 1998; Szeto et al. 1992). The breathing pattern of healthy term infants is fractal (Larsen et al. 2004; Cernelc et al. 2002). Frey et al. showed that also the breathing pattern of preterm infants has fractal properties but a lower memory, in respect to the ones born at term, that tend to increase with postnatal age (U Frey et al. 1998).

COMPARISON AMONG THE DIFFERENT TECHNIQUES FOR LUNG FUNCTION TESTING IN NEWBORNS

In Table 1-1 comparison among the available techniques for lung function testing in newborns is reported. The measured parameters, some advantages and disadvantages of each technique are summarised.

Table 1-1 Summary of the non-invasive available techniques for assessing lung function in newborns.

Technique	obtained information	Advantages	Limitation			
Electrical impedance		Non-invasive	Low spatial resolution			
tomography	regional	Available at bedside	Inaccurate morphology			
EIT	aereation	Allows continuous monitoring	Inflenced by fluid content			
-		Non-invasive	Low spatial resolution			
Lung Ultrasound (LUS)	regional	Available at bedside	Inaccurate morphology			
	aereation	Allows continuous monitoring	No contineous monitoring			
		Simple not invasive				
Flowmators	relative	Allows continuous monitoring	Affected by leaks			
Flowineters	volume changes	Augulable at hadside	Affected by integration drift			
		Available at bedside	Critical and nations encoding calibration			
Respiratory inductive		Available at beuside				
pietnysmography		No connection to airway opening				
RIP	- absolute -	Allows continuous monitoring	Chest wall motion described by 2 degrees of freedom			
	volume change;	No connection to airway opening				
Optoelectronic	thoraco-abdominal	No subject-specific calibration	Cumbersome and expensive set up			
nlethysmography	asyncronies	No drift	No avaialable at bedside			
OEP		No assumption about chest wall	Affected by motion			
<u>SEI</u>		cinetics	Aneceed by motion			
		Allows continuous monitoring				
Rapid thoraco-						
abdominal compression	Forced		Risk of alveolar collapse			
RTC	expiratory		No continuous monitoring			
Forced deflation	curves		Require sedation			
technique			·			
			Affected by leaks			
Gas dilution	Absolute volume;		No continuous monitoring			
technique - multiple breath	Ventilation	Non invasive	No avaialable at bedside			
washout	inhomogeneity (VI)		VI influenced by breathing nattern			
Whole-body	Absolute volume	Non-invasive	No avaialable at bedside			
nlethismography	Rrs and specific Rrs	Allows continuous monitoring	Affected by box humidity and temperature			
pictilishiography	his and speeme his	Allows continuous monitoring	No continuous monitoring			
Single occlusion	Rrs, Crs		Requires stable FELV			
tecnique		Non-invasive	Assumption of rapid pressure equilibration			
SOT			Assumption of rapid pressure equilibration			
			Affected by Muscle activity			
Multiple-occlusion			No continuous monitoring			
tecnique	Crs	Non-invasive	Requires stable EELV			
мот			Assumption of rapid pressure equilibration			
			Affected by muscle activity			
Interrupter technique	Rrs	Non-invasive	Requires rapid equilibration of pression			
<u></u>			No continuous monitoring			
Multiple			Low success rate with high respiratory frequencies			
interrupter technique	Rrs, Crs	Non-invasive	Assumption of a homogeneous respiratory model			
MIT			No continuous monitoring			
High-speed		Non-invasive	Very affected by non-linearities			
interrupter technique	Rrs(f), Xrs(f)		No continuous monitoring			
HIT		Avaiable at beuside	No continuous monitoring			
Esophageal			Discomfort for the infants			
manometry + flow	Rrs, Crs	All	PES and PPL equivalence is questioned in preterm			
measurement + multiple	Estimation of WOB	Allows continuous monitoring	affected by non linearity and tidal recruitment			
linear regression (MLR)			Needs of lung and chest wall relaxation curves for WOB computation			
Forced						
oscillation		Availble at bedside	requires a sealed connection with the infants or measurement of chest wall			
technique	Rrs(f), Xrs(f)	Allows continuous monitoring	displacement			
EOT		Flexible	uispiacement			
Diaphrometic						
oloctromucaranhu	Edi		expensive catheter, infections associated with use of catheters			
electromyography		Allows continuous monitoring				

1.3.2 FORCED OSCILLATION TECHNIQUE (FOT)

The forced oscillation technique (FOT) has been introduced by Dubois in 1956 (Dubois et al. 1956). Because of technological barriers, it has attracted little attention of the clinic and scientific communities until the 90's when, thanks to the technology progresses, FOT become interesting as a potential non-invasive technique to derive specific information on respiratory system mechanics. Since it does not require any collaboration by the patients, this technique is particularly interesting to study infants' lung.

The respiratory system is studied by the principles of linear systems analysis. A forced pressure oscillation is applied at the respiratory system while recording pressure and flow signals. The mechanical properties are measured in terms of impedance Z_{rs} , which is the complex ratio between the applied pressure (P) and the resulting volumetric flow rate (\dot{V}) at the frequencies contained in the forcing signal.

The real part of Z_{rs} is the resistance of the respiratory system (R_{rs}) , and describes the dissipative mechanical properties of the respiratory system reflecting airway and tissue resistance, whereas the imaginary part, or reactance (X_{rs}) , is related to the energy storage capacity and thus determined jointly by the elastic and the inertive properties represented by the compliance (C_{rs}) and by the inertance of the respiratory system (I_{rs}) . The inertive properties become progressively more important with increasing frequencies.

$$Z_{rs} = \frac{P}{\dot{V}} = R_{rs} + iX_{rs} \tag{1.3}$$

where *i* is the imaginary unit and

$$X_{rs} = -\frac{1}{2\pi f C_{rs}} + 2\pi f I_{rs}$$
(1.4)

Since the respiratory system, especially in diseased conditions, may be strongly non-linear the requirement of linearity is satisfied by the use of small-amplitude oscillations. On the other hand the amplitude of the forcing signal should be large enough to guarantee a satisfactory signal-to-noise ratio. Pressure oscillations of $\sim 2 \text{ cmH}_2\text{O}$ amplitude are normally used.

In studying oscillatory mechanics, the respiratory system can be considered as a generalized two-port system in which one port is the airways opening and the other one the body.

Depending on where pressure and flow are measured it is possible to calculate the input impedance (Z_{in}) or the transfer impedance (Z_{tr}) of the respiratory system.

To obtain Z_{in} the stimulus is imposed to the airway opening and the response analysed by combining flow and pressure at the same site. Z_{tr} is computed considering flow and pressure measured at different sites (airway opening and body surface) with stimulus imposed to airway opening or to body surface.

$$Z_{in} = \frac{P_{AO}}{\dot{V}_{AO}}; \qquad \qquad Z_{tr} = \frac{P_{BS}}{\dot{V}_{AO}} \qquad or \qquad Z_{tr} = \frac{P_{AO}}{\dot{V}_{BS}} \tag{1.5}$$

where P_{AO} and \dot{V}_{AO} are respectively the pressure and flow measured at the airways opening while P_{BS} and \dot{V}_{BS} are respectively the pressure and flow measured the body surface. If the measured system is linear, the two expressions above for Z_{tr} must be equivalent (Peslin et al. 1986).

Since Z_{in} and Z_{tr} are affected differently by the parallel elements of the respiratory system, they can be selected or combined to obtain different information on the airway and tissue impedance. Z_{in} is more affected by airways shunt and is not reliable in presence of leakage at

the connection with the patient but it is more used then Z_{tr} as it requires a less complicate set up.

MEASUREMENT SETUP

Different FOT equipment has been developed to measure Z_{in} and Z_{tr} depending on the sites of application of the driving signal and recording of the mechanical response.

International standards and recommendations have been published for FOT set up to measure Z_{in} in adults (Oostveen et al. 2003) and children (Beydon et al. 2007). This is the easiest and most commonly used FOT configurations.

Forcing signal is generated by a loudspeaker and applied to the airway opening of the subject where flow and pressure are measured. A bias flow is maintained to minimize rebreathing while a bias tube offers a low-resistance pathway to atmosphere for the spontaneous breathing and a high-resistance pathway to atmosphere for the forcing signal. For children the dead space represented by the mouthpiece, bacterial filter and flowmeter should be less than 50 to 70 ml.

Although this setup is very simple, it has the inconvenience of being affected by the adverse effect of the movement of the cheeks and extrathoracic airways that, resulting mechanically in parallel with the intrathoracic respiratory system, constitutes a shunt pathway that influence the measure of Z_{in} . Supporting subjects' cheeks results in an increased impedance of the upper airways and therefore in a decreased influence on Z_{in} .

In an alternative the subject's head is enclosed in a chamber (the head generator technique), so that the oscillatory pressure is applied both at the airways opening and around the upper airway walls, thus minimizing the upper airway wall shunting (R. Peslin et al. 1985). However this set up is bulky and not suitable for clinical application.

Loudspeakers are not suitable for measurements at low frequencies, therefore reciprocating piston-pumps actuated by servo-controlled linear motors or proportional solenoid valve incorporated in a close loop system are used.

During mechanical ventilation the forced oscillation generator is connected in parallel with the ventilator to measure Z_{in} (Navajas et al. 2001). Therefore the generator should: not interfere with the ventilation (thus it should have a high impedance at the breathing frequencies); be able to withstand the high positive pressures and fast pressure transients delivered by the ventilator; be able to generate pressure oscillations with a nearly constant amplitude during the whole respiratory cycle and be suitable for the use in an intensive care unit.



Figure 1-5 Schematic representation of the set-up to measure Z_{in} by FOT during spontaneous breathing (A) and mechanical ventilation (B).

Less studies concerning Z_{tr} have been published probably because its measurement is more difficult.

In adults three different setups have been proposed.

Traditionally Z_{tr} has been assessed by applying the forcing signal to the body surface using a modified partial body plethysmograph with one or more loudspeaker mounted on its walls with the pressure measured inside the box (P_{BS}) and the flow at mouth (\dot{V}_{BS}) (Lutchen et al. 1998). The sealing of the box around the neck is critical: leaks must be avoid while not compressing the upper airways. Moreover, the reliability of the results at frequency greater than 15-20Hz is limited by the non-uniform pressure distribution around the chest (Oostveen et al. 1989).

Another approach permits simultaneous measurements of Z_{tr} and Z_{in} (R Peslin et al. 1985). The stimulus is applied at the mouth by loudspeaker while the subject is in a flow-type plethysmographic chamber, breathing through a mouthpiece. Mouth flow (\dot{V}_{AO}) and pressure (P_{AO}) and box pressure (P_{BS}) are measured. The frequency response of the body box must be accurately identified in order to correct the effect of the compressibility of the gas enclosed into the box on the assessment of \dot{V}_{AO} and the hypothesis of a single compartment chest wall behavior must be verified. A limiting disadvantage of this set up is the direct transmission of pressure changes through the cheeks that represents a parallel shunt of the lung.

Recently, a new method, based on the combination of FOT with the optoelectronic plethysmography (OEP) has been presented to measure simultaneously Z_{tr} and Z_{in} (Aliverti et al. 2001). The forcing signal is applied to the airway opening using a loudspeaker while P_{AO} and \dot{V}_{BS} are measured. \dot{V}_{BS} is obtained as the time derivative of V_{BS} measured by OEP (see paragraph 1.3.1). This technique does not require any body box, can be used in different postures and allows the study of mechanical behaviour of several chest wall compartments (i.e. upper rib cage, lower rib cage and abdominal).



Figure 1-6 Schematic representation of three proposed set-up to measure Z_{tr} by FOT.

FORCING SIGNAL

A variety of stimulus' shape can be adopted.

Different oscillatory frequencies provide different information about the mechanical properties of the respiratory system. Briefly, high frequencies provide information about upper airways while low frequencies provide information about the mechanical properties of the tissues and of lung periphery. In particular input impedance measured over the 0.1 to 10 Hz bandwidth reflects different phenomena as parallel time constant heterogeneity, airway wall distensibility, parenchymal viscoelasticity, expiratory flow-limitation and collateral ventilation (Kaczka et al. 2011).

However when applied during normal breathing, the forcing signal must have a frequency at least one order of magnitude higher than breathing frequencies (f>3-4 Hz for adults) to be able to separate the stimulus response from the activity of the breathing muscles.

When these signals include components in the range of the breathing frequencies, the measurement can be performed on fully relaxed or paralyzed patients using the Optimal Ventilation Techniques (OVW) that uses a stimulating waveform whose frequency contents has been studied to simultaneously sustain ventilation and stimulate the patient (Lutchen et al. 1993).

Both single-frequency and composite signals have been used in research and clinical practice:

i) Single-frequency FOT is used in the tracking of rapid changes in Z_{rs} , for example those occurring within the respiratory cycle, or for continuous monitoring. A single sinusoid allows a high Signal/Noise ratio but only a single value of transfer function can be calculated (the one at the stimulus frequency). To characterize the frequency spectrum requires several measurements at different stimulus frequencies making the technique time demanding. Moreover every single frequency component of the spectrum will be estimated in different times, introducing errors in the estimation of the whole spectrum.

ii) By using a white noise, instead, it is possible to obtain the entire frequency spectrum, but the S/N ratio is lower and the result affected by harmonic distortion.

iii) Impulse oscillation permits to obtain the frequency spectrum over a broad frequency range in the same moment but the measure is strongly affected by non-linearity.

iv) Composite signals are a compromise and are used to explore the frequency dependence of Z_{rs} . Using a stimulus whose frequency components are not integer multiples or linear combination of others reduces the impact of crosstalk and harmonic distortion. In particular such a signal is able to completely eliminate the bias introduced by a system describe by a second order equation and therefore this family of waves is called Non-Sum-Non-Difference of order 2 (Suki et al. 1992).

ANALYSIS OF THE RESPIRATORY IMPEDANCE

Despite the diagnostic potential of FOT, there has been relatively little effort to incorporate its use into routine clinical practice (Kaczka et al. 2011). Beside technical difficulties related to FOT set up development especially for infants, this can be due to the fact that several studies are mainly focused on interpreting the impedance data on mathematical models with non-immediate clinical application of the results.

Different approaches have been explored to extract information and indices from FOT measurements.

1) Patterns of frequency dependence.

The shape of the real and imaginary part as a function of the forcing frequency can be analysed, looking for specific pattern of abnormalities. In the frequency range 2-30Hz, the healthy respiratory system exhibits a largely frequency-independent Rrs whose major component is airway resistance. Conversely, Xrs undergoes the transition from negative values (when the elastic reactance dominates) to positive values increasing with frequency (when the inertial reactance dominates). At the characteristic resonant frequency, where Xrs crosses zero, the elastic and inertial forces are equal in magnitude and opposite.

The study of the frequency dependence of impedance has been proposed for different clinical application. In particular resonant and anti-resonant-peak frequencies have been proposed as diagnostic indices both in adults and infants (U. Frey et al. 1998; U. R. S. Frey et al. 2000; Chalker et al. 1992).

2) Inverse modelling of impedance

By assuming a lumped parameter model of the airways and tissue it is possible to derive mechanical properties of the respiratory system estimating the model parameters that best fit the measured impedance. It could allow the identification of the source of the abnormality but the result depends critically by models used.

The first interpretative model proposed and widely used afterward, consider the respiratory system composed by a single airway connected to a single alveolus inside an elastic chamber modelled by an equivalent T-network circuit (Peslin et al. 1986).



Figure 1-7 Six element lumped parameter model of the respiratory system. An electric resistance (*Raw*) and inductance (*Iaw*) represents the mechanical properties of the airways accounting for the viscous pressure loss and the inertial mass of the gas contained in the tracheo-bronchial tree. A capacitance (*Cg*) models the elastic behaviours of the air while a series of resistance (*Rt*), inductance (*It*) and capacitance (*Ct*) represents viscosity losses, inertial mass and elastic properties of the lung and chest wall tissues.

From the model shown in Figure 1-7 we obtain:

$$Z_{in} = Z_{aw} + \frac{Z_g \cdot Z_t}{Z_g + Z_t}; \qquad \qquad Z_{tr} = Z_{aw} + Z_t + \frac{Z_{aw} \cdot Z_t}{Z_g}; \qquad (1.6)$$

For frequencies less than 20 Hz the effect of Z_g is negligible and Z_{in} can be reasonably approximated as composed by the series of airways and tissues impedances, obtaining:

$$Z_{in} = R_t + R_{aw} + j\left(\omega(I_t + I_{aw}) - \frac{1}{\omega C_t}\right) = R_{rs} + j\left(\omega I_{rs} - \frac{1}{\omega C_t}\right)$$
(1.7)

Data from healthy lungs have shown a behaviour very similar to a RLC series model supporting the above equation (Dubois et al. 1956; Michaelson et al. 1975; Rotger et al. 1995). Nonetheless, such a model is too simple to characterize dynamic mechanical behaviour for most pathologies of the respiratory system.

Measurements of lung impedances performed on healthy animals showed that at low frequencies airway resistance remains fairly constant (Hantos et al. 1990) while parenchymal tissue resistance decreases near-hyperbolically with increasing frequency approaching a plateau around 4 Hz and showing the behaviour of a viscoelastic material (Lutchen et al. 1993; Suki et al. 1994).

To account for these findings Hantos et al. (Hantos et al. 1992) proposed a new model composed by a homogeneous airways compartment containing an airway resistance (R_{aw}) and inertance (I_{aw}) leading to a viscoelastic, constant-phase tissue compartment that displays a stress relaxation response in which pressure decays as a power-law in time. For this model, the predicted lung impedance as function of the angular frequency (ω) is given by:

$$\widehat{Z_L(\omega)} = R_{aw} + j\omega I_{aw} + \frac{G - jH}{\omega^{\alpha}} , \qquad \alpha = \frac{2}{\pi} \tan^{-1}\left(\frac{1}{\eta}\right) , \qquad \eta = \frac{G}{H}$$
(1.8)

where R_{aw} , I_{aw} , G H are the parameters of the model with G and H representing tissue damping and tissue elastance. Tissue resistance, defined as $Rt=G/\omega\alpha$, decreases quasi-hyperbolically, with the degree of frequency dependency determined by α that, in turn, depends by tissue hysteresivity η . Studies on healthy lung have validate the use of this model to separate the properties of airways and tissues against data obtained with the alveolar capsules results (Lutchen et al. 1994; Petak et al. 1993)

Both the described models represent the respiratory system as serial compartments representing homogenous airways and tissue.

Especially under pathologic condition, non-uniform airways constriction/obstruction and/or non-uniform alteration of the tissue compliance result in airway-tissue pathways with heterogeneous time-constants. This leads to an additional impedance's frequency-dependence that is not due to viscoelasticity alone (Lutchen et al. 1994).

Respiratory system's models can be complicated in the attempt to account for these phenomena.

The homogeneous airway compartment can be divided into two equal halves by an additional shunt airway compliance parameter to account for non-rigid airway walls while, to compensate for parallel time-constant inhomogeneity, the homogeneous airways model can be modified to contain two separate Raw pathways, both leading to identical constant phase tissues.



Figure 1-8 Models accounting for airways resistance heterogeneity (A) and for the shunting of oscillatory flow into non-rigid airway walls (B).

However a more accurate description of lung mechanical behaviour may be obtained allowing for stochastic variability in Raw or H by distributing parallel heterogeneity across an arbitrary number of parallel pathways. For example, considering a probability density function of airways obstruction, P(Raw), with lower (Raw,min) and upper bounds (Raw,max), the impedance can be estimated as follows:

$$\widehat{Z_L(\omega)} = \left[\int_{R_{aw,min}}^{R_{aw,max}} \frac{P(R_{aw})}{R_{aw} + j\omega I_{aw} + \frac{G-jH}{\omega^{\alpha}}} \, dR_{aw} \right]^{-1} \tag{1.9}$$

However, while increasing the complexity of the model to account for additional mechanisms may significantly improve the fit to the data, the resulting parameters may become statistically unreliable. Moreover, obtaining a model with the best statistical fit to the data does not imply that all of the physiological mechanisms contributing to the frequency dependence of Z have been account for (Kaczka et al. 2011).

This approach has been proved useful to understand physio-pathological phenomena and may provide useful information for diagnosis of patologies such as asthma (Frey 2005).

3) Within breathe changes of input impedance.

An alternative method of analysis of the measured impedance was firstly proposed by Peslin et al, and consists in evaluating the within breath change (Peslin et al. 1993). In fact taking advantage of the a priori knowledge of the frequency content of the forcing signal it is possible to study the time course of Z_{rs} tracking its rapid changes and still obtain a reasonable estimate of its spectra contents (Kaczka et al. 1995; Kaczka et al. 1999).

This approach is potentially valuable for a wide range of clinical applications. It has been proved able to identify expiratory flow limitation (EFL) in COPD patients and to quantify the degree of EFL, to detect obstructive sleep apnea, and the effects of deep inspirations on airway constriction (Jensen et al. 2001; Dellacà et al. 2006).

It has been suggest to monitor the subject condition, also at home, and to titrate therapy. In particular the time course of Z_{rs} at 5Hz has been used to assess the airflow obstruction and to titrate the level of CPAP therapy in order to maintain upper airways patency in patients with sleep apnea and severe obstructive and restrictive respiratory patients (Navajas et al. 1998; Farré et al. 2000). Recently it has been proposed also for PEEP optimization (1.4).

1.4 LUNG VOLUME RECRUITMENT AND PROTECTIVE VENTILATION

Ventilatory support has the goal to help infants in reaching correct gas exchange. Different factors (ventilation, surface of exchange, concentration gradient, diffusion capacity) play a role in this. Concentration gradient can be increased using higher FiO_2 , however this can lead to oxidative stress. Ventilation can be increased by increasing the driving pressure (PIP-PEEP) however this can lead to mechanical stress to the tissue. In principle if the exchange surface is optimized (the lung is recruited and not overdistended) then correct blood oxygenation can be obtained with lower FiO_2 and PIP requirements. However, in preterm infants obtaining and maintaining correct lung recruitment from resuscitation in the delivery room to discharge is a difficult task as different factors have to be taken into account together with the changes in the respiratory system due to growth.

A major clinical challenge in the management of preterm newborns in an acute phase is to avoid alveolar collapse promote by the lack of surfactant and the presence of lung fluid.

Each alveola has its own opening and closing pressure. The sigmoid shapes of both the inflation and deflation pressure-volume curve (see paragraph 1.1.1) implies a Gaussian distribution of opening and closing pressures of alveoli with the closing pressure distribution shifted towards lower pressure compared to the opening one (Crotti et al. 2001). Therefore the pressure required to fully open the alveolai is higher than the one need to keep them opened. Even if needed to open the alveolai, once they are opened, maintaining such high pressure is unnecessary and can injure the lung (Crotti et al. 2001; Gaver et al. 1990; Servillo et al. 1997).

A number of strategies named <u>recruitment manoeuvres</u> are used at birth or later on during ventilator support to expand, or re-expand, collapsed lung tissue increasing the pressure applied to the lung, and decreasing it still maintaining enough pressure, to prevent subsequent derecruitment allowing ventilation with a lower pressure in the context of a more open lung. Because of viscoelastance and other time-dependant phenomena the tendency of previously collapsed lung units to open depends both on the applied transpulmonary pressure and duration of its application.

Various recruitment manoeuvres exist, including sustained lung inflation (SLI), intermittent sighs, and stepwise increases in PEEP and/or PIP. The two most commonly used approaches in infants are SLI, consisting in the application on a continuous high pressure (20-25 cmH₂O) at the airways opening for a determined period, and incremental + decremental PEEP trial, consisting of step of increasing/decreasing PEEP, with a fixed step width and duration. Which is the best technique to recruit the lung (not only the type of the manoeuvre but also time and pressure settings) is currently unknown and most likely it may depend on the specific circumstance. The availability of a tool for monitoring lung recruitment and distension during these manoeuvres is of great importance. In fact it could permit the identification of a strategy that will lead to correct lung recruitment minimising the stress applied to the lung during the process. Moreover, a tool that is available also in a clinical environment would give the possibility to personalise the manoeuvre on the base of each lung response.

After the lung has been recruited, it is crucial to keep it open during the whole respiratory cycle. It is important to prevent alveolai derecruitment without increasing the stress applied to the tissue by applying the lower <u>end-expiratory pressure</u> that maintains the lung open (te Pas, Siew, Wallace, Kitchen, Fouras, R. a Lewis, et al. 2009). Therefore, depending on the ventilator support used, nCPAP, PEEP or CDP have to be set appropriately.

Even if there are a restrict range of end-expiratory pressure used for newborns, there is not a single optimal pressure value equal for all the infants. Moreover with lung maturation, increased surfactant production, progress of pathology and tissue remodelling, the lung characteristics changes dramatically and alveolar recruitment could not be the principal determinant of the ventilated areas of the lung but other phenomena as airways collapse and obstruction could play a major role. In this contest recruitment manuvers would not be much useful but the application of a pressure can be fundamental to keep open the airways. The importance of a ventilatory strategy customized according to the single patient's pathophysiology, reveals the need for a method able to assess the lung response to changes in PEEP, CDP or nCPAP to guide setting.

Currently in clinical practice PEEP, CDP and nCPAP are usually adjusted to optimize arterial oxygenation, inflation of the lung on the patient's chest radiograph and subjective assessment of chest wall movement. Even if gas exchange can be used to indirectly monitor lung recruitment or airways collapse, it is difficult to detect pressure-induced over-distension and tidal recruitment (Richard et al. 2004)

CT is the preferred tool for assessing lung aeration (Gattinoni et al. 2001; Rouby et al. 2003; Vieira et al. 1998; Malbouisson et al. 2001) but is not available at the bedside, it is not adequate for a continuous monitoring and it is associated with high doses of radiation.

A different strategy to identify to guide pressure setting is needed and although PEEP, CDP and nCPAP share the same rationale (moving the working point along the P-V curve), different specificity due to the different modality of ventilation has to be taken into account.

PEEP AND CDP TITRATION

Respiratory mechanic has been extensively studied for the definition of a safe range of ventilatory pressures. In fact it can provide information about lung recruitment and distention and the optimal end expiratory pressure value may result from a compromise between maximal recruitment and minimal overdistension.

<u>Static PV-curve</u>. The static PV curve and in particular the UIP and LIP points give information about lung recruitment and distension (see paragraph 1.1.1).

It has been shown that choosing a PEEP or CDP level around the maximal point of curvature on the deflation limb of the pressure-volume curve results in effective lung recruitment and improvement in oxygenation both from a theoretical (Hickling 2001) and experimental point of view (PEEP: (Rouby et al. 2003; Crotti et al. 2001; Muscedere et al. 1994); CDP: (Goddon et al. 2001; Luecke et al. 2003)).

However the static P-V curve cannot be measured in very sick preterm ventilated infants and the static compliance does not reflect the lung condition during ventilation (Adams et al. 2001; Stahl et al. 2006; Katz et al. 1981; Hickling 2002).

<u>Dynamic compliance (C_{dyn})</u>. A clinically feasible way to set the PEEP is based on maximizing the C_{dyn} (Suarez-Sipmann et al. 2007), or similarly minimizing the elastance (Ers) of the respiratory system (Ers = 1/C_{dyn}) (Carvalho et al. 2007; Carvalho et al. 2006), during a decremental PEEP titration.

 C_{dyn} is also affected by intratidal recruitment that can occur because of high tidal volumes and inappropriate levels of PEEP (Malbouisson et al. 2001). Therefore the estimation of intra-tidal changes in C_{dyn} by measurement of the slope of the tidal P-V curve (Nève et al. 2000; Ranieri et al. 2000; Terragni et al. 2003; Gama de Abreu et al. 2003), or by quantifying the percentage of non-linearity in elastance (Bersten 1998; Kano et al. 1994), or by computing the ratio between the compliance obtained from the slope of the last 20% of the tidal P-V curve and the mean tidal P-V slope (Fisher et al. 1988) have been proposed to individuate a lung protective PEEP level that minimised intra-tidal recruitment.

Even though C_{dyn} has the advantage of being continuously provided by the ventilator, its estimation is strongly affected by non-linearities, which may be relevant in diseased lungs, and by the activity of the respiratory muscles, requiring sedation of the patient in presence of spontaneous breathing or the use of an esophageal baloon.

During HFOV C_{dyn} cannot be measured in a standard way but it has been shown that there is an inverse relationship between lung compliance and the ratio of pressure swings at the distal and proximal ends of the endotracheal tube. This measurement has been proposed as an indicator of the optimal CDP during HFOV (van Genderingen et al. 2001; van Genderingen et al. 2002).

<u>Oscillatory mechanics.</u> More recently, new strategies based on the forced oscillation technique (FOT, see paragraph 1.3.2) have been studied.

Bellardine Black et al. (Bellardine Black et al. 2007) showed, in animals ventilated by an enhanced ventilator waveform with an inspiratory profile with 5 frequency components between 0.2 and 8 Hz, that the optimal range of PEEP identify by CT correspond to the one that minimize the frequency dependence of respiratory resistance and low-frequency elastance, indexes reflective of the mechanical heterogeneity. However the disadvantages of this approach are the interference with the ventilation, the presence of errors due to non-linearity and crosstalk between the different frequencies and the impossibility to determine the presence of tidal recruitment.

Pillow et al. (Pillow et al. 2004) applied FOT to preterm lambs on HFOV. They exploit a pseudorandom excitation signal (2 to 20 Hz) and the constant phase model (to separate the airway and tissue contribution to total Zrs) to evaluate the effect of recruitment manoeuvres and surfactant administration. They found that recruitment manoeuvres and surfactant administration mostly affected tissue resistance. Moreover the MAP that minimized tissue impedance was the one that minimized pressure transmission to the lung parenchyma and therefore it should also minimizes lung injury. FOT measurements were performed disconnecting the animals from the ventilator and connected it to a loudspeaker making of this measurement difficult in clinical practice. A disadvantage of this approach is that the respiratory

system has to verify the hidden assumption of the constant phase model (linearity, homogeneity of the lung and stationarity). These hypothesis are not verified in a pathological lung.

Dellacà et al. proposed a different approach, a single frequency overimposed on conventional ventilation. The choice of a single frequency allows high temporal resolution and to follow intratidal changes of X_5 . Therefore they can study the change of X_5 with PEEP at end expiration avoiding the influence of intra-tidal recruitment/derecruitment. They showed that reactance at 5Hz can be used to monitor recruitment/derecruitment during ventilation in two different porcine models of lung collapse (Dellaca et al. 2009) and allows the definition of an optimal PEEP in agreement with CT data (Dellacà et al. 2011). They suggest that FOT may provide a noninvasive bedside tool for PEEP titration. The same group shows also that in a lavage model of lung injury a PEEP optimization strategy based on maximizing Xrs leads to higher PEEP values but lower driving pressure than clinically selected PEEP, leading to attenuated the signs of ventilator induced lung injury (Kostic et al. 2011). Finally they found that optimizing PEEP by end-expiratory reactance minimized intra-tidal recruitment/derecruitment with the potential to minimize cyclic mechanical stress on lung tissue. Moreover they suggest that end-inspiratory X could be useful to optimize V_T (Zannin et al. 2012). Finally, FOT has been proposed also for CPD optimization during HFOV as an optimal CDP close to the point of maximal curvature of the deflation limb of quasi-static pressure-volume curve can be identified by measuring Zrs during a decreasing continuous distending pressure trial in rabbits (Dellacà, Zannin, et al. 2013). More details about this approach are given in the following chapter.

NCPAP AND NPEEP TITRATION

In principle lung mechanics could play the same role in optimizing pressure during noninvasive than during invasive ventilation. However it is more difficult to assess lung mechanics during these modalities. Moreover during this modality the activity of the infant plays a major role on the determination of EELV. In fact the absence of an ETT permits to the infant to actively elevate EELV also trough flow-breaking action of the larynx (see paragraph 1.1.2). Infant's efforts could compensate a low pressure increasing WOB. A longer time of monitoring could be required to identify the optimal CPAP just on lung mechanics bases as short term changes could be mask by the increased infant's activity.

Therefore together with lung mechanics also other indices could be considered to guide pressure titration.

<u>Oscillatory mechanics.</u> With the current CPAP device for infants, continuous Zin monitoring is made difficult by 1) the present of leakage at the infant connection that prevent reliable flow measurement at the mouth and 2) the fact that adding a flowmeter will increase the dead space and therefore the respiratory load of the infants. Even if now Zin can be measure for a short time, technological improvements are required to permit Zin monitoring during this modality.

Ztr (see paragraph 1.3.2) can be a valid alternative however even if it is possible to obtain Ztr using the traditional set up in 15wks old infants (Jackson et al. 1999) a new set up needs to be defined for application in small and sick newborns.

<u>WOB and fatique indices.</u> Two studies suggest the use of indices of respiratory efforts obtained from esophageal and gastric pressure when setting CPAP in infants with severe bronchiolitis (Essouri et al. 2011) and upper airways obstruction or BPD (Khirani et al. 2013). They found that there was a CPAP level associated with the greatest unloading of the respiratory muscles at which these indices reach a minimum. These are the only studies that suggest a method to guide CPAP titration. However this method was suggest not to individualize the treatment for each infant but for research purpose in order to identify the best mean level of CPAP to treat an homogeneous group of infants, because of the invasiveness of the measure.

WOB at different CPAP level in preterm babies but it is not clear if it was possible to identify an optimal CPAP level (Courtney et al. 2011). The disadvantages of these methods are connected to the need of gastric and/or esophageal pressure measurement that makes harder the use of this approach in clinical practice.

<u>Breathing pattern analysis.</u> Changes in volume can be monitor easier than pressure during non-invasive ventilation by RIP and EIT (see paragraph 1.3.1). EELV, V_T , Ti:Te, breathing frequency thoraco-abdominal synchrony, and ventilation distribution measure change with CPAP level in preterm babies (Miedema et al. 2013; Elgellab et al. 2001). However, whether the analysis of these parameters permits to identify an optimal CPAP level is still to be verify.

<u>Electrical activity of Diaphragm (Edi)</u>. Emeriaud et al. found that the diaphragm remains partially active during expiration in intubated and mechanically ventilated infants and that removal of PEEP affects this tonic activity (Emeriaud et al. 2006). They suggest that Edi could be useful for the management of PEEP in intubated and infants treated with CPAP (Beck & Sinderby 2004).

<u>Variability</u>. Variability of breathing pattern is related to both breathing control and lung mechanics (see paragraph 1.3.1). The application of pressure might modify EELV, lung mechanics, the input to the neural centers that arrives from the stress receptor and consequently the variability of the breathing pattern. Goldman et al. showed that the application of a pressure at the airways opening modify the multifractal properties of the intra breath interval in children (Goldman et al. 2008). Preterm babies could be more sensible to pressure levels as they have strong reflexes that play a large role in the control of breathing, immature control of breathing and they have to actively elevate EELV above the resting volume of the respiratory system. In newborns, variability of breathing pattern parameters has been found to be modified by assisted ventilation (Emeriaud et al. 2010). However, it is not known whether CPAP level influences breathing pattern variability in infant and if it can play a role in CPAP titration.

MECHANICAL

2 INVASIVE VENTILATION

Although nowadays the tendency is to avoid invasive ventilation to the majority of the infants, mechanical ventilation is indispensable for the survival of patients with the most complex clinical condition and lungs difficult to ventilate. However excessive tidal volumes and inadequate lung recruitment may contribute to mortality and development of BPD by causing ventilator-induced lung injury (see paragraph 1.2.1). There is a diffuse awareness of the importance of a protective ventilation strategy for newborns as well as that the key for it is recruiting the lung and keep it opened (see paragraph 1.4). However how to implement it in the clinical practice remains controversial and setting of ventilatory parameters remains a very difficult task.

Because of the great inter-patient heterogeneity and the continuous changes due to growth and pathology in the functionality of infants' respiratory system, it is impossible to identify a common strategy (even for subgroups of patients with similar characteristics) and only the customization of the treatment would realise real protective ventilation strategy. In order to achieve this, parameters, that could track changes in the respiratory function during ventilator setting titration and be measured non-invasively in the clinical practice, should be identified.

Nowadays ventilatory parameters are set primarily evaluating arterial oxygenation, auscultation and assessment of chest wall movement as indirect indicators of lung recruitment. However a strategy based on lung mechanics would have the advantage of providing information about both recruitment and distention therefore it could guide the choice of the ventilator settings toward a compromise between maximal recruitment and minimal overdistension.

Only two studies tried to individualise ventilatory settings during conventional mechanical ventilation in human newborns on lung mechanics bases. In the first study (Mathe et al. 1987), newborns were taken off the ventilator and slowly inflated with 10 ml/kg oxygen over 20 to 30 s. The inspiratory limb of the pressure-volume curves of the total respiratory system was measured and the optimal PEEP was defined as the airway pressure at the point of the curve above which it became abruptly linear and steeper: this is the minimum level of PEEP which enabled MV to take place in the linear zone. However the invasiveness of this approach discouraged more investigation from this method as it is not likely to enter in the clinical practice. Later, Gauthier et al. (Gauthier et al. 1998) studied within breath changes of Rrs and Xrs at 20Hz assessed by FOT at different levels of PEEP in term infants affected by bronchiolitis (PNA=2-8wk; BW=3.3-4.3kg). They conclude that measurements of Zin are informative in regard to the pathophysiological mechanisms occurring in bronchiolitis during mechanical ventilation,

and they may be helpful in setting the level and assessing the effect of PEEP. Despite the encouraging results, differences between the issue address in this study and PEEP optimization for preterm infants with acute or chronic diseases, made the adaptation of this method to this group of patients difficult and explains the lack of other studies on this argument. In fact, the set up used in this study was the traditional loudspeaker based FOT set up that is not suitable for small preterm newborns and for clinical practice in general. Moreover this study addresses the issue of PEEP optimization for infants at term with an airway disease while to optimize lung recruitment it is important to obtain information on parenchyma and peripheral airways.

Only other few studies have been performed on infants under the first month of life using FOT. Considering only studies on preterm ventilated infants the number is further reduced.

The first report in intubated infants appeared in 1983 (Dorkin et al. 1983). In this study Dorkin et al. used an oscillatory flow from 4 to 40 Hz, generated by a loudspeaker, to characterize the respiratory system of 6 intubated infants (GA= 31-36 wk;PNA= 1-7d;BW=1.6-2.3kg) with RDS. Later on, Sullivan et al. (Sullivan et al. 1991) measured respiratory impedance in 9 ventilated infants (7 with RDS and 2 with chronic lung disease; GA= 29-38 wk; PNA= 1-61d;BW=0.8-3.3kg) who were classified into two groups based on the presence or absence of a resonance phenomenon (zero crossing of the Xrs) between 6 and 16 Hz, which was associated to the duration of ventilatory therapy. These two studies analysed the frequency dependency of the impedance disconnecting the infants from the ventilator and connected to a device that provided a stimulus within a wide range of frequencies.

Despite the great need of means to measure lung function in mechanically ventilated newborns, after these two old studies there have been no other studies applying FOT to this population. This is probably related to the lack of a set up easy to use and of direct clinical impact of these measurements. However the improvement in technology (that let see the possibility to implement FOT into a ventilator making it available in clinical practice) together with the recently proposed approach to FOT (intra-breath analysis and attention on relative changes in lung impedance rather than on its absolute values as function of the stimulation frequency) have led us to develop a new set up in order to apply this technique to the studies of the respiratory system of intubate newborns. Once the set-up has been validated in vitro, in order to evaluate if FOT can provide a tool to optimize the treatment for these patients, we applied it to the studies of clinical intervention that could modify EELV and its distribution like PEEP and prone positioning.

As PEEP is the ventilatory parameter that impacts on how many alveoli remain open at endexpiration, we first applied FOT to the study of the changes in lung mechanics during PEEP titration. Different group of patients (RDS, evolving BPD and healthy lung) have been considered to understand if their response to PEEP is different and whether and how Zin measurement could guide the choice of PEEP setting in these different patients. Moreover as especially during the first days of life the respiratory system undergoes dramatic changes we studied also how the relationship between PEEP and impedance evolves during the first week of life in the same infant and how the optimal PEEP from a mechanical point of view changes during this period in comparison with the clinical one.

Animal studies have shown that prone positioning leads to a greater regional recruitment and a more homogeneous distribution of the stress applied to the parenchyma (Broccard et al. 2000; Valenza et al. 2005). As preterm infants are very exposed to injury from oxygen toxicity and aggressive mechanical ventilation, prone positioning could be a valuable tool for improving respiratory support. Therefore we studied how Zin is affected by posture in infants with RDS and BPD.

The data obtained from these studies have provided important information also for understanding physiological mechanisms and for tuning algorithms and the approach to Zin measurement. Finally, the results of these studies emphasize the importance of customization and continuous adaptation of the treatment to the respiratory system condition. They provide a justification for the efforts required to integrate FOT into a mechanical ventilator in order to make Zin measurement possible in clinical practice (see paragraph 4.2.1). This will open the way to clinical studies evaluating the impact of this measure on clinical outcome of these small patients.

2.1 NEW APPROACH TO OPTIMIZE LUNG VOLUME

How reported in paragraph 1.4, Dellacà et al. proposed an alternative approach for optimizing PEEP on lung mechanics basis consisting in within-breath measurements of Zrs at a single frequency.

The rationale for the use of Xrs for the evaluation of lung volume recruitment and distention is the following. Because inertance is mainly due to the endotracheal tube and to proximal airways, its contribution is not likely to change significantly with PEEP and the trend of reactance is mainly due to changes in lung compliance. In the simplifying hypothesis that the alveolar units can be modelled by compliant elements connected to each other in parallel, the measured Xrs results from the sum of the compliance of the single alveolar units which are reached by the oscillations and therefore ventilated. Therefore the total compliance and Xrs (equation 1.4) depend on the number of open alveoli and on the compliance of the single alveolar unit that decrease with distention.

Figure 2-1 shows how Xrs changed during lung volume recruitment and derecruitment. During an incremental PEEP trial Xrs increases if recruitment of alveoli occurs. However in the same time, the compliance of the already open alveoli decreases with alveolar distention and eventually this effect predominates, causing Xrs to decrease again. During a decremental PEEP trial, the lung has already been fully recruited (or nearly so) at the commencement of the trial. As PEEP is reduced from its highest level, the alveolar compliance increases as the distention of the tissue is decreased. Only when the PEEP level falls below closing pressure of some alveoli end-expiratory collapse occurs, thus reducing the number of aerated alveoli and the total alveolar compliance. However, this effect is opposed by the increasing alveolar compliance at lower alveolar volume. Xrs changes with PEEP are often characterized by a hysteretic behaviour between incremental and decremental trials that demonstrates that new alveolar units were recruited as PEEP was increased.

Dellacà et al. studied the change of reactance with PEEP at end expiration to avoid the influence of intra-tidal recruitment/derecruitment. In particular they showed that, in a surfactant-depletion model of ALI, monitoring Xrs at 5Hz during a decremental PEEP trial allowed the identification of the lowest PEEP able to prevent lung derecruitment, with high sensitivity and specificity when compared with CT (Figure 2-1). They also demonstrated how reactance measured at end-expiration at 5 Hz, could be used to monitor recruitment/derecruitment regardless model and distribution pattern of lung collapse (Dellaca et al. 2009).



Figure 2-1. Relationship between PEEP during the inflation-deflation PEEP trial and respiratory system reactance (Xrs), percentage amount of non-aerated tissue (VtissNA%), and partial pressure of oxygen (PaO2). Values are mean and SD. The arrows identify the inflation and deflation limbs of the curve. The circles identify the optimum PEEP defined as the maximum Xrs value reached during the deflation limb. *p\0.05 compared to the previous step. Modified from (Dellacà et al. 2011).

The effects of repeated PEEP optimization on Xrs basis on oxygenation, lung mechanics, and histologic markers of lung injury have been compared with the results obtained by applying the ARDSnet protocol based on oxygenation alone in a porcine surfactant-depletion lung injury model over a 12-hour ventilation period (Kostic et al. 2011). The FOT-based optimization strategy resulted in the selection of higher PEEP levels than the ARDSnet protocol. Higher levels of PEEP resulted in higher dynamic compliance in the FOT group leading to lower pressure amplitude and lower or comparable levels of plateaux pressure. Moreover the PEEP optimization strategy based on Xrs resulted in a better PaO_2/FiO_2 ratio and in reduced histopathologic evidence of VILI compared to the ARDSnet protocol.

As FOT can be performed with high temporal resolution and without changing lung volume, during CMV it can be used to assess lung mechanical properties both at end-inspiration and end-expiration (Zannin et al. 2012). Optimizing PEEP on Xrs basis minimized intra-tidal recruitment/derecruitment with the potential to minimize cyclic mechanical stress on lung

tissue. The measurement of intratidal changes in Xrs has the potentials to be used not only to optimize and individualize PEEP but also to evaluate the tidal recruitment and distension associated to different pressure amplitudes or tidal volumes.

The advantages of this approach in comparison with the other FOT based optimization strategies are: i) it does not interfere with ventilation; ii) as it is not based on models, the respiratory system does not have to satisfy the models' hypothesis; iii) it is not affected by errors due to non-stationarity (changes during the breath-cycle) and crosstalk between the different frequencies and iv) permits the identification of tidal recruitment. Also compared to the use of Cdyn in monitoring lung volume recruitment and derecruitment, the use of Xrs offers important advantages. First, since FOT requires very small lung volume changes, the measurement can be performed at a specific lung volume, minimizing the artefacts due to nonlinearities of the respiratory system. On the contrary Cdyn is calculated over a whole breath, and this large volume change invalidates the hypothesis of linearity on which the calculation of Cdyn is based, especially in diseased lungs. Second, Xrs is not affected by spontaneous breathing while Cdyn requires sedation or esophageal pressure measurement. Third, since Xrs may be measured with a high time resolution, it allows the assessment of within-breath changes of lung mechanics to monitor intra-tidal recruitment and over-distension, both of which have a role in ventilator-associated lung injury while Cdyn is affected by tidal recruitment.

These reasons make Xrs measurement particularly interesting for application in preterm newborns.

2.2 METHODS

FOT has been identified as a promising technique as it has been shown that permits to optimise PEEP in an animal model of RDS.

However to apply FOT in infants adequate set up and methods for data elaboration have to be developed to meet the specific requirement related to this group of patients. In fact, the very low flow and volume and the high breathing frequency add technical issues and constraints for the experimental set up and the data processing. In fact the traditional loudspeaker based FOT set up is not suitable for the study of ventilated preterm infants, as the additional compliance represented by the loudspeaker when connected to the ventilation circuit interferes with the ventilator trigger and the delivery of a proper ventilator waveform.

2.2.1 FOT MEASUREMENT IN PRETERM NEWBORNS DURING CMV

OSCILLATION GENERATED EXTERNALLY TO THE VENTILATOR

FOT measurements are usually performed by using loudspeakers enclosed in a rigid box to generate the forcing signal superimposed to conventional mechanical ventilation. The limitation of this solution is that the loudspeaker and the gas enclosed in the box and in the additional tubing represents an additional compliance to the inspiratory line of the ventilator. In a preliminary study, we found that the compressible volumes added to the ventilator circuits led to a slower rise in pressure during inspiration impairing patient-ventilator interaction. For this reason, we generated the oscillations using a 20 ml glass syringe, the piston of which was moved by a servocontrolled linear motor. The syringe was placed in parallel to the mechanical ventilator. The position of the motor was controlled in closed loop, using a PID controller, the parameters of which were tuned using Zigler and Nicolson method. A digital-to-analog board (NI-DAQ USB-6221, National Instruments, Austin, TX) mounted on a personal computer was used

to generate the signal which, amplified, controlled the motor. The amplitude of the driving signal was adjusted in order to have a peak-to-peak amplitude of the pressure swing of ~2 cmH₂O.

The pressure signal was measured by a differential pressure transducer (PXLA0075DN, Sensym, Milpitas, CA). The flow signal was measured by a mesh-type heated pneumotachograph (Hans Rudolph 8410A, resistance = $0.6 \text{ cmH}_2\text{O*s/I}$ at 0.27 I/s, dead-space volume = 1.3 ml) coupled with a differential pressure transducer (PXLA02X5DN, Sensym, Milpitas, CA). All the signals were sampled by the same A/D-D/A board used to control the motor and recorded on a personal computer. A program developed in LabView permits data visualization, Z computation at the stimulus frequency and data storage.

We verified that the dead space and the resistance added by this set up to the ventilator circuit was compliant with the international standards of infants lung function testing (U. Frey et al. 2000). The common mode rejection ration of the flow channel, which may be critical with high impedances, like those of the infants, was \geq 50 dB up to 20 Hz. Drift of the pressure sensor was negligible while drifts related to temperature and water vapour condensation were prevented by heating the pneumotachograph. A calibrated syringe was use to calibrate flow before each measurement. Signal was sampled at 600Hz. Leaks were constantly monitored during the measurements. The accuracy and the frequency response of the sensors were assessed in vitro measurements (see the following paragraph).



Figure 2-2 Glass syringe based FOT set up.

The advantage of this set up is that permits Zin measurements in ventilated infants independently by the ventilator used; does not require correction for non-linearity of the sensors and permits simultaneous pressure and flow sampling. The disadvantages are that it requires disconnection of the patients to connect the syringe and the sensors to the ventilation circuits, it adds dead space and time and technical staff is needed to predispose everything for the measurement.

OSCILLATION GENERATED INSIDE THE VENTILATOR

Stephanie ventilator (Stephan, Germany) gives the possibility to over-impose the HFOV waveform to the SIPPV one. Therefore the ventilator can be used to ventilate the infant with SIPPV and simultaneously generate the stimulus signal for FOT setting the peak to peak pressure at 2cmH₂O for HFOV with a sinusoidal waveform. Moreover flow and pressure measurements are provided as analog outputs by the ventilator. This permits to avoid the used of an external flowmeter that adds dead space to the circuit.

However pressure is not measured at the mouth, the flowmeter is not linear and when HFOV and SIPPV are used together there is a small shift between the set PEEP and the measured one as the oscillations interfere with the controller. These issues were solved by measuring the pressure externally, compensating for the non-linearity of the flowmeter and providing real time PEEP measurement to the clinician so that he could adjust the settings to obtain the desired PEEP when he turns on the oscillations.

Pressure was measured by a differential pressure transducer (PXLA0075DN, Sensym, Milpitas, CA) at the inlet of the ETT. The correction to compensate for the non-linear response of the Stephanie pneumotacograph was provided by the manufactor.

A homemade electronic board was developed to sample the signals and send them to the computer. A program developed in LabView permits data visualization, Z computation at the stimulus frequency and data storage. PEEP is continuously computed from pressure measured at the mouth and displayed on the computer so that the clinician knows the actual PEEP value and can adjust the settings when he turns on the oscillation or wants to change the PEEP value.

As pressure measurements involved the same circuits than the set up described before and flow measurement were performed by a commercial NICU ventilator, also this set up results compliant with the international standards of infants lung function testing. Signal was sampled at 300Hz and leaks were constantly monitored during the measurements. Moreover, we tested the accuracy of the measure at different frequencies (see the following paragraph).



Figure 2-3 FOT set up.

The advantage of this set up is that permits Zin measurements in ventilated infants in an easy way without disconnected the patient from the ventilator, adding dead space to the circuit or requiring technical staff.

2.2.2 DATA PROCESSING

Pre-processing of the acquired signals (calibration, synchronization of the signals) has been managed by acquisition software implemented in LabVew (National Instruments Corporation, Austin, TX, US). Data elaboration has been performed using special purpose calculus routines implemented in Matlab (MathWorks Inc, Natick, MA, US) while all the statistical analysis have been carried out by using the tools of a commercial software for statistical elaboration and analysis (Sigmastat 9, Systat Software Inc., San Jose, CA, US)

Generally, the values of several breathing pattern parameters and lung mechanics at end expiration/inspiration were computed for each breath. The mean values, standard deviation and scaling exponent according to the protocol were computed for each subject and each condition.

CHOICE OF FORCING SIGNAL

Different stimulus shapes and frequency components can be adopted depend on the phenomena of interest (see paragraph 1.3.2).

We choose a sinusoidal stimulus as, for tracking changes over time in respiratory mechanics the use of a single frequency has the advantage to maximize signal to noise ratio (SNR). The chosen amplitude of the signal is approximately of $2\text{cmH}_2\text{O}$ as should be large enough to guarantee a satisfactory SNR, but not too large so as to avoid discomfort for the subject, nonlinear behaviour of the respiratory system and synchronization between breathing and input signals.

The respiratory system behaves like a low pass filter, so low frequencies are more sensitive to peripheral phenomena such as alveolar recruitment and distension. However, the forcing frequency must be high enough not to be affected by the breathing rate of the infants. In animals, previous studies have showed that 5Hz is a good compromise, with Xrs measured at that frequency showing a high correlation with lung volume recruitment/derecruitment (Dellaca et al. 2009) and being able to identify the lowest PEEP preventing lung collapse during a decremental PEEP trial in a surfactant depletion model of RDS (Kostic et al. 2011). However the breathing frequency of preterm infants it is higher and the power spectrum of the breathing signal has still a significant component at 5Hz. On the other hand, as also the resonant frequency of infants' respiratory system is higher, even higher frequency could still provide information about lung periphery. Moreover at 5Hz measurement of Zin at end inspiration are often not possible as the inspiratory time is too short to ensure at least a complete sinusoidal cycle at endinspiration. For these reasons, we use a stimulus frequency of 5Hz for all the studies but we performed also measurement with stimulus at higher frequencies (10Hz and 15Hz) to test if the use of these frequencies is a best compromise. The values of 10Hz and 15Hz were chosen among the other because these are the frequencies normally used for HFOV and available in neonatal ventilators.

IMPEDANCE CALCULATION

Pressure and flow signals can be elaborated both in the time and in the frequency domain in order to compute impedance. Both these methods have been tested in order to evaluate the relative advantages and disadvantages for application in newborns.

<u>Frequency domain.</u> The Z_{rs} can be estimated using the spectral approach by Fourier analysis (Michaelson et al. 1975):

$$Z_{RS} = \frac{G_{ff}}{G_{pf}} \tag{2.1}$$

Where G_{pf} is the cross-spectrum between the pressure and flow signals, G_{ff} is the autospectrum of the flow signal.

The estimates of G_{pf} and G_{ff} is obtained by the Welch's averaged periodogram method, i.e. dividing the signal into overlapping windows (in order to avoid power leakage at the borders of the single window), computing the periodogram for each window and finally averaging all the periodograms.

The influence of different parameters (length of time blocks, overlapping, windowing, low-pass and/or high-pass filtering) is to be considered for the data processing (Lorino et al. 1993).

The principles outlined before apply to one-input-one-output linear system not contaminated by external noise or interference. As this condition is unlikely in biological system, the coherence function γ^2 is used to provide an index of causality between the input and the output of a linear system and thus how much the output is influenced by factors other than the input:

$$\gamma^{2} = \frac{|G_{pf}^{2}|}{G_{ff} \cdot G_{pp}} , \quad \gamma^{2} \in [0,1]$$
 (2.2)

The value of γ^2 is less than 1 if the output is the result of more than one input, if the system is nonlinear, or if the system is contaminated by extraneous noise (e.g. electrical noise). Therefore, proper threshold may be used to evaluate the goodness of the spectral estimate of Z_{rs} .

Some advantages of the impedance computation by spectral analysis are: the speed of measurement and simultaneous determination of the magnitude and phase of Z_{rs} at each frequency of interest; it does not require any hypothesis or a priori knowledge about the stimulating signal and can be applied with non-sinusoidal stimuli.

However, the time-dependent changes of the respiratory mechanics cannot be tracked and analysed with a high time-resolution. As the mechanical indices measured by FOT change during a test and inside each single breath, a proper evaluation of the variability of these indices at different time scales requires a continuous analysis in the time domain rather than a spectral estimate of the magnitude and phase of Z_{rs} at different frequencies.

<u>Time domain.</u> A possible alternative to study Z_{rs} as a function of time and still obtain a reasonable estimate of its spectral content takes advantage of the a priori knowledge of the frequency content of the forcing signal (Kaczka et al. 1995; Kaczka et al. 1999). This solution may be used to track rapid changes in Z_{rs} , as those occurring in the respiratory cycle.

Flow and pressure are modelled in the time domain using trigonometric approximations:

$$\dot{V}(t_n) = a_0(t_n) + \sum_{k=1}^k a_k(t_n) \cdot \cos(2\pi f_k t_n) + \sum_{k=1}^k b_k(t_n) \cdot \sin(2\pi f_k t_n) + \varepsilon_{\dot{V}}(t_n)$$
(2.3)

$$P(t_n) = c_0(t_n) + \sum_{k=1}^k c_k(t_n) \cdot \cos(2\pi f_k t_n) + \sum_{k=1}^k d_k(t_n) \cdot \sin(2\pi f_k t_n) + \varepsilon_P(t_n)$$
(2.4)

where t_n is the sampling index representing the time of the nth sample and the coefficients $a_k(t_n), b_k(t_n), c_k(t_n)$ and $d_k(t_n)$ are real functions of time that determine the linear oscillatory response for flow and pressure at each specified frequency f_k and time. The error terms, $\varepsilon_{\dot{V}}(t_n)$ and $\varepsilon_P(t_n)$ account for both modelling and measurements errors and include measurements noise and non-linear components of pressure and flow.

Since the trigonometric coefficients reflect the steady-state magnitudes and phases of the flow and pressure signals, once the parameter vector has been estimated, the respiratory system impedance (Z_{rs}) can be calculated at each discrete frequency as:

$$Z_{rs}(f_k) = \frac{c_k - jd_k}{a_k - jb_k}$$
(2.5)

One common and efficient approach used to calculate Z_{rs} in the time domain, under the hypothesis of linearity, is the use of a recursive least square algorithm. At a given time instant, we consider a time window of N samples of flow and N samples of pressure, where N is the minimum number of data required to include one complete period of the component of the forcing signal with the lowest frequency. Thus, two linear systems of N equations can be obtained, one for flow and one for pressure. Either system is expressed in matrix notation as:

$$Y = X\theta + \varepsilon \tag{2.6}$$

where for flow:

$$\boldsymbol{Y} = \begin{bmatrix} \dot{\boldsymbol{v}}(t_1) \\ \dot{\boldsymbol{v}}(t_2) \\ \vdots \\ \dot{\boldsymbol{v}}(t_N) \end{bmatrix}, \qquad \boldsymbol{\theta} = \begin{bmatrix} \boldsymbol{\varepsilon}_1 \\ \boldsymbol{\theta}_1 \\ \boldsymbol{\theta}_2 \\ \boldsymbol{\theta}_2 \\ \vdots \\ \boldsymbol{\theta}_k \\ \boldsymbol{\theta}_k \end{bmatrix}, \qquad \boldsymbol{\varepsilon} = \begin{bmatrix} \boldsymbol{\varepsilon}_{\dot{\boldsymbol{v}}}(t_1) \\ \boldsymbol{\varepsilon}_{\dot{\boldsymbol{v}}}(t_2) \\ \vdots \\ \boldsymbol{\varepsilon}_{\dot{\boldsymbol{v}}}(t_N) \end{bmatrix}$$
(2.7)

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while for pressure:

$$\boldsymbol{Y} = \begin{bmatrix} P(t_1) \\ P(t_2) \\ \vdots \\ P(t_N) \end{bmatrix}, \qquad \boldsymbol{\theta} = \begin{bmatrix} c_0 \\ c_1 \\ d_1 \\ c_2 \\ \vdots \\ c_k \\ d_k \end{bmatrix}, \qquad \boldsymbol{\varepsilon} = \begin{bmatrix} \varepsilon_P(t_1) \\ \varepsilon_P(t_2) \\ \vdots \\ \varepsilon_P(t_N) \end{bmatrix}$$
(2.8)

for both:

$$\boldsymbol{X} = \begin{bmatrix} 1 & \cos(2\pi f_1 t_1) & \sin(2\pi f_1 t_1) & \cos(2\pi f_2 t_1) & \sin(2\pi f_2 t_1) & \cdots & \cos(2\pi f_K t_1) & \sin(2\pi f_K t_1) \\ 1 & \cos(2\pi f_1 t_2) & \sin(2\pi f_1 t_2) & \cos(2\pi f_2 t_2) & \sin(2\pi f_2 t_2) & \cdots & \cos(2\pi f_K t_2) & \sin(2\pi f_K t_2) \\ \vdots & \vdots \\ 1 & \cos(2\pi f_1 t_N) & \sin(2\pi f_1 t_N) & \cos(2\pi f_2 t_N) & \sin(2\pi f_2 t_N) & \cdots & \cos(2\pi f_K t_N) & \sin(2\pi f_K t_N) \end{bmatrix}$$
(2.9)

The goal is to estimate the parameter vector θ for the flow and pressure segments. To accomplish this, a least-squared estimate of θ can be computed as:

$$\boldsymbol{\theta} = (\boldsymbol{X}^T \boldsymbol{X})^{-1} \boldsymbol{X}^T \boldsymbol{Y} \tag{2.10}$$

Then, the moving window is shifted ahead of one sample and the procedure is reiterated. Some advantages of the impedance computation by spectral analysis are: possibility of intrabreath analysis of impedance's changes; it is not necessary the assumption that the system under analysis has achieved a dynamic oscillatory steady state and high temporal resolution.

AUTOMATIC COMPUTATION OF THE MEAN ZIN

Sinusoidal stimulus at 5Hz is not separable in the frequency domain from the frequency content of the breathing signal; therefore we separate the signal in the time domain considering the oscillation at end-expiration and at end-inspiration when the inspiratory time was long enough. At these points the SNR is high enough to guarantee accurate results. Moreover in order to evaluate lung recruitment, the impedance at end expiration is the one of interest as reflects the number of alveoli that remains open during all the respiratory cycle.

While the implementation of this is straightforward for the analysis in the time domain, for the frequency domain intra breath impedance values can be obtained computing the spectra of the flow and pressure signals using a rectangular windows of lengths equal to the period of the stimulus (Farré et al. 1997). For real time application, as the computation of Zin in time domain is much faster than in the frequency one with no other disadvantages, within breath input impedance has been estimated by using the least mean square based approach. An automatic algorithm has been developed to identify end expiration to compute the mean Zrs value in each period of interest: i) the time course of lung volume was obtained by integrating V'_{AO} and by removing the linear drift. ATPS (ambient temperature and pressure saturated) / BTPS (body temperature and pressure saturated) were not applied as the air in the ventilator circuit is heated and humidified ii) from this trace breathing parameters were computed and end-inspiration and end-expiration points identified; iii) in general a fixed number of breathes was selected from the last part each period of interests; iv) breaths in which impedance tracings showed spikes or oscillations due to ETT closure or measurements noise were discarded.

CORRECTION FOR ENDO-TRACHEAL TUBE (ETT) IMPEDANCE

In infants, measuring pressure at the tip of the ETTs is not possible as their internal diameters are very small. Therefore the pressure is measured at the inlet of the ETT and the impedance value obtained by FOT accounts also for the impedance of the ETT.

This is not an issue when studying impedance changes in the same infants without replacing its ETT during the measurements. In fact, as the impedance of ETT is constant, possible changes are related to modification of the respiratory system mechanics.

However, as different ETT diameters are used according to the dimension of the trachea, in comparing measurements between different infants or in the same infant after replacement of ETT, it introduces a difference that it is not due to respiratory system condition.

A measure of the ETT impedance can be obtained in an in vitro experiment using a mechanical analogue of the infants' respiratory system. The mechanical analogue is connected to the ETT and the pressure is measured both at the inlet and at the end of the ETT. From the measure of the pressure across it and the flow passing through it, it is possible to compute its impedance. For each study performed the impedance of these tubes has been characterized using the same pressure stimulus and set up used in the study.

Impedance data obtained in infants are then corrected by subtracting Rrs and Xrs values for the ETT with the correspondent diameter. This approach has been validated by Dorkin et al. (Dorkin et al. 1983).

2.2.3 IN VITRO VALIDATION

OSCILLATION GENERATED EXTERNALLY TO THE VENTILATOR

A mechanical model of the infant respiratory system was realized by small cross section glass tubes connected in parallel to represent both resistance and inertance in series with a glass bottle that was chosen to represent lung compliance. The characteristics of tubes and bottle were chosen in order to obtain values of resistance, inertance and compliance similar to the one of the infants' lung as measured by Dorkin et al. (Dorkin et al. 1983). This bench model of the infant lung was used to tune the servo-controller parameters and to test the frequency response and the accuracy of the measurement set-up vs a traditional loudspeaker-based set up. Since the performance of the motor can vary depending on the pressure that it has to stand, the measurements were performed at three different levels of positive end-expiratory pressure (PEEP): the lowest, the average and the highest values used in the clinical practice (2 cmH_2O , 6 cmH_2O , 10 cmH_2O).

Table 2-1 shows impedance data measured by our FOT set-up at different PEEP levels. The percentage differences with respect to the values obtained using the reference set-up are also reported.

-			
PEEP	2 cmH ₂ O	6 cmH ₂ O	$10 \text{ cmH}_2\text{O}$
R5Hz	114.84 ± 3.78	112.72 ± 4.41	108.21 ± 3.21
	(-2.8 ± 0.3%)	(-4.1 ± 0.2%)	(-3.2± 0.2%)
X5Hz	-75.21 ± 7.59	-73.62± 6.28	-70.56 ± 4.38
	(-1.9± 0.3%)	(-3.6 ± 0.2%)	(-1.7 ± 0.3%)
R11Hz	121.90 ± 1.95	116.94 ± 1.49	112.10 ± 1.38
	(-1.3 ± 0.1%)	(-2.1 ± 0.1%)	(0.4 ± 0.2%)
X11Hz	-10.75 ± 2.14	-9.13 ± 2.28	-8.02 ± 1.97
	(8.1 ± 1.2%)	(-14.6 ± 1.1%)	(9.0± 1.1%)
R19Hz	128.74 ± 2.04	123.26 ± 1.24	116.69 ± 0.67
	(-8.8 ± 0.1%)	(2.0 ± 0.1%)	(9.6 ± 0.1%)
Х19нг	25.60 ± 2.40	29.32 ± 1.09	31.12 ± 0.49
	(13.9 ± 0.4%)	(-4.8 ± 0.8%)	(-11.4 ± 0.5%)

Table 2-1. Resistance (R) and reactance (X) measured at different PEEP levels and percentage differences with
respect to the reference.

R_{5Hz}: R measured at 5 Hz, X_{5Hz} : X measured at 5 Hz, R_{11Hz}: R measured at 11 Hz, X_{11Hz} : X measured at 11 Hz, R_{19Hz}: R measured at 19 Hz, X_{19Hz}: X measured at 19 Hz. R and X are in cmH₂O*s/l.

The difference between the measurements performed on the bench model using our set-up and a standard loudspeaker-based one was below 10% for Rrs and below 15% for Xrs in the whole range of frequencies of interest (5-20 Hz) and the performance of the measurement system was not affected by the pressures generated by the ventilator. Moreover the higher percentage error in Xrs at 11Hz and 19Hz are due to low Xrs values at these frequencies and correspond to a low absolute error (< 3.5cmH₂O).

OSCILLATION GENERATED INSIDE THE VENTILATOR

A bench model of the infant lung similar to the one described previously was used to test the frequency response and the accuracy of the Stephanie ventilator based set-up vs a traditional loudspeaker and linear pneumotacograph based set up (Table 2-2).

		R _{5Hz}	X _{5Hz} R _{10Hz}		X _{10Hz}	R _{15Hz}	X _{15Hz}	
_		(cmH ₂ O*s/I)	(cmH ₂ O*s/l)	(cmH_2O*s/I)	(cmH ₂ O*s/I)	(cmH ₂ O*s/l)	(cmH ₂ O*s/l)	
	Ref	95.7	-56.5	104.3	-12.4	107.4	3.7	
	Set up	91.7	-53.8	100.9	-10.7	103.5	2.2	
	err	4.1	-2.7	3.4	3.4 -1.7 3.9	3.9	1.5	
	err %	4.3	4.7	3.3	13.9	3.6	41.2	

Table 2-2 Resistance (R) and reactance (X) measured at different frequency with the Stephanie ventilator and with a traditional set up (Ref) in order to test the accuracy and the frequency response of the measurement system.

The absolute difference between the measurements performed on the bench model using this set up and a standard loudspeaker-based one was below 5 cmH₂O*s/l for Rrs and Xrs at all the frequencies.

2.3 RELATIONSHIP BETWEEN RESPIRATORY IMPEDANCE AND PEEP IN MECHANICALLY VENTILATED NEONATES

Surfactant deficiency combined with the poor outward elastic recoil of the chest wall makes premature infants susceptible to alveolar instability, airways collapse and atelectasis, leading to lung volume derecruitment and increased lung opening pressure.

The application of positive end-expiration pressure (PEEP) is widely used to prevent the collapse of alveoli and terminal airways at end-expiration in Respiratory Distress Syndrome (RDS). Unfortunately there is also evidence that an inappropriate use of PEEP may cause alveolar overdistension, with reduced compliance, carbon dioxide retention (Field et al. 1985) and increased risk of developing VILI (Jobe et al. 2008).

Nowadays PEEP titration in clinical practice in newborns relies on monitoring oxygen saturationas an indirect and crude indicator of lung recruitment.

In the pioneering study of Suter et al (Suter et al. 1975), the authors argued that PEEP should be tailored on each individual patient identifying the lowest pressure providing the highest lung compliance.

Recent studies provided further evidence that PEEP could be successfully optimized in acute lung injury or acute RDS (ALI/ARDS) at the bedside by either maximising dynamic compliance (C_{dyn}) (Dargaville et al. 2010; Suarez-Sipmann et al. 2007) or minimising dynamic elastance (Edyn) (De Luca et al. 2012; Pillow et al. 2004) of the respiratory system during a decremental PEEP trial. However, the estimation of C_{dyn} (and E_{dyn}) is affected by respiratory muscles activity, which prevents its use in non-paralysed patients (De Luca et al. 2012).

Moreover preterm infants are prone to develop Bronchopulmonary Dysplasia (BPD) and require mechanical ventilation for long periods in the phase of evolving BPD. While the rationale for PEEP titration in RDS is to identify the lowest level of PEEP able to counteract lung volume de-recruitment, in evolving BDP PEEP should be set at the minimum level that counteracts airways collapse, which is likely to occur because of airway walls remodelling, interstitial fluids accumulation and reduced elastic recoil of the lung. Therefore the assessment of lung mechanics would be of great value also in evolving BPD but with a different aim, i.e. identifying airway collapse.

The lack of adequate bedside tools for the assessment of respiratory mechanics in ventilated infants is nowadays the major limiting factor for the individualized tailoring and continuous adjustment of PEEP in these critical patients.

A possible answer to this need is provided by the forced oscillation technique (FOT). FOT can be used to identify lung volume recruitment-derecruitment during both conventional (Dellaca et al. 2004; Dellaca et al. 2009) and high frequency oscillatory ventilation (Pillow et al. 2004). Moreover, it has been recently shown that reactance (Xrs) can guide PEEP titration by identifying the lowest PEEP that maintains lung volume recruitment minimising lung mechanical stress in an experimental model of ALI (Dellaca et al. 2009; Kaczka et al. n.d.), leading to a more protective ventilation strategy compared to an oxygenation-based approach (Kaczka et al. n.d.).

To our knowledge, FOT has been applied to ventilated preterm newborns in very few studies (Dorkin et al. 1983; Jobe et al. 2001), and in none of them respiratory system impedance has been studied at different PEEP settings.

The aim of the present study was to apply a new set-up designed to apply FOT on mechanically ventilated newborns during an incremental/decremental PEEP trial in order to evaluate the feasibility of bedside FOT measurements and to characterize the impedance-PEEP relationships in newborns with healthy lungs, with RDS and chronically ventilated infants that developed BPD.

2.3.1 STUDY PROTOCOL

All measurements were performed in the Neonatal Intensive Care Unit of Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico in Milan. The study had been approved by the local Ethics Committee and informed parental consent had been obtained prior to the studies.

All infants assisted by conventional mechanical ventilation, in hemodynamically stable conditions, regardless of the gestational age, postnatal age and the cause of respiratory failure, were eligible for the study. Infants with severe intracranial hemorrhage (III and IV) and/or malformations were excluded.

All the infants were studied in the supine position. Infants were intubated with uncuffed endotracheal tubes (size 2.5-3.5 mm i.d.) and treated with Synchronized Intermitted Positive Pressure (S-IPPV) ventilation (Babylog 8000 plus, Drager, Lubeck, Germany or Leoni Plus, Heinen & Lowenstein, Germany) with volume guarantee ($V_T = 5 \text{ ml/kg}$) and PIP=24±2cmH₂O. All ventilatory parameters but PEEP were kept constant at the clinically set values, while the FiO₂ was adjusted in order to maintain oxygen saturation (SpO₂) between the clinical limits (86-94%). At first PEEP was lowered to 2 cmH₂O for 1 minute, then it was increased to 10 cmH₂O and subsequently decreased to 2 cmH₂O in steps of 1cmH₂O. Preliminary measurements showed that one minute allows the major impedance changes to stabilize, but we cannot exclude the occurrence of dynamics phenomena with longer time scales. Since we wanted to minimize the duration of the maneuver and especially the time spent at sub-optimal PEEP levels, each step lasted 1 minute.

Sinusoidal oscillations at 5Hz with a \sim 2 cmH₂O peak-to-peak amplitude (see paragraph 2.2.2) were delivered for all the duration of the trial and superimposed on the ventilator waveform. The total duration of the study never exceed 30 minutes.

A detailed description of the measurement set-up is reported in paragraph 0. Flow and pressure data were digitized at 600 Hz and stored on a personal computer. SpO₂, blood pressure and heath rate were continuously monitored non-invasively to evaluate how the manoeuvre was tolerated by the patient.

Data analysis

Zrs was computed using a least squared method and corrected by subtracting Rrs and Xrs values for the ETTs (see paragraph 2.2.2) (Dorkin et al. 1983)(Dorkin et al. 19

Oscillatory elastance (E_{FOT}) was obtained from Xrs as follows:

$$E_{FOT} = -2\pi f_{osc} X_{rs}; \tag{2.11}$$

Where fosc is 5Hz.

 E_{dyn} was estimated by fitting P_{AO} and \dot{V}_{AO} to the equation of motion of the respiratory system:

$$P_{AO} = E_{dyn}V + R_{rs}\dot{V}_{AO} + EEP;$$
(2.12)

where V is volume obtained by integration of \dot{V}_{AO} Rrs is the resistance of the respiratory system and EEP is the end-expiratory pressure.

Statistical analysis

Significance of differences in population characteristics and clinical parameters among groups was tested by Mann-Whitney test. Two-way ANOVA for repeated measurements was used to test the significance of differences in impedance data among groups and within each group among PEEP levels. Multiple comparisons after ANOVA were performed using the Holm-Sidak method. Differences were considered statistically significant for p<0.05. Spearman's correlation was performed to test whether there was a statistical dependence between impedance and clinical parameters and ρ was used to test the correlation strength.



Figure 2-4. Experimental tracings. Right panel: pressure, flow, resistance, and reactance. For each PEEP step the last 5 breaths are reported. Resistance and reactance are reported as mean and SD of these 5 breaths. Left panel: enlargement of one PEEP step (5 cmH₂O). The grey areas represent the data which were discarded.

2.3.2 RESULTS

A total of 28 newborns were studied (Table 2-3).

		Gender	GA (wk)	Birth weight (g)	PNA (d)	Weight (g)	PEEP (cmH ₂ O)	FiO ₂	PO ₂ (mmHg)	PCO ₂ (mmHg)
DDC		F	29	1200	1	1220	4	24	47.9	21.2
KDS	VLBW/	F M	28	1200	1	1230	4	24	47.8	31.3
	LBW	M	29	1490	1	1400	4	33 27	21.5	/1.1
		M	29	1490	2	1400	5	27	55.0 41.8	03.0 40.5
		M E	24	2410	1	1640	4	21	41.8	40.5
		г	34 22	2410	5	2300	5	27	32 27 7	40.0
		M	20	2310	1	1255	5	40	57.7	59.7
		M	29	1400	2	1840	3	21	47.1	25.0
		IVI	52	1770	Z	1840	4	20	44./	33.9
		2F:6M	31.0 (3.7)	1630 (685)	2.0 (1.7)	1640 (785)	5.0 (1.0)	22.2 (10.8)	37.7 (11.1)	40.6 (22.8)
	FIRW	м	26	845	4	755	4	21	563	44.7
	LLD W	M	20	890	7	850	4	21	27.5	59.3
		M	25	550	1	640	4	30	59.2	34.2
		F	20	590	4	565	15	35	50.1	13.8
		M	24	660	7	700	4.J 5	30	39.7	43.8 66.5
		M	23	650	2	570	5	24	59	52.1
		M	23	700	2	620	45	24	50.9	41.3
		M	23	565	1	580	4.4	25	53.2	56
		1F:7M	25.5 (3.0)	655 (195)	3.0 (4.0)	630 (152)	4.7 (0.7)	24.5 (5.5)	52.0 (12.7)	48.4 (15.1)
				. ,						
		3F:13M	27.0 (5.2)	890 (942)	2.0 (3.0)	850 (955)	4.5 (1.0)	27.0 (5.7)	44.7 (16.5)	44.7 (17.9)
BPD		F	24	650	30	810	5.5	40	22.6	59.8
		F	25	570	20	882	5	55	35.1	75.5
		M	24	670	27	880	4	35	44.1	78.1
		F	25	430	50	895	4	35	30	93.6
		M	23	600	42	950	4	26	48.3	58.1
		F	24	460	28	745	4	35	36.4	91.6
		M	24	520	35	905	5.7	45	33.2	67.6
		4F:3M	24.0 (0.7)	570 (162)	30.0 (13.0)	882 (75)	4.0 (1.4)	35.0 (8.7)	35.1 (11.4)	75.5 (26.5)
			. ,	. ,	. ,	. ,	. ,		. ,	
H-L		М	36	2540	15	2610	4	21	30.3	56.1
		М	37	2950	19	3600	4	21	39.3	52.3
		М	34	2380	4	2100	4	21	47.1	39.5
		М	35	2600	3	2370	5	21	47.3	43.5
		F	37	2520	22	2700	4	21	50.2	46.9
:		1F:4M	36.0 (2.2)	2540 (202)	15.0 (16.0)	2610 (622)	4.0 (0.2)	21.0 (0.0)	47.1 (11.0)	46.9 (10.7)

Table 2-3: Patients' characteristics

ELBW = Extremely Low Birth Weigh, VLBW/LBW = Very Low Birth Infants/Low Birth Infants, BPD= bronchopulmonary dysplasia, H-L= healthy lung, GA = gestational age, BW = birth weight, PNA = post natal age. For each group summary data are expressed as mean(IQR). PO₂ and PCO₂ values were obtained from the closest blood gas analysis prior the beginning of the study, FiO₂ values were the baseline settings at the clinical PEEP.

Infants were divided into three groups: infants with RDS (n=16), chronically ventilated infants (for more than 20 consecutive days at the time of measurement) that developed BPD (n=7; BPD was defined as in (Jobe et al. 2001)), and infants ventilated for surgical pathologies that did not affect the respiratory system (controls, n=5).
The set up and the manoeuvre were well tolerated by all the infants and did not induce clinically relevant changes in SpO₂, heart rate or arterial blood pressure. At the highest PEEP, the Peak Inspiratory Pressure was limited by the set value in 9 patients, but without affecting V_T significantly.



Figure 2-5 Respiratory system resistance (Rrs) and reactance (Xrs) in the control (panel A), RDS (panel B) and evolving BPD (panel C) groups. Data are expressed as mean and SD. Open symbols represent the incremental PEEP trial and closed symbols the decremental one.

Figure 2-5 shows the relationships of PEEP with Rrs and Xrs for the three groups of patients.

In the control group Rrs was $36\pm15 \text{ cmH}_2\text{O*s/L}$ at PEEP=2cmH₂O and slowly decreased with increasing PEEP. Xrs was $-27\pm7 \text{ cmH}_2\text{O*s/L}$ at PEEP=2 cmH₂O, significantly higher than in both BPD and IRDS (p<0.01) infants, and it was almost unaffected by PEEP.

In infants with evolving BPD, Rrs was not significantly different than in controls (p=0.213), while Xrs was significantly lower than in controls at all PEEP levels. At low PEEPs Rrs was highest (Rrs at PEEP=2cmH₂O during increasing PEEPs was 121±42 cmH₂O*s/L) and Xrs most negative (Xrs at PEEP=2cmH₂O during increasing was -95±13 cmH₂O*s/L). As PEEP was increased, a sudden change was observed with a rapid drop in Rrs and a steep increase in Xrs. As PEEP was further increased, Rrs showed a smaller and gradual decrease while Xrs was stable with no negative swings.

In the RDS group, Rrs values were not significantly different than in the control group and was independent from PEEP, while Xrs was significantly lower than in controls and it reduced with increasing PEEP. As it can be observed in Fig. 3-B, in this group Xrs presented big error bars that increased with PEEP. This is due to the fact that in different infants Xrs decreased differently with PEEP. By evaluating individual graphs of Xrs vs. PEEP in RDS patients we identified different patterns and we tried to correlate them with anthropometric variables. In Figure 2-6 the change of Xrs between its maximum value (Xmax) and the value at 10 cmH₂O was used as an indicator of possible lung overdistension and plotted vs birth weight (BW).



Figure 2-6. Relationship between body weight and the difference between the maximum in Xrs and the value of Xrs at PEEP = 10 cmH₂O.

Figure 2-6 shows a strong hyperbolic correlation with body weight with a marked difference occurring for patients with BW lower than 900g. As this is very close to the commonly used threshold on body weight of 1000 g used to define the Extremely Low Birth Weight newborns, we adopted that definition and performed the analysis separately for ELBW (n=8) and Very Low or Low Birth Weight (VLBW/LBW, n=8).

ELBW infants had a significantly lower GA and a significantly better PaO_2 at baseline compared with VLBW/LBW infants. Rrs was not significantly different between the two groups (p=0.379), while Xrs was significantly higher in the VLBW/LBW than in the ELBW group at each PEEP (see Figure 2-7).



Figure 2-7 Respiratory system resistance (Rrs) and reactance (Xrs) in ELBW (triangles) and VLBW/LBW (squares) sub-groups. Open symbols, dotted line: inflation trial; closed symbols, dashed line: deflation trial

In VLBW/LBW newborns Xrs presented quite a flat pattern, similar to that observed in the control group, but with significantly lower values In ELBW infants Xrs decreased with increasing PEEP, suggesting that these infants may be more prone to lung tissue distension. Moreover, for these infants the Xrs-PEEP relationship presented a marked hysteresis, with higher Xrs values during the decremental than during the incremental PEEP series. Moreover, we found a significant correlation between baseline values of Xrs and oxygenation (ρ =-0.44 vs. FiO₂), which was particularly strong in ELBW infants (ρ = -0.843 vs. FiO₂ and ρ = 0.762 vs. PaO₂/FiO₂).

Figure 2-8 shows elastance estimated from Xrs (E_{FOT}) and intra-tidal elastance (Edyn). A good agreement can be observed between E_{FOT} and Edyn in identifying the differences among groups and in describing the relationship between lung mechanics and PEEP.



Figure 2-8 Comparison between respiratory system elastance measured by FOT (EFOT, left panel) and dynamic elastance (Edyn, right panel) in controls (A), in the IRDS infants (B) and in the evolving BDP group (C). Open symbols, dotted line: inflation trial; closed symbols, dashed line: deflation trial.

2.3.3 DISCUSSION

The present study reports respiratory system resistance and reactance during an incremental/decremental PEEP trial in infants subjected to mechanical ventilation. Both measurements and procedures were well tolerated by all infants indicating that FOT is feasible even in very small and severe infants. The main findings were: i) impedance in preterm newborns is representative of lung mechanics and very sensitive to its changes with PEEP in ventilated infants; ii) the relationship between impedance and PEEP differed in infants with different lung diseases, suggesting that oscillatory mechanics during mechanical ventilation can provide useful information for tailoring the ventilator settings according to the pathophysiological characteristics of the patient.

Comparison with other studies

To our knowledge this is the first time that Zrs has been measured during mechanical ventilation as a function of PEEP in a population of small and severe newborns. Only one study reported Zrs at different PEEP settings (Gauthier et al. 1998) but in infants affected by bronchiolitis.

Dorkin et al (Dorkin et al. 1983) studied 6 ventilated infants with RDS in the first week of life over the range of 4-40 Hz at zero end-expiratory pressure. They reported impedance data for only 4 infants who showed an average Rrs of about 40 cmH₂O at 4Hz. This value is very similar to the one that we found (40±20 cmH₂O*s/L at lowest PEEP) in our VLBW/LBW patients group who were also very similar in term of GA, weight at measurement and days of life.

Effects of PEEP on respiratory input impedance

PEEP affected Rrs and Xrs differently in different groups of infants. In the control group Rrs slightly decreased with PEEP, likely as a consequence of the increased lung volume at higher end-expiratory pressures. Lung volume and airway resistance are related by an inverse relationship resulting from the effect of the elastic recoil of lung tissue which increases with increasing lung volumes and dilates the airways reducing airflow resistance (Jordan et al. 1981). The relationship between Xrs and PEEP was quite flat, suggesting that, for the range of PEEPs applied in this study, the lungs did not either collapse or reached the upper flat part of the pressure-volume relationship. In this group Xrs was higher compared with the other groups likely because these infants have more mature lungs with larger aerated volumes and Xrs is positively related to body size and lung maturation (Pillow et al. 2001; J J Pillow et al. 2005).

In the RDS group Rrs showed a negative PEEP dependence, similar to the one of controls, while Xrs presented two peculiar features: a significant reduction with increasing PEEP and hysteresis between increasing and decreasing PEEPs

This shape of the relationship between Xrs and PEEP is very similar to the one already observed in a surfactant depletion model of RDS (Dellacà et al. 2011), and it has been associated with the occurrence of lung volume recruitment and de-recruitment and with an increasing distension applied to the lung tissues with increasing pressures. The maximum value of Xrs in the deflation limb of the PEEP trial was associated to the lowest PEEP able to maximise alveolar recruitment, as confirmed by CT scan (Dellacà et al. 2011).

Interestingly, in this group, the point of maximum Xrs was, in average, 5 cmH₂O, the most commonly applied value for these babies.

The average Xrs pattern is the result of different individual behaviours. In ELBW infants Xrs was significantly lower than in VLBW/LBW infants and markedly decreased with PEEP, being significantly higher at the lowest PEEP level than at several steps between 5 and 10 cmH₂O, possibly because at these PEEPs they were ventilated in the upper flat part of their lung pressure-volume curve.

A further different behaviour was found in infants with evolving BPD. In these patients Rrs was significantly higher and Xrs significantly more negative at PEEP=2 cmH₂O than at higher PEEP levels with a sudden and marked change occurring in average at PEEP=4 cmH₂O.

The combination of the high Rrs and the negative Xrs values at low PEEP levels are compatible with central airways narrowing which can be reversed by the application of a relatively low level of PEEP. Even if the small number of patients suggest caution in interpreting the data, this behaviour may be related to the physio-pathological features of BPD, which affects mostly the mechanics of peripheral airways with the thickening of the mucosa layer and the accumulation of interstitial fluids due to the inflammatory processes and with the lower elastic recoil due to the alteration of the alveolarization processes. All these factors reduce the transmural pressure across airway walls leading to excessive airway narrowing at low lung volumes. If this interpretation was correct, the role of PEEP in these patients would be to keep the airways

dilated. Even if further studies larger population of patients are needed to confirm these results, the continuous assessment of the effects of PEEP on impedance data might provide useful information also for the management of these patients.

Comparison between EFOT and Edyn/Cdyn

Previous studies used tidal elastance, or its inverse C_{dyn} , to identify the optimal PEEP in ventilated subjects (Suarez-Sipmann et al. 2007; Carvalho et al. 2007). These parameters have the advantage that they are displayed by most mechanical ventilators, but they are strongly affected by the spontaneous breathing of the patients, resulting poorly reliable in triggered ventilation modalities. On the contrary FOT is independent from the patient respiratory efforts.

If compute E_{dyn} manually selecting breaths showing minimal patients efforts, we found a good agreement between E_{FOT} and E_{dyn} . However some differences exist between the two parameters: i) in the present study E_{FOT} was computed at end expiration, while E_{dyn} reflects the average properties over the large volume variation required to perform the measurement, ii) elastance is frequency dependent, especially in presence of heterogeneities, therefore some of the differences in the absolute values can be explained by the difference between the frequencies at which elastance was evaluated: 5 Hz for E_{FOT} and the breathing frequency (roughly 1 Hz) for E_{dyn} .

Limitations of the study

It is possible that the duration of each PEEP step (1 minute) was not always enough to allow for the lung volume to equilibrate. However, based on preliminary measurements, we found that one minute is a good compromise between allowing enough time for major changes to complete and keeping the manoeuvre short without exposing the infants to non-optimal PEEP levels for too long. This short time prevented us to measure changes in SpO₂and the FiO₂ given the longer time needed by these parameters to stabilise. For this reason, future studies are required to relate changes in lung mechanics to changes in blood oxygenation.

Finally, a greater number of subjects would be necessary to establish whether FOT is representative of lung mechanics, in particular in healthy and evolving BPD infants.

In conclusion, FOT has been recently validated in animal models for the detection of lung volume recruitment/derecruitment and for the identification of the optimal level of PEEP, defined as the lowest value that maintains lung recruitment (Dellaca et al. 2004; J J Pillow et al. 2005). This study supports the use of FOT for monitoring and optimising mechanical ventilation at bedside also for ventilated newborns. Considering that it would be easy to implement FOT in modern mechanical ventilator by modifying the software without the need of external dedicated hardware, this technique could provide a useful tool for improving individualisation and tailoring of mechanical ventilation, allowing a better implementation of the concept of protective lung ventilation in preterm newborns.

2.4 Positional effects on lung mechanics in ventilated preterm infants with acute and chronic lung disease

Prone positioning has been used to improve oxygenation in adult patients undergoing mechanical ventilation for severe hypoxemia and acute respiratory failure since 1974 (Fernandez et al. 2008; Guérin et al. 2013; Mancebo et al. 2006; Pemperton et al. 1974). Several studies investigated the rationale for prone positioning and its possible role in protective ventilation (Lamm et al. 1994; Pelosi et al. 2002). Animal studies showed that prone position reduces ventilation induced lung injury, secondary to greater recruitment and a more homogeneous distribution of the stress applied to the parenchyma (Broccard et al. 2000; Nishimura et al. 2000; Valenza et al. 2005). These mechanisms may explain the survival advantage of approximately 10% in severely hypoxemic adult patients (Sud et al. 2010).

As preterm infants are more exposed than adults to injury from oxygen toxicity and aggressive mechanical ventilation, prone positioning has been investigated as a potential tool for improving respiratory support in neonatal respiratory distress syndrome (RDS). A meta-analysis of data from randomized and quasi-randomised trials incorporating 133 preterm infants receiving mechanical ventilation (Balaguer et al. 2013) showed that prone positioning was associated with a significant improvement in oxygenation, ventilation and thoraco-abdominal asynchrony compared with the supine position. However, the magnitude of this improvement was very small with no evidence of improvements in clinically relevant outcomes (Balaguer et al. 2013).

The reason for the modest response to prone positioning in preterm newborns is likely related to the structural differences in the infant respiratory system compared with the adult one, such as the incomplete growth of alveoli and bronchi, the under-developed airway cartilage, small airway and intercostal muscles (Adams et al. 1994; Muller et al. 1979) the more compliant chest wall and the smaller effect of the gravitational force (Hough et al. 2011; Hough et al. 2010) due to the smaller geometrical distance between the dorsal and the ventral regions.

To our knowledge, only two studies investigated the effect of prone positioning on pulmonary mechanics in mechanically ventilated neonates. While one found an improvement in resistance and work of breathing in the prone versus supine position during the breaths supported by the ventilator (Mendoza et al. 1991), the other did not find any difference in terms of resistance but reported a significant improvement in static compliance and work of breathing in the prone versus the supine position during unsupported breaths (Mizuno et al. 1999).

The difficulty in assessing lung mechanics in small preterm newborns is a major factor preventing a better understanding of the effect of prone positioning. However the forced oscillation technique (FOT) allows an accurate assessment of lung mechanics without interfering with the spontaneous breathing activity of the patient, and it has been successfully applied to the study of ventilated preterm newborns (Dellacà, Veneroni, et al. 2013).

The aims of our study were, in mechanically ventilated preterm infants: 1) to study the short term effect of supine and prone position on the mechanical properties of the respiratory system assessed by FOT; 2) to evaluate whether it is possible to identify a sub-group of infants, with acute or chronic lung disease, that benefit most from prone positioning.

2.4.1 STUDY PROTOCOL

All measurements were performed in the Neonatal Intensive Care Unit of Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, University of Milan. The study was approved by the local Ethics Committee and informed parental consent was obtained for each infant.

Preterm infants assisted by conventional mechanical ventilation, either with RDS or with evolving bronchopulmonary dysplasia (BPD) were included in the study. Based on data reported

in previous studies (Mendoza et al. 1991; Mizuno et al. 1999) we estimated that, to get a statistical power of 0.8, the sample size must be of six infants for the RDS group and of eight for the BPD group.

RDS was diagnosed on the basis of typical clinical and radiographic signs: tachypnea, dyspnea, cyanosis in room air together with the typical chest radiographic finding of a uniform reticulogranular pattern "ground-glass appearance" accompanied by peripheral air bronchograms.

Infants ventilated for more than 20 consecutive days at the time of measurement, who later on received the diagnosis of BPD [defined as in (Jobe et al. 2001)], were defined as infants with "evolving BPD". Exclusion criteria were the presence of severe intracranial haemorrhage (III/IV) and/or malformations.

All infants were nasally intubated (endotrachal tube size 2.5-3.5 mm, Portex, Smiths Medical, St Paul, MN, USA) and mechanically ventilated (Babylog 8000 plus, Drager or Leoni Plus, Heinen & Lowenstein) in synchronized intermittent positive pressure (S-IPPV) mode with volume guarantee with a target tidal volume (V_T) of 5 ml/kg.

Infants were studied for 15 min periods in both the supine and prone positions in a computergenerated random sequence. At the end of the first 10 min of each period, SpO_2 , heart rate (HR) and transcutaneous blood gases (PtcO₂ and ptcCO₂) were recorded. Immediately after these measurements, forced oscillations at 5 Hz were applied for the remaining 5 min while flow and pressure data were continuously recorded The ventilator settings were kept constant at the baseline values, if needed FiO₂ was adjusted by the attending clinician in order to maintain SpO_2 between the clinical limits (86-94%).

A detailed description of the measurement set-up is reported in paragraph 2.2.1. Flow and pressure data were digitized at 600 Hz and stored on a personal computer. Since uncuffed tubes were used, the degree of leaks at baseline and after each change in posture was monitored using the ventilator and kept below its threshold. SpO₂, HR, PtcO₂ and ptcCO₂ were measured by Philips IntelliVue X2 and Linde Medical Instruments Microgas, respectively.

<u>Data analysis</u>

For each posture, the values of Rrs and Xrs at end–expiration were obtained as the average values of 20 breaths free from artefacts and corrected for the contribution of the endotrachel tube as described in 2.2.2.

The time course of lung volume was obtained by integrating V'ao and by removing the linear drift. From this trace, V_T , respiratory rate (RR), duty cycle (Ti/Ttot) and minute ventilation (MV = $V_T \cdot RR$) were computed.

Statistical analysis

Data was tested for normality using the Kolmogorov-Smirnov test and expressed as mean and standard deviation (SD). Differences between supine and prone positions are expressed as mean (95% CI). Significance of difference between postures was tested by two-way ANOVA for repeated measures using posture and patient's group (RDS or evolving BDP) as factors. Multiple comparison after ANOVA was performed by Holm-Sidak test. Differences were considered statistically significant for p<0.05.

Pearson correlation was performed to test whether there was a statistical dependence between the posture related changes in Rrs, Xrs, $ptcCO_2$, $PtcO_2/FiO_2$ and minute ventilation, baseline ventilatory settings, body weight or endotracheal tube size.

2.4.2 RESULTS

Eighteen neonates (Table 2-4) were enrolled in the study: nine infants with RDS and nine with evolving BPD. Seven out of the evolving BPD group developed severe BPD and two developed moderate BPD. In two infants with RDS and two infants with BPD it was not possible to obtain

reliable measurements of ptcCO₂ and ptcO₂. The ventilator settings at the time of the study, expressed as mean (SD), are also reported in Table 2-4Table 2-4 Patients' demographic characteristics and ventilatory settings..

		GA (wk)	BW (g)	PNA (d)	Weight (g)	Gender	PEEP (cmH₂O)	Vt (ml)	FiO₂
		26	940	7	760	F	5.0	4.3	21
		28	1180	2	1120	F	5.0	4.5	21
		27	565	1	580	Μ	4.5	3.5	25
		24	750	7	645	F	4.5	6.5	30
PDS		29	1440	1	1360	Μ	4.0	5.2	21
KD3		26	820	7	920	F	5.0	4.8	21
		25	850	7	765	Μ	5.0	6.5	30
		25	890	7	850	Μ	4.0	5.5	27
		29	1400	5	1355	Μ	5.0	6.5	21
	Mean (std)	27 (2)	982 (297)	5 (3)	928 (289)	4F - 5M	5.0 (0.4)	5.0 (1.1)	24 (4)
		25	430	50	895	F	4.0	4.0	35
		23	470	32	725	F	5.0	5.0	25
		25	815	21	1020	Μ	4.4	5.5	21
avalving		24	460	28	745	F	4.0	4.4	35
evolving		24	520	35	905	Μ	5.7	6.5	45
БРО		24	650	29	915	Μ	5.0	6.0	21
		24	750	32	840	F	5.0	5.2	21
		24	670	27	880	Μ	4.0	5.0	35
		27	660	40	1200	М	5.0	6.1	30
	Mean (std)	24 (1)	603 (138)	33 (8)	903 (143)	4F - 5M	4.7 (0.6)	5.3 (0.8)	30 (8)
all infants	Mean (std)	25 (2)	792 (297)	19 (15)	916 (221)	8F - 10M	4.7 (0.5)	5.3 (0.9)	27 (7)

Table 2-4 Patients' demographic characteristics and ventilatory settings.

Data is expressed as mean (SD). Abbreviations: GA, gestational age; BW, birth weight; PNA postnatal age; PEEP, positive end expiratory pressure; V_T, tidal volume; FiO₂, fraction of inspired oxygen.

Breathing pattern and oxygenation parameters for each posture are shown in Table 2-5.

		RR (breaths/min)	Ti/Ttot (%)	Minute Ventilation (ml/min)	FiO ₂	[§] ptcO₂ (mmHg)	[§] ptcCO₂ (mmHg)
RDS	supine	55 (17)	36.8 (7)	312 (131)	24 (5)	54 (19)	61 (14)
	prone	54 (12)	36.8 (5)	305 (110)	25 (4)	55 (17)	57 (16)
evolving	supine	49 (5)	38.7 (6)	379 (156)	36 (13)	49 (17)	70 (12)
BPD	prone	48 (5)	35.6 (3)	350 (122)	35 (12)	53 (13)	64 (10)

Table 2-5: Breathing pattern and oxygenation parameters for each position.

Data is expressed as mean (SD). Abbreviations: RR, respiratory rate; Ti/Ttot, percentage of inspiratory time; FiO₂, fraction of inspired oxygen; PtcO₂, transcutaneous partial pressure of oxygen; PtcCO₂, transcutaneous partial pressure of carbon dioxide. §=calculated only on 7 infants.

 FiO_2 was significantly higher in neonates with evolving BPD compared to the RDS group (p=0.02). However, in both groups there were no statistically significant differences for any of these parameters between the two postures.



Figure 2-9. Lung mechanics and gas exchange in prone versus supine position. Rrs, Xrs, PtcO2/FiO2 and ptcCO2 in the supine (S) and prone (P) position. Individual data (open symbols) and mean (SD) values (closed symbols) for all patients are reported. * p< 0.05 between P and S.

The reproducibility for both Rrs and Xrs in our population, evaluated as the variability of the values computed over several breaths, was $7 \text{ cmH}_2\text{O*s/l}$.

Figure 2-9 shows Rrs, Xrs, PtcCO₂ and PtcO₂/FiO₂ in the supine and prone position for all infants. Xrs was, on average (95% Cl), -2.6 (-8.9, 3.6) cmH₂O*s/l lower, PtcCO₂ was 4 (-0.1, 9.7) mmHg higher and PtcO₂/FiO₂ -0.1 (-0.3, 0.2) mmHg lower in the supine than in prone position (n.s.). However the statistical power that we had was not high enough to exclude that we failed in detecting differences in these parameters. The behavior of Rrs was similar in all patients: on average it was 9.8 (1.3, 18.3 as 95% Cl) cmH₂O*s/l higher in the supine compared with the prone position (p=0.02).

Differences in Rrs, Xrs, $PtcCO_2$ and $PtcO_2/FiO_2$ between prone and supine position in the subgroups of infants with evolving BPD and RDS are shown in Figure 2-10.



Figure 2-10. Lung mechanics and gas exchange in prone and supine position in infants with RDS and BPD. Rrs, Xrs, $PtcO_2/FiO_2$ and $PtcCO_2$ in the prone (P, gray) and supine (S, black) position in the BPD group, in the RDS group and for all patients. Data is reported as mean and SD. * p< 0.05 between P and S.

On average the evolving BPD group presented greater values of Rrs (p=0.04) and ptcCO₂ (n.s.) and lower values of Xrs (n.s.) and PtcO₂/FiO₂ (p=0.04) than the RDS group. In the evolving BPD group Rrs was, on average, 15.9 (3.6, 28.3; 95% Cl) cmH₂O*s/l lower in the prone than in the supine position changing from 69.0 ± 27.4 to 53.0 ± 16.7 cmH₂O*s/l, (p=0.01), while in the RDS group it was 3.7 (-9.2, 16.5) cmH₂O*s/l lower (n.s.) changing from 47.7 ± 20.6 to 44.0 ± 15.6 cmH₂O*s/l (n.s.). In the evolving BPD group PtcO₂/FiO₂, was on average 0.2 (-0.1, 0.5) mmHg higher in the prone position versus the supine position. The difference was not significant but the statistical power was not high enough to exclude that we failed in detecting a difference in oxygenation because of the small sample size (see discussion). There was a significant correlation between posture related changes in Xrs and Rrs (r= -0.656, p=0.003) and between changes in PtcO₂/FiO₂ and PtcCO₂ (r= -0.560, p=0.04), but we did not find any correlation between changes in lung mechanics, PtcO₂ or PtcCO₂ both considering the whole population or the two subgroups separately. Finally, we found no correlation between baseline ventilator settings, body size or endotracheal tube diameter and any outcome.

2.4.3 Discussion

The main findings of this study are: i) Rrs was decreased in the prone versus supine position in preterm infants receiving mechanical ventilation, ii) changing posture did not affect Xrs significantly, iii) the difference in Rrs between the supine and prone position was greater in

evolving BPD than in RDS infants; iv) we did not find any statistically significant correlation between changes in lung mechanics and transcutaneous blood gas parameters.

Both experimental protocol and set-up were well tolerated by all infants. We did not observe changes in transcutaneous blood gasses and saturation due to the dead space added by the measuring apparatus or to the onset of oscillations. We did not experience any problem due to the change in posture including accidental extubation, bleeding or leaks. In all cases we managed to have leaks below the detection threshold of the ventilator at baseline and after changing position. Moreover, since at 5 Hz the impedance of the leak is very high compared to the impedance of the patient, possible residual leak should have had only a limited impact on the quality of the measurements.

As changes in the sleep state or movements or changes in body position may affect lung mechanics, all measurements were performed during quite sleep of the newborn. Quite sleep was defined as closed eyes, regular respiration, and absence of eye and gross body movements.

All babies were supine prior to the beginning of the study, therefore it is possible that the initial posture could have influenced the results. However, a posteriori analyses showed that there were no differences in outcomes between the babies who were randomized to begin in the supine vs. prone position.

In our study we found only moderate changes in lung function in the prone versus the supine position, in contrast with the marked effects observed in adult patients (Fernandez et al. 2008; Guérin et al. 2013; Mancebo et al. 2006; Pemperton et al. 1974).

This is likely due to the important structural and mechanical differences between the infant's and adult's respiratory system. In particular, in adult patients gravitational forces play an important role in determining the effect of positional changes on lung mechanics, while in infants, due to the smaller size and lower weight, it is likely that the nondependent structures exert less compression on the dependent lung regions. This has been recently confirmed by two studies in which ventilation distribution, assessed by electrical impedance tomography, was found to be independent from gravity in infants either during spontaneous breathing, continuous positive airway pressure (CPAP) or mechanical ventilation (Hough et al. 2011; Hough et al. 2010).

Effect of posture on lung mechanics and gas exchange

In the present study we found that Xrs (which is related to the elastance of the respiratory system) was not affected by changes in posture, while Rrs was significantly decreased in the prone position.

The effect of body position on lung mechanics that we observed is in agreement with previous studies. Numa et al, found a significant improvement in Rrs in the prone position in infants and children with obstructive diseases (Numa et al. 1997). Mendoza et al. (Mendoza et al. 1991)found no differences in dynamic compliance and a significant reduction in resistance in the prone position in a population with similar characteristics to our evolving BPD group. Schrod et al., in infants with similar characteristics to our RDS group, did not find any difference in terms of dynamic compliance (Schrod et al. 2002). Finally, in very low birth weight infants with chronic lung disease, Mizuno et al. (Mizuno et al. 1999) observed that before feeding the prone position resulted in a reduction in pulmonary resistance by about 20%, which is similar to what we observed in our population of infants with evolving BPD, however in their study this difference was not statistically significant.

Differently from previous studies (Balaguer et al. 2013), we did not find any change in blood gas with changing posture. When the analysis was limited to the evolving BPD group on average we observed an improvement in oxygenation (although not significant). However we did not observe any correlation between the reduction in Rrs and changes in blood gasses. The lack of changes in blood gases with positioning may be related to the short monitoring time as changes in blood gas could have required a longer time to establish after lung mechanics had changed.

Moreover, we expect that during mechanical ventilation changes in lung mechanics would not impact much on blood gasses because the resistive load is overcome by the ventilator and not by the respiratory muscles of the newborn.

Our results suggests the lack of significant alveolar recruitment/derecruitment when changing posture (Dellacà et al. 2011; Dellaca et al. 2009; Kostic et al. 2011) and an increase of airway diameters at the level of the central airways (Lutchen et al. 1997), probably due either to a slight increase in absolute lung volume in the prone position or to the effect of the weight of the lung tissue and the mediastinum on the elastic recoil forces exerted by the lung parenchyma to the external wall of the airways, or a combination of both.

Even if the reduction of Rrs in the prone position observed in the evolving BPD group was not associated to a clinical meaningful improvement of blood gasses, it could be interesting to consider this option in patients with long time constants of the respiratory system. These patients require long times for expiration and are at high risk of developing dynamic hyperinflation and high values of intrinsic PEEP (PEEPi). By reducing Rrs, prone position could reduce the time constants of the respiratory system, permitting to empty the lung in a shorter time and therefore reducing the risk of potentially harmful values of PEEPi.

Limitations of the study

Preliminary measurements showed that major changes in lung mechanics stabilize within 1 minute. However we acknowledge that we may have neglected phenomena with a longer time scale such as redistribution of fluids, adaptation of breathing pattern and changes in tissue oxygenation and CO₂ concentration. It has been recently demonstrated that breathing pattern parameters and oxygenation stabilize within 30 minutes of change in body position (Gouna et al. 2012). Therefore this study design does not allow drawing conclusions about the effect of prone position for a longer time.

Another weakness of this study is that arterial blood gasses were not available, therefore we used trascutaneous measurements (available only on 14 patients) which take longer to equilibrate and may not accurately match arterial blood gasses, especially in the older babies.

In the evolving BPD group there is a single outlier that influences the mean difference of Rrs between prone and supine position, however this difference is still statistically significant even without this subject. The strength of our negative results is limited by the small sample size. In fact, while the sample size has been computed to detect differences in Rrs, based on the statistical power of the tests computed on our data we cannot exclude that we failed in detecting differences in Xrs and blood gassed. With our sample size and with the observed variability in the parameters, we can conclude that, if we failed in detecting a difference between prone vs. supine position, this difference is expected to be anyway below 13% for Xrs, 0.36 mmHg for ptO₂/FiO₂ and 7.0 mmHg for ptCO₂, with a power of 80%. Therefore we think that, even though further studies with larger numbers of patients would clarify the role of prone positioning, the present study still provides provocative results about the effect of posture on lung mechanics in ventilated infants.

In conclusions, our results suggest that prone vs. supine positioning does not lead to significant changes in short-term lung function assessed by FOT in mechanically ventilated infants with RDS. In patients with evolving BPD prone positioning is associated to lower Rrs values and could have a role in the clinical management of highly obstructed patient as it reduce the time constants of the respiratory system. However further studies are needed to confirm these data and to evaluate the effects of prone positioning in non-intubated preterm infants during spontaneous breathing or non-invasive respiratory support.

2.5 CHANGES IN RESPIRATORY IMPEDANCE DURING THE FIRST WEEK OF LIFE IN MECHANICALLY VENTILATED EXTREMELY PRETERM NEWBORNS

It is a common experience that Extremely Low Gestational Age Newborn (ELGAN), treated with prenatal steroids and postnatal surfactant, after initial stabilization, tend to have a period of clinical stability with minimal need for respiratory assistance and medications that usually ends by the third day of life followed by clinical deterioration secondary to hemodynamic changes and/or reduced pulmonary function (Eden et al. 2010).

During these first days of life the lung mechanical properties of newborn infants change very quickly as they adapt to extra-uterine life. Different phenomena, including absorption of the foetal lung fluid, modification of the lung parenchyma, increased number of air-filled alveoli, and surfactant related changes in surface tension, contribute to the change (Drorbaugh et al. 1963). During this time ELGAN infants with respiratory failure are treated with surfactant, oxygen therapy and mechanical ventilation. Therefore also other phenomena, related to the effects of the treatments on the immature and growing lung and the development of pathology, could modify the lung mechanical properties of these infants.

Some studies have suggested that optimizing the treatment during the first day of life is of great importance. In fact lung damage may result if there aren't changes in the ventilator management to reflect the ones in lung compliance. Morris et al. have pointed out that early ventilation strategies during the apparently stable period have significant implications on long term morbidity in patients with respiratory distress syndrome (RDS) (Morris et al. 2006).

However setting optimal ventilation parameters is difficult and primarily evaluated by arterial/capillary blood gases, chest radiography, auscultation and assessment of chest wall movement.

For these reasons, a tool to track changes in lung mechanics during this critical period and to provide information on how to adjust PEEP accordingly is of great importance.

Recently, it has been shown that the use of forced oscillation technique (FOT) allows identifying an optimal open-lung PEEP minimizing mechanical stress to the lungs (Kostic et al. 2011). Moreover FOT has been successfully applied to study changes in lung mechanics vs PEEP in ventilated preterm newborns with different pathologies (Dellacà, Veneroni, et al. 2013).

The aims of the present study were: 1) To evaluate the relationship between PEEP and lung mechanics in preterm infants and how this relationship is evolving during the first week of life; 2) To characterize the optimal mechanical PEEP settings in the first week of life in mechanically ventilated extremely preterm newborns in comparison to the clinically set PEEP by current clinical approach.

2.5.1 STUDY PROTOCOL

All measurements were performed in the Neonatal Intensive Care Unit of Uppsala University Children Hospital, Uppsala, Sweden. The study was approved by the local Ethics Committee and informed parental consent was obtained for each infant (D:nr 99092).

Preterm infants assisted by conventional mechanical ventilation starting from the first day of life were included in the study. Exclusion criteria were the presence of major congenital anomalies, hemodynamic instability, seizures, or ongoing sepsis or meningitis.

All infants were intubated and mechanically ventilated (Stephanie ventilator, Fritz Stephan GmbH, Germany) with synchronized intermittent positive pressure (S-IPPV) mode.

PEEP was increased by 2 cmH₂O above the clinically set PEEP (PEEP_c) and then decreased by four 5-minute steps of 1 cmH₂O. At each step sinusoidal waveforms at 5, 10 and 15Hz were overimposed in sequence on ventilation waveform (Figure 2-11). Measurements were performed thrice for each infant, approximately during the 1^{st} , 3^{rd} and 7^{th} day of life. If needed

 FiO_2 was adjusted during the trial by the attending clinician in order to maintain SpO_2 between the clinical limits (85-90%).

A detailed description of the measurement set-up is reported in paragraph 0. Flow and pressure data were digitized at 300 Hz and stored on a personal computer. Saturation (SpO₂) and heart rate (HR) were measured by Philips IntelliVue X2.

Data analysis

For each PEEP level and at each frequency, the values of Rrs and Xrs at end-expiration were obtained as the average values of 10 breaths free from artefacts and corrected for the contribution of the endotracheal tube as described in 2.2.2.

The optimal mechanical PEEP (PEEP_x) was defined as the one at which the maximum of Xrs was reached (Dellacà et al. 2011).

Statistical analysis

Data was tested for normality using the Kolmogorov-Smirnov test and expressed as mean and standard deviation (SD). One-way ANOVA for repeated measurements was used to test the significance of differences in impedance among PEEP levels for each day; among different days at the same PEEP step of the protocol; at PEEP_c before, during and after the PEEP trial and in parameters at PEEP_c and PEEP_x among the days. Multiple comparisons after ANOVA were performed using the Holm-Sidak method. Signed t-test was used to compare parameters at PEEP_c and PEEP_c and PEEP_c statistically significant for p<0.05.

Spearman's correlation was performed to test whether there was a statistical dependence between impedance and clinical parameters and ρ was used to test the correlation strength.



Figure 2-11. Experimental protocol and tracings. Upper panel: graphic representation of the PEEP sequence. Lower panel: enlargement of pressure, flow, resistance, and reactance traces during some breathes at one PEEP step. The grey areas represent the inspiration phase.

2.5.2 RESULTS

A total of eight ELGAN newborns (GA=24.3±1.5wks, BW=641±156g) were studied (Table 2-6).

N of infants	GA (wk)	BW (g)	Gender	Apgar 1min	FiO ₂ max	days MV (d)
1	23.4	582	М	2	43	37
2	23.1	467	F	5	26	89
3	27.9	964	М	5	21	13
4	24.7	775	М	1	21	*
5	24.1	578	М	6	36	63
6	24.1	597	М	4	43	52
7	23.7	553	М	3	30	22
8	23.7	615	М	4	21	30

Table 2-6. Patients' characteristics

GA = gestational age, BW = birth weight, FiO₂ max= maximal FiO₂ during the first week of life; days MV=number of days of mechanical ventilation before discharge * died before extubation.

Postnatal age (PNA), ventilatory parameters and arterial blood gas analysis at the time of measurements are shown in Table 2-7.

	1 st day	3 rd day	7 th day
PNA (h)	15±7	60±41	155±42
PEEP (cmH ₂ O)	4.5±0.5	4.6±0.7	5.0±0.0
PIP (cmH ₂ O)	15.0±1.6	16.0±1.6	19.0±2.6
FiO ₂	23±5	25±5	24±5
pO ₂	7.1±1.2	7.8±1.5	5.8±0.9
pCO ₂	4.8±0.8	5.7±1.1	6.5±1.5

Table 2-7. PNA, ventilatory settings and arterial gas concentration at the time of measurements.

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Figure 2-12 shows Xrs as function of PEEP at 5, 10 and 15Hz during the different days of the study. Xrs at all the frequencies presents similar trend with PEEP. With respect to 10Hz and 15Hz, Xrs at 5Hz is more sensible to changes in PEEP. Therefore for this study we report data at 5Hz.



15Hz (squares).

Figure 2-13 shows the mean Rrs and Xrs values vs PEEP for the 1st, 3rd, 7th day of life. No statistically significant differences were found at the same PEEP step of the protocol among the different days in Rrs and Xrs but for Xrs at PEEP_c after the trial between the 3rd and the 7th day (-112±25, -90±22 cmH₂O*s/l respectively, p=0.04).

Rrs tends to decrease with PEEP in all the day. On the 1st day Xrs decreases significantly with PEEP. There is a statically significant difference between Xrs at PEEP_c during the descending limb and after the trial (-93±21, -103±20 cmH₂O*s/l respectively; p = 0.024). On the 3rd day, the range of variation of Xrs with PEEP is reduced with respect to the first day (20 vs 28 cmH₂O*s/l) but there is still a statistically significant difference between some PEEP levels. There is a statically significant difference between some PEEP levels. There is a statically significant difference also between Xrs at PEEP_c at the beginning of the trial or during the descending limb and after the trial (-98±24, -98±25 and -112±25 respectively cmH₂O*s/l; p = 0.010). On 7th day there is no a statistically significant decrease of Xrs with PEEP and there is a similarity of values measured at the beginning and at the end of the trial at PEEP_c.



Figure 2-13. Respiratory system resistance (Rrs) and reactance (Xrs) in the first, third and seventh day of life of ELBW infants asfunction of PEEP. PEEP is calculated as the mean of the PEEP applied to the infants. Data are expressed as mean and SD. *p<0.05 with the lower PEEP; § p<0.05 with the higher PEEP; ° p<0.05 with the higher PEEP – 1cmH₂O.

Comparison between $PEEP_c$ and $PEEP_x$ is given in Table 2-8 (*p<0.05). $PEEP_c$ is statistically different from $PEEP_x$ only on the 1st day (p<0.001).

For each day the variability in terms of standard deviation of the PEEP value is greater for $PEEP_x$ than for the $PEEP_c$. Day by day changes are also greater for $PEEP_x$ on the 1^{st} day is statistically different from the one on 7^{th} day (p=0.003). Both the $PEEP_c$ and $PEEP_x$ show an increase with time indicating that these infants require increased ventilatory support few days after birth. No statistically significant differences were found in term of oxygenation and resistance. On the first day of life, the application of $PEEP_x$ results in a significantly higher reactance value than the one associated with $PEEP_c$. Even if it does not result in a better oxygenation, it comports less stress to the tissues.

There are no statistical differences in Rrs, Xrs and SpO_2/FiO_2 among the different days at $PEEP_c$ and at $PEEP_x$.

	1 st day		3 rd	day	7 th day	
	PEEP _c	PEEP _x	PEEP _c	PEEP _x	PEEP _c	PEEP _x
PEEP (cmH ₂ O)	4.5±0.5*	3.0±0.8	4.6±0.7	3.6±0.9	5.0±0.0	4.5±1.4 [§]
SpO ₂ /FiO ₂	4.2±0.6	4.1±0.6	3.7±0.8	3.9±0.8	4.0±0.7	4.0±0.5
R (cmH ₂ O*s/l)	44.6±13.5	48.6±13.0	47.6±17.0	49.8±21.8	56.4±18.4	57.4±18.9
X (cmH ₂ O*s/l)	-96.5±16.8*	-78.8±17.0	-98.4±23.9	-93.0±28.7	-86.9±16.1	-78.4±22.9

Table 2-8. Comparison between PEEP_c and PEEP_x.

*p<0.05 with PEEP_x; § p<0.05 with 1^{st} day.

Xrs at PEEP_x and at PEEP_c on the 1st day correlate with the maximum FiO₂ during the first week (r=-0.83; p=0.005). Xrs evaluated at PEEP_x and at PEEP_c on the 7rd day correlates with days of invasive ventilations (r=-0.82, p=0.01; r=-0.79, p=0.02 respectively) (Figure 2-14).



Figure 2-14. Correlation between the maximum value of Xrs in the 7th day of life and the total day of mechanical ventilation before discharge.

2.5.3 Discussion

The main findings of this study were: i) monitoring changes in lung mechanics with PEEP in the first days of life in ventilated preterm newborns could provide useful information about the changes the respiratory system is undergoing; ii) the respiratory system is more prone to overdistention on the 1st day of life than on the 7th day; iii) the optimal mechanical PEEP differs from the clinical one on the 1st day of life and there are no differences between them in term of oxygenation. These results suggest that oscillatory mechanics during mechanical ventilation can provide useful information for improving the tailoring of ventilator settings according to the patho-physiological characteristics of the patient.

Effects of PEEP on respiratory input impedance

Rrs slightly decreased with PEEP, likely as a consequence of the increased lung volume at higher end-expiratory pressures. Lung volume and airway resistance are related by an inverse relationship resulting from the effect of the elastic recoil of lung tissue which increases with increasing lung volumes and dilates the airways reducing airflow resistance (Jordan et al. 1981).

The significant decrease of Xrs with PEEP on the 1st day suggests that the lung is easily overdistended by PEEP and this could result in an increased risk of injuring the tissues. On the 1st and 3rd day, the difference in Xrs at PEEP_c between the beginning and the end of the trial suggests that PEEP is helpful to prevent lung volume de-recruitment. In fact Xrs at PEEP_c at the end of the trial is lower than at the beginning and this could be explained by alveolar derecruitment while the infant was ventilated at lower PEEP. The slight worsening of Xrs on the 3rd day could be related to the turnover of surfactant, the immature surfactant homeostatis and production. All these babies received only one dose of surfactant, administered some minutes after birth before the onset of MV. We could speculate that probably, due to the inhomogeneity of the new lung, over-distended parts of the lungs are established during the first surfactant instillation (Goldsmith et al. 1991). The re-expansion of the lesser ventilated parts of the lungs occurs with time, when signals to the surfactant producing cells (pneumocytes type II) establish a more functional homeostatis of surfactant. This might be more difficult in the more immature lungs of ELGA and these parts might stay occluded and malfunctioning for a longer period.

We can further speculate that the different lung behaviour between the 7th and the 1st day could be linked to the reabsorption of foetal fluid that reduces the interstitial oedema resulting in a less rigid tissue. In fact Xrs of lung tissue is reduced when the scaffold of the extravascular matrix is put under tension by interstitial oedema (Dellacà et al. 2008). In this condition the applied pressure is dangerously more transmitted to the tissue. Reducing PEEP, decreases the overdistantion of the tissue and results in a great increase in Xrs that could mask alveolar derecruitment. In these conditions, longer duration of PEEP steps and monitoring of changes in Xrs during time at the same PEEP level could permit a better interpretation of Xrs.

On all days, the high error bars, due to high inter-subject variability, underline the need for customization of the treatment.

On the first day of life we found a statistically significant difference between $PEEP_c$ and $PEEP_x$. No differences in SpO_2/FiO_2 but in Xrs between $PEEP_c$ and $PEEP_x$ in the first day of life underline the need of lung function monitoring as gas exchange is not enough to understand lung condition. Even if there was no statistically significant difference between $PEEP_c$ and $PEEP_x$ in the other days of measurements, there are differences on individual bases that suggest that the availability of these measurements at bedside could improve the customization of the treatment. The higher inter-patient and inter-day variability of $PEEP_x$ with respect to $PEEP_c$ may be a consequence of a higher level of customization of the treatment.

Both $PEEP_c$ and $PEEP_x$ increase during the first week. Different reasons could account for this, however because of the difference between the $PEEP_c$ and $PEEP_x$ in the 1st day of life we can

speculate that VILI could be among these reasons. The combination of incompletely differentiated cells and an immature supporting structure lends to a unique vulnerability of the epithelial lining of the extremely preterm lung, easily injured by high and/or mal-distributed tidal volume. The possible too high PEEP_c applied on this day, instead of leading to further recruitment could have caused mechanical injury. Further studies of FOT adjustments of ventilation during the first week of life, should address this question.

Finally the correlation between Xrs and the total days of mechanical ventilation suggests that Xrs values can be predictive of clinical outcomes, even if confirmation on a high number of subjects is required.

Different frequencies

Measurements were repeated at 5, 10 and 15 Hz for all PEEP levels. These frequencies were chosen as they are those available on the ventilator for HFOV. As the respiratory system behaves like a low pass filter, low frequencies are more sensitive to peripheral phenomena such as alveolar recruitment and distension. However they are also more affected by the breathing signal. We were able to obtain reliable Zin vs PEEP curves at all the frequencies even if the row data at 5Hz are noisier. The obtained curves show the same trend as a function of PEEP but with Zin at 5Hz being more sensible to PEEP changings.

Limitations of the study

One limit of this study is the small number of patients. However the study is still ongoing and we are presently collecting more data to strengthen our results and achieve adequate statistical power. Another limitation of the study is the absence of transcutaneous blood gases (PtcO₂ and ptcCO₂) during the first days of life as the risk of skin injure does not permit these measurements. The limited range of pressures covered (clinical PEEP±2 cmH₂O) and the fixed value of PIP during all the trial could have limited alveolar recruitment and influenced the optimal mechanical PEEP. However this was done to prevent exposing these extremely preterm infants' lung to injurious high or low pressures during this early postnatal stage. Finally, especially in the first day of life, when the presence of more fluid results in higher time constants, step duration could have been insufficient to asses all the changes induced by PEEP. The length of the step was chosen to avoid to exposed infants for long time to insufficient or high PEEP levels.

In conclusion, the evaluation of mechanical lung condition by FOT can help increasing the level of individualization of the PEEP value on each patient and adapting PEEP according to the changes in lung condition with time. Moreover these measurements can potentially be useful to evaluate the clinical course of the infants and the impact of treatments. Future studies will be addressed at evaluating whether setting the mechanically optimal PEEP could be beneficial in term of clinical outcome and whether measurements of lung mechanics by FOT during the first week could be predictive of clinical outcomes.

3 NON-INVASIVE VENTILATION

Recently, there is an increasing interest in non-invasive ventilation not only for weaning from mechanical ventilation (MV) but as an alternative to MV in the attempt to reduce VILI and the incidence of BPD. New modalities have been proposed in order to overcome the limits of the present technology and to prevent intubation. However there is still a need of studies investigating the mechanisms of functioning of these new forms of supports together with means to optimize non-invasive supports in general in order to avoid MV to the majority of the infants (Morley & Davis 2008).

Nasal continues positive airways pressure (nCPAP) is the most diffused form of non-invasive support. It consists in applying a distending pressure to the respiratory system with the rationale of maintaining alveolar recruitment and airways patency. It is currently been evaluated as primary form of support for preterm infants instead of intubation and MV (see paragraph 1.2.2). Efforts are being directed to improve its efficacy combining it with surfactant administration and evaluating different modality of pressure generation. Also new interface with the infant have been designed to obtain less rigid and heavy nasal cannula that fit correctly the infant's nares without injuring them and minimising the dead space and therefore WOB required to the infant.

A new modality that is going further in the direction of a less invasive interface with the infant is called humidified high-flow nasal cannula (HHHFNC). It is emerging in a variety of clinical situations, thanks to its ease of use, better infants' tolerance, improved feeding and bonding. It consists in the delivery of flow exceeding patients' inspiratory flow (clinical range: 2-8 l/min) through not sealed nasal cannula. The working mechanisms of HHHFNC are not yet fully understood but the washout of the upper airways and the provision of a distending pressure are considered the most relevant ones (Locke et al. 2012; Spence et al. 2007; Dysart et al. 2009; Frizzola et al. 2011). However the pressure is generated in a different way in respect to nCPAP and it is not monitored. If it can play a role similar to nCPAP, requiring the same WOB to the infant is still to be verified. Therefore we compared the effect CPAP and HHHFNC on lung function in term of breathing pattern, resistance, dynamic compliance and WOB when the same level of distending pressure is not the only mechanisms of functioning, it can be used also to generate a pressure with comparable effects to nCPAP.

The drawback of this tendency toward gentler interface with the infants is the increase difficulty in monitoring breathing signals. Therefore, even if in principle lung mechanics could play the same role in optimizing end expiratory pressure during these modalities than during invasive ventilation the impossibility to monitor flow at the mouth prevent the use of Zin to optimise pressure with the current technology. A possible alternative could be the measurement

transfer impedance by FOT, but new methods has to be developed and validated before its measurement is possible in small and sick newborns (see Future perspectives). Moreover during this modality the activity of the infant plays a major role in the determination of EELV. In fact the absence of an ETT permits to the infant to actively elevate EELV also through flow-breaking action of the larynx (see paragraph 1.1.2). Infant's efforts could compensate a low pressure increasing WOB. A longer time of monitoring could be required to identify the optimal CPAP just on lung mechanics bases as short term changes could be masked by the increased infant's activity. Therefore not only lung mechanics but also other indices could be fundamental to guide pressure titration.

Fatigue indices, electrical activity of diaphragm, analysis and variability analysis of the breathing pattern have been used to assess changes in lung function caused by the application of a pressure (see paragraph 1.1.2). However, whether the analysis of these parameters permits to identify an optimal pressure level is still to be verified. Fatigue indices are unsuitable for clinical practice, as require esophageal and/or gastric pressure measurement. Drawbacks of measurements of the electrical activity of diaphragm are linked to the need of expensive catheters. On the other hand, variability analysis of breathing pattern has the advantage to be performed on different signals related to the respiratory system that can be measured noninvasively and it has increasingly been applied to the study of the respiratory system in infants. Information about control of breathing as well as mechanical properties of the thoracopulmonary system can be obtained (see paragraph 1.3.1). The application of pressure might modify EELV, lung mechanics, the input to the neural centres that arrives from the stress receptors, gas concentrations and consequently the variability of the breathing pattern. Goldman et al. showed that the application of a pressure at the airways opening modifies the multifractal properties of the intra breath interval in children (Goldman et al. 2008). Preterm babies could be more sensible to pressure levels as they have strong reflexes that play a large role in the control of breathing, immature control of breathing and they have to actively elevate EELV above the resting volume of the respiratory system. In newborns, variability of breathing pattern parameters has been found to be modified by assisted ventilation (Emeriaud et al. 2010). However, it is not known whether nCPAP level influences breathing pattern variability in infants and if it can play a role in nCPAP titration. To test this hypothesis, we applied variability analysis to the study of lung condition at different level of nCPAP in preterm infants during their first day of life. In particular we looked at long term correlation present in EELV, V_T and RR by detrended fluctuation analysis (DFA). Between the different methods proposed to assess variability DFA has the advantages of non-requiring the stationarity of the data and distinguishing between intrinsic fluctuation generated by a complex system (exhibit long-range correlation) and those caused by external stimuli acting on the system (local effect, having characteristic time scales). For this first study we measured lung volume changes by OEP as, even if it is not suitable for clinical practice, it has the advantages not to be subject to drift, to provide different breathing pattern indices including EELV without assuming only two degree of freedom for the highly deformable infant chest wall or requiring a subject specific and difficult calibration. Moreover it has been shown that it permits accurate measurements in infants (Dellaca et al. 2010). However, other indices and correlation between parameters (Entropy, multifractal analysis, cross entropy, etc.) applied to signals that can be more easily obtained in clinical practice as volume changes by RIP or mouth pressure during nCPAP and Edi (see Future perspectives) will need to be explored.

3.1 HHHFNC: EFFECTS ON RESPIRATORY MECHANICS AND WORK OF BREATHING

Despite Nasal Continuous Positive Airway Pressure (nCPAP) being the most common noninvasive respiratory support for preterm newborns (Donn et al. 2006; Courtney et al. 2007; de Winter et al. 2010), it still presents issues in clinical application, such as the risk of nasal trauma and difficulty in keeping the device correct positioning (Dani et al. 2009b; Manley, Dold, et al. 2012b; Fischer et al. 2010b). In contrast, High Flow Nasal Cannula (HHHFNC) offers ease of use, better tolerance and improved feeding and bonding (Dani et al. 2009b; Manley, Dold, et al. 2012b; Lee et al. 2012b; Manley, Owen, et al. 2012b; Spentzas et al. 2009b) and it is being increasingly used in neonatal ICU (NICU) in a variety of clinical situations (Sreenan et al. 2001b; Campbell et al. 2006; Shoemaker et al. 2007; Woodhead et al. 2006a; Holleman-Duray et al. 2007b; Miller et al. 2010b), most recently as a primary approach to neonatal respiratory distress syndrome (RDS) (Yoder et al. 2013b).

Even though the working mechanisms of HHHFNC are not yet fully understood, the washout of the upper airways (leading to a reduction of the physiological dead space) and the provision of a distending pressure are considered the most relevant ones (Locke et al. 2012; Spence et al. 2007; Dysart et al. 2009; Frizzola et al. 2011). While the first is specific of HHHFNC, the application of a distending pressure is in common with nCPAP. However, while in a nCPAP system pressure is developed by the device within the system, in HHHFNC pressure is generated within infant's nasal-pharyngeal cavity across the leaks between cannula and patient's nares (Hasan et al. 2011), depending both on flow-rate and amount of leakage (Spence et al. 2007). Unlike nCPAP, in HHHFNC the level of pressure generated can't be monitored (Dani et al. 2009b; Manley, Dold, et al. 2012b) and, most importantly, the retropharyngeal pressure (P_{rp}) is likely to show larger within-breath changes due to the effects of the breathing flow. In HHHFNC, even small changes in flow may result in a marked within-breath variation of in the applied pressure, influencing, in turn, breathing pattern and work of breathing (WOB). To date, lung mechanics and WOB in HHHFNC and nCPAP have been compared in a single study that did not measure the P_{rp} generated by HHHFNC (Saslow et al. 2006).

In this study we aim to evaluate in a population of preterm infants with RDS: 1) work of breathing, breathing pattern, lung mechanics and gas exchange during HHHFNC compared to nCPAP when the same level of continuous positive airway pressure is provided, and 2) whether the different mechanisms of developing the P_{rp} have a significant impact on these variables.

3.1.1 EXPERIMENTAL SET UP

From simultaneous pressures and flow/volume measurements it is possible to obtain information about the mechanical properties (resistance and dynamic compliance) of the respiratory system and its subsystems and computing the WOB (1.3.1).

Therefore we developed a set up that permits simultaneous measurement of P_{PL} , retropharyngeal pressure (P_{rp}) and tidal volumes.

Lung tidal volumes were measured using respiratory inductance plethysmography in AC mode (Bioradio 150 CleveMed, Cleveland, Ohio, USA).

Direct comparison of tidal lung volume changes measured by a face-mask pneumotachography (8410A Hans Rudolph, Kansas City, MO, USA) over several spontaneous breathes was used to compute the calibration coefficients (M and K). RIP lung volume changes (V_L) then were obtained as:

 $V_{L}=M^{*}(K^{*}RC+AB)$

with RC and AB being the signals coming from the abdominal and thoracic bands, respectively.

A neonatal oesophageal balloon, connected to a pressure transducer (DCXL Series, Honeywell, New Jersey), was placed in the lower third of oesophagus to estimate intrapleural pressure from the oesophageal pressure. Catheter length of introduction was estimated by the sum of distances between xyphoid process of the sternum and ear lobe and between ear lobe and nasal bridge. Correct position of the oesophageal pressure was confirmed by evaluation of the pressure waveform and, when possible, by the occlusion technique (Baydur et al. 1982).

A 6 Fr feeding catheter with 4 narrow holes at the distal extremity was coupled with a pressure transducer to measure P_{rp} . The depth of insertion was estimated by measuring the distance between the tip of nose and the ear lobe. To minimise the influence of the secretion on P_{rp} measurements, a 40 mL/h airflow via a micro-infuser was applied through the catheter after verifying that this airflow did not influence P_{rp} measurements. All the sensors are compliant with the international standards of infant lung function testing.



Figure 3-1 Set up for WOB and lung mechanics computation.

3.1.2 STUDY PROTOCOL

All measurements were performed in the Neonatal Intensive Care Unit of Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico in Milan. The study was approved by the Institutions Human Ethics Committee and informed parental consent had been obtained prior to the study.

Preterm infants between 28^{+0} and 32^{+6} weeks gestational age requiring FiO₂ > 0.3 to maintain peripheral oxygen saturation (SpO₂) between 88-93% were enrolled. They were randomly commenced on either nCPAP or HHHFNC and they were studied after stabilization within a postnatal age of 96 hours. Infants were not studied if they were deemed too unstable by the treating clinical team, had an intraventricular haemorrhage or major congenital abnormalities.

SpO₂, transcutaneous partial pressure of oxygen ($PtcO_2$) and carbon dioxide ($PtcCO_2$) and heart rate were continuously measured using the Philips IntelliVue X2 and Linde Medical Instruments Microgas.

Tidal changes in lung volume, P_{es} and P_{rp} were measured using the set up described before.

The study design was a randomized cross-over trial. Each infant was treated with both nCPAP (SiPAP, Viasys, Healthcare Inc., Palm Springs, CA, USA) and humidified HHHFNC (Precision Flow-Vapotherm, Stevensville, USA) applied in a random order.

During nCPAP pressures of 2, 4 and 6 cmH₂O were applied for 15-min in a randomized sequence. As it was not possible to adjust HHHFNC in real time to provide for each newborn similar distending pressure, during HHHFNC flows rates of 2, 4 and 6 L/min were applied to all infants with the aim of selecting a posteriori, for each infant, the flow rates in which P_{rp} matched the values applied during nCPAP.

For HHHFNC, nasal prongs size was chosen according to the infant's weight based on the indications provided by the manufacturer.

For the purpose of this study, mouth air leaks where avoided by gently closing the mouth during data collection.

<u>Data analysis</u>

SpO₂, P_{es}, P_{rp} and V_L were continuously recorded at 200 Hz for the last 5-min at each nCPAP/HHHFNC setting using a custom-built digital acquisition system. PtcO₂ and PtcCO₂ were collected at the end of each protocol step. From these recordings the following were computed:

Breathing pattern. The following indices were computed: respiratory rate (RR), tidal volume (V_T) , minute ventilation, percentage contribution of the rib cage to V_T (%RB), inspiratory and expiratory asynchrony indices (IAI and EAI respectively), labored breathing index (LBI), and pressure time product (PTP). IAI and EAI were defined as the fractions of inspiratory/expiratory time during which the abdomen and the ribcage move in opposite directions.

LBI is defined as the sum of the integrals of absolute values of the derivatives of rib cage (RC) and abdominal (AB) volume signals divided by the integral of the derivative of the sum signal over the duration of inspiration.

Lung mechanics. Resistance (R) and dynamic lung compliance (C_{dyn}) were estimated by fitting the transpulmonary pressure ($P_L=P_{rp} - P_{es}$) and flow signals to the equation of motion of the respiratory system by the least-squares method.

Work of breathing. The work of breathing (WOB) and its components were estimated from P_{es} and lung volume changes measured by RIP as described in Saslow et al. (Saslow et al. 2006). The total WOB was divided into its elastic (eWOB) and resistive (rWOB) components. Note that, as it was not possible to obtain an accurate passive pressure-volume relationship for the chest wall, in eWOB the contribution of the chest wall has been neglected. Total WOB, rWOB and eWOB were further divided into the inspiratory (WOB_i) and expiratory (WOB_e) components. WOB was also divided to account for the contribution associated to upper (WOB^{up}) and the lower respiratory system (WOB^{lo}). WOB^{up} was calculated considering the difference between pressure at the end of the nasal cannula and P_{rp} . The pressure at the end of the nasal cannula was considered constant and equal to the P_{rp} at zero flow. WOB^{lo} was calculated using the transpulmonary pressure. WOB^{up} was considered totally resistive, while WOB^{lo} was further divided into its resistive and elastic components.

In order to better compare the effect of nCPAP and HHHFNC, the comparison was performed at the same level of P_{rp} . In particular each parameter was evaluated at a P_{rp} as close as possible to 2 and 4 cmH₂O. For this reason, we considered the subgroup of 15 infants (infants n° 1, 3, 5, 10, 15 reported in Table 3-1 were excluded) in which it was possible to obtain P_{rp} close both to 2 and 4 cmH₂O during HHHFNC. A minimum of 15 breaths free from artifacts were selected and the analyses were performed on each breath.

Statistical analysis

Sample size estimation was based on finding a clinically significant difference in work of breathing between NCPAP and HHHFNC. Calculations (Sigmaplot 11.0, Systat Software, Inc.), indicated that 14 subjects would be sufficient to reject the hypothesis of equivalence with 80% probability using an alpha of 0.05, given that means differed by at least 40%. Mean and standard deviation were taken from.[21] To account for potential patients that cannot be included in the comparison (see above), we recruited 20 subjects.

In order to compare the effects of nCPAP and HHHFNC at the same P_{rp} pressure, data points for which both techniques provided a similar P_{rp} (max difference < 1 cmH₂O) were selected.

ANOVA on Ranks for repeated measurements was used to test the significance of differences among the six conditions of ventilation support. Multiple comparisons after ANOVA were performed using the Tukey test. Differences were considered statistically significant for p < 0.05.

3.1.3 RESULTS

Patients' characteristics

Twenty infants were enrolled from December 2011 to June 2012 with a mean (SD) GA 31+3 (1+1) weeks, PNA 53 (27) hours and birth weight 1433 (375) g. Detailed patients demographics are reported in Table 3-1.

Patient	Gender	GA (wks+days)	PNA (hrs)	BW (g)	Study weight (g)	Apgar score I'/V'	Surfactant dose	Intubation and MV
1	F	32 ⁺⁰	10	1495	1495	9/9	1	no
2	М	32 ⁺⁰	23	1415	1360	7/8	0	no
3	М	32 ⁺⁰	93	1420	1260	3/7	1	yes
4	F	30 ⁺⁵	48	1490	1450	8/8	0	no
5	М	30 ⁺⁵	78	1560	1390	8/9	1	no
6	М	30 ⁺⁶	50	1600	1480	6/9	1	no
7	М	30 ⁺⁶	55	1490	1400	7/8	1	no
8	М	30 ⁺⁶	96	980	950	3/3	1	yes
9	F	29 ⁺⁴	29	1050	920	8/9	1	no
10	М	33 ⁺⁰	13	1780	1780	8/9	0	no
11	F	32 ⁺⁴	35	1680	1680	5/9	0	no
12	М	32 ⁺⁴	37	2340	2280	6/8	0	no
13	М	31 ⁺⁰	52	1370	1340	7/9	1	no
14	F	31 ⁺⁵	39	1650	1520	6/8	0	no
15	F	31 ⁺⁵	42	1490	1440	6/8	0	no
16	М	31 ⁺⁰	84	1870	1777	10/10	1	no
17	F	31 ⁺⁰	89	1690	1480	9/10	1	no
18	М	31 ⁺⁰	94	1120	1015	6/9	0	no
19	F	29 ⁺⁰	68	1260	1070	8/8	0	no
20	F	31 ⁺⁰	35	1450	1440	8/9	0	no
	9F/11M	$31^{+3}(1^{+1})$	53 (27)	1522 (361)	1433 (375)	7/8	10/20	2/20

Table 3-1. Patients' demographic.

GA: gestational age; PNA: post natal age; BW: birth weight; MV mechanical ventilation. Surfactant refers to the total number of doses administrated before the study. Intubation and mechanical ventilation refer before to the study. The summary for GA, PNA, BW and weight at the study is expressed as the mean (SD).

Prior to commencement of non-invasive respiratory support the patients had a Silverman score of 5 (5;6) and a FiO_2 requirement of 0.3-0.6. After recruitment, patients were randomized to either NCPAP or HHHFNC. At the time of the study FiO_2 was 0.21-0.25 for both the modalities. Thirteen infants were receiving NCPAP at 4-6 cmH₂O, while the other seven were on HHHFNC at

4-6 l/min. All ventilation modalities/settings were well tolerated by all infants. No interventions, including FiO_2 adjustments, were required to maintain SpO_2 in the range 88-93%, suggesting that, by the time of the study, most of the patients had improved. This consideration explains relatively high values of compliance were found in few patients.



Figure 3-2. Experimental tracings: retropharyngeal pressure (P_{rp}), oesophageal pressure (P_{es}), transpulmonary pressure (P_L), lung volume changes (V_L), abdominal and thoracic contributions to lung volume changes (Vab and Vrb) of a representative infant during nCPAP and HHHFNC at a pressure of end-expiration P_{rp} of 2 cmH₂O.

Generated end-expiratory pressure

Figure 3-2 shows experimental traces of a representative infant during nCPAP and HHHFNC at an end-expiratory P_{rp} of 2 cmH₂O. V_T was similar in the two modalities while P_{rp} and P_{es} present higher intra-tidal variations during HHHFNC.

The relationship between flow rate in HHHFNC and the level of end-expiratory P_{rp} is shown in Figure 3-3. There was a poor correlation between the two, even when flow values were corrected for infants weight (P_{rp} =0.3+0.7*V'; r2=0.37). The maximum P_{rp} recorded was 7 cmH₂O.



Figure 3-3. Linear regression between flow rate divided by infants' weight and end-expiratory P_{rp} in HHHFNC (P_{rp} = 0.3 + 0.7*V'; r2 =0,37).

nCPAP at settings of 2, 4, 6 cmH₂O allowed to actually achieve an end-expiratory pressure of 2, 4 and 6 cmH₂O at the level of the retro-pharynx. HHHFNC was able to achieve an end-expiratory P_{rp} of 2 cmH₂O in all 20 infants, while a maximum P_{rp} of 4 cmH₂O was obtained in 15 infants and 6 cmH₂O in 5 infants. For this reason, the comparison was limited to 15 infants at P_{rp} of 2 and 4 cmH₂O. During HHHFNC, P_{rp} of 2 cmH₂O was reached in 4 infants with 2 l/min and in 11 infants with 4 l/min while P_{rp} of 4 cmH₂O was reached in 4 infants with 4 l/min and in 11 infants with 6 l/min.

Comparison between NCPAP and HHHFNC

Detailed comparisons between HHHFNC and NCPAP can be found in Table 1. No statistically significant difference was found between HHHFNC and NCPAP on breathing pattern parameters, gas exchange and respiratory mechanics.

RR and %RC were lower and IAI was higher during HHHFNC than NCPAP but without reaching statistical significance.

Increasing P_{rp} from 2 to 4 cmH₂O produced similar effects during the two modalities: a significant reduction in RR and a slight increase in both V_T and PtcO₂.

Figure 4 shows WOBi, divided into WOBup and the component due to the lower part of the respiratory system. At a P_{rp} of 4 cmH₂O the inspiratory WOB^{up} was significantly higher during HHHFNC than NCPAP. However we did not observe any significant difference in terms of WOBi, because WOB^{up} contributes only in a small part (16%) to the total.

Table 3-2: Pressure swings, breathing pattern, lung mechanics, gas exchange and work of breathing during HHHFNC and nCPAP at 2 and 4 cmH₂O of end-expiratory retropharyngeal pressure.

Variable	Variable unit of measure		HFNC	NCPAP	HFNC
Pressures					
Prp at EE	cmH₂O	2.1 (1.9;2.4)	1.9 (1.7;2.2)	3.8 (3.3;4.2)	3.8 (3.6;4.1)
ΔPrp	cmH₂O	1.3 (1.1;2.4)	1.7 (1.2;2.4)	1.2 (1.1;2.0)	2.0 (1.5;3.1)
Δpes	cmH₂O	4.5 (3.9;6.4)	4.9 (4.2;6.4)	4.4 (3.2;6.0)	5.7 (4.1;7.4)
ΔPtr	cmH₂O	4.1 (3.2;5.9)	4.2 (3.4;5.4)	3.3 (2.7;5.3)	4.3 (3.3;6.5)
Breathing pattern					
Ti	S	0.41 (0.32;0.47)	0.42 (0.37;0.53)	0.43 (0.35;0.61)	0.56 (0.45;0.64)
Те	s	0.40 (0.31;0.50)	0.53 (0.40;0.66)	0.47 (0.34;0.75)	0.58 (0.47;0.76)
RR	breaths/min	76 (63;98)	63 (53;80)	69 (46;89)	53 (44;66)
V _T	ml	5.77 (4.76;6.75)	5.29 (3.46;7.95)	6.89 (4.33;8.41)	7.54 (3.97;10.17)
MV	L/min	439 (263;514)	336 (221;391)	366 (274;480)	402 (221;531)
Vrc	ml	0.57 (0.14;0.85)	0.30 (0.06;0.93)	0.64 (-0.04;1.43)	0.39 (-0.25;1.67)
Vab	ml	4.66 (4.12;6.02)	4.45 (3.02;7.83)	4.51 (4.13;6.85)	6.03 (3.81;8.64)
%RC	%	11 (3;16)	4 (1;15)	9 (-2;26)	11 (-6;16)
%AB	%	89 (84;97)	95 (85;100)	91 (74;102)	89 (83;106)
IAI	%	34.7 (29.3;39.7)	37.2 (32.0;51.5)	31.0 (23.7;55.0)	33.8 (21.5;61.9)
EAI	%	34.3 (32.5;40.4)	41.3 (35.1;51.3)	34.8 (27.1;58.8)	40.0 (24.6;63.7)
LBI		1.07 (1.05;1.10)	1.10 (1.06;1.18)	1.07 (1.05;1.15)	1.09 (1.04;1.18)
РТР	cmH ₂ O*s	0.82 (0.66;0.99)	0.94 (0.68;1.12)	0.71 (0.54;1.13)	1.13 (0.86;1.53)
Mechanical properties					
R	cmH ₂ O*s/I	47.3 (31.7;61.5)	48.3 (35.6;76.9)	43.6 (27.1;67.8)	44.8 (28.4;66.9)
Cdyn	ml/cmH₂O	2.96 (1.84;4.32)	2.76 (1.16;3.56)	2.80 (1.76;5.03)	2.75 (1.59;4.55)
Gas exchange					
SpO2	%	93 (90;98)	91 (90;97)	96 (91;98)	95 (92;97)
ptO2	mmHg	56.0 (51.0;69.0)	56.0 (47.5;69.5)	62.0 (54.5;73.0)	61.0 (57.0;72.0)
ptCO2	mmHg	40.0 (34.0;42.0)	41.0 (36.5;43.5)	41.0 (31.5;44.0)	40.0 (36.5;42.0)
WOR J Dieutining	cmH O	2 22 (1 80.2 67)	2 52 (1 96.2 11)	2 15 (1 17.2 50)	2 70 (1 87.4 33)
		2.32(1.89, 3.07) 1 00 (0 52.2 12)	2.32 (1.30,3.11) 0.92 (0.45·1.19)	2.13 (1.47,3.30) 0.94 (0.72·1.67)	0.89 (0.62.1.90)
rWOB; / VT	cmH ₂ O	1 19 (1 09.1 89)	1 53 (1 31.1 95)	1 31 (1 08.1 97)	1 74 (1 13.2 43)
rWOBe / VT	cmH ₂ O	1.30 (0.75:2.16)	1.49 (0.97:2.10)	1.17 (0.84:1.53)	1.42 (0.87:1.86)
rWOB / VT	cmH ₂ O	2.51 (1.82;4.02)	3.05 (2.39;4.19)	2.90 (1.94;3.35)	3.30 (2.18;4.43)
rWOBi ^{lo} / Vт	cmH₂O	0.86 (0.63;1.04)	0.96 (0.65;1.36)	0.93 (0.52;1.37)	0.82 (0.59;1.52)
rWOBe ^{lo} / VT	cmH₂O	0.68 (0.44;1.26)	0.76 (0.48;1.42)	0.82 (0.52;1.19)	0.69 (0.41;1.03)
rWOB ^{lo} / Vτ	cmH₂O	1.39 (1.13;2.17)	1.75 (1.29;2.79)	1.65 (1.00;2.62)	1.71 (1.07;2.72)
rWOBi ^{up} / Vт	cmH₂O	0.52 (0.24;0.71)	0.60 (0.33;0.86)	0.35 (0.21;0.56)	0.54 (0.48;0.99) *
rWOBе ^{up} / Vт	cmH₂O	0.34 (0.16;0.62)	0.55 (0.42;0.81)	0.30 (0.21;0.61)	0.73 (0.38;1.08) *
rWOB ^{up} / Vт	cmH₂O	0.67 (0.49;1.52)	1.02 (0.82;1.67)	0.65 (0.49;1.09)	1.57 (0.85;2.09) *

$Prp = 2 cmH_2O$

$Prp = 4 cmH_2O$

 P_{rp} : retropharyngeal pressure; EE: end expiration; P_{es} : oesophageal pressure; P_L : transpulmonary pressure; Ti: inspiratory time; Te: expiratory time; RR: respiratory rate; V_T : tidal volume; MV: minute ventilation; Vrc and Vab: rib cage and abdominal volume change; %RC and %AB: percentage contribution of the rib cage and abdomen to V_T ; IAI and EAI: inspiratory and expiratory asynchrony indices; LBI: labour breathing index; PTP: pressure time product; R: lung resistance; C_{dyn} : dynamic compliance; SpO₂ oxygen saturation; PtCO₂ and PtcCO₂: transcutaneous partial pressure of oxygen and carbon dioxide; WOB_i: inspiratory work of breathing; eWOB: elastic work of breathing; rWOB, rWOB_i and rWOB_e: total, inspiratory and expiratory resistive work of breathing; rWOB^{IO} , rWOB^{IO} and rWOB_e^{IO}: total, inspiratory and expiratory resistive work of breathing associated to the lower respiratory system; rWOB^{up} , rWOB_i^{up} and rWOB_e^{up}: total, inspiratory and expiratory resistive work of breathing associated to the upper respiratory system. Data are expressed as median (IQR). * p<0.05. Figure 3-4 shows WOB_i, divided into the resistive component due to the upper airways (from airway opening to the pharynx) and the component associated to the lower part of the respiratory system. At a P_{rp} of 4 cmH₂O the inspiratory WOB^{up} was significantly higher during HHHFNC than nCPAP. However, since this difference represented less than 13% of the total (elastic + resistive) WOB_i, no significant difference was observed in terms of total WOB_i. At a P_{rp} of 4 cmH₂O the expiratory WOB^{up} was also significantly different between nCPAP and HHHFNC. However, since the expiratory work is often at the expense of the elastic recoil energy stored in the respiratory system during inspiration, it is unlikely to have affected the respiratory energetics of the patient.



Figure 3-4. Left panel: Inspiratory work of breathing divided into the resistive component due to the upper airway (plain colour) and to resistive (striped) and elastic (white) components due to the lower part of the respiratory system. Right panel: resistive expiratory WOB with the resistive WOB due to the upper airway highlighted (plain colour). Data are presented as mean and standard deviation.

3.1.4 DISCUSSION

This study compares the effects of HHHFNC and nCPAP on breathing pattern, gas exchange, lung mechanics and WOB in premature infants with mild to moderate RDS at equivalent applied retropharyngeal pressures. At the P_{rp} compared, there was no difference in gas exchange, WOB and lung mechanics, suggesting similar efficacy between nCPAP and HHHFNC.

Generated end-expiratory pressure

In our study, during HHHFNC it was possible to reach an end-expiratory P_{rp} of at least 4 cmH₂O in 75% of the infants, but rarely over 5 cmH₂O. This result suggests that HHHFNC as currently applied in the clinical settings provides lower continuous distending pressures than those commonly used in nCPAP. Moreover, as in this study the infant's mouth was closed and HHHFNC flow rate was increased up to 6 l/min, it is likely that the pressures reported here are even higher than those commonly applied.

Similar to previous studies, potentially dangerous high applied pressures were never reached throughout the study. However, since during HHHFNC the generation of an airway pressure depends on the flow passage through the leaks between cannula and nares, in case of nares obstruction or occlusion, higher pressures could be reached, especially when applying high flow rates (Locke et al. 2012; Lampland et al. 2009). We found a very poor correlation between the HHHFNC flow rate and P_{rp}, consistent with previous reports if HHHFNC in preterm infants (Dani

et al. 2009b; Manley, Dold, et al. 2012b). The linear regression between flow and pressure was different from those reported previously (Wilkinson et al. 2008), meaning that, in addition to the wide inter- and intra-subject variability in the amount of pressure developed at a given flow rate (Lampland et al. 2009), there may also be variability due to prong type and experience of the attending personnel.

Comparison between nCPAP and HHHFNC

No statistically significant differences were found either in gas exchange, breathing pattern, thoraco-abdominal asynchronies or WOB between the two modalities, in agreement with previous studies (Frizzola et al. 2011; Saslow et al. 2006; Boumecid et al. 2007).

In particular, Saslow et al. (Saslow et al. 2006) found no significant difference between HHHFNC (at either 3, 4 or 5 l/min) and nCPAP at 6 cmH₂O in terms of phase angle (thoraco-abdominal asynchrony), RR, V_T or WOB. However, differently from the present study, they found a statistically significant improvement in dynamic compliance during HHHFNC at 5 l/min compared to nCPAP at 6 cmH₂O, despite a reduction of end-expiratory pressure estimated from P_{es} .

In our study, as distinct from the previous ones (Saslow et al. 2006; Boumecid et al. 2007), the comparison between the two modalities was performed at the same P_{rp} , in order to highlight possible differences due to the different principles of developing and applying pressure.

Whichever ventilatory support was used, as P_{rp} was increased, only RR varied significantly, while the other parameters were only slightly affected. This result suggests that changing the airway pressure did not produce a common response among patients and, therefore, it highlights the need for individualised tailoring protocols for both modalities. The lack of differences at different P_{rp} levels might also explain the agreement of our results to previous studies (Saslow et al. 2006) in which the comparison was performed regardless the pressure actually applied.

Upper airways resistance during HHHFNC

Although no difference was found between nCPAP and HHHFNC in total WOB, at a P_{rp} of 4 cmH₂O the WOB_i was slightly higher during HHHFNC than nCPAP. In particular, the fraction of WOB_i associated to the resistive load of the upper airways was significantly higher during HHHFNC. The WOB_e^{up} was also higher during HHHFNC, however, the WOB_e is often at the expense of the eWOB, suggesting there was no increase in breathing effort.

The difference in rWOB^{up} between HHHFNC and nCPAP is likely related to the different mechanisms of generating pressure. During HHHFNC, P_{rp} is created by the resistance offered through the free space between nares and cannula to the exhaled flow. This resistance can be described as a leak, in which the pressure is related by a quadratic function of the flow that passes between cannula and nares. When airflows become significant, even a small increase in flow can result in a relevant variation in the applied pressure.

During inspiration, the air coming from the cannula is partly inspired into the lung; the remaining is exhaled through the leakage and contributes to developing a P_{rp} . Conversely, during expiration, the flow exhaled through the leakage is higher as it results from the expiratory flow from the lung that adds to the flow from the cannula. Therefore, the P_{rp} generated during HHHFNC is not constant during the breathing cycle but tends to be lower during inspiration, providing less inspiratory support, and to increase during expiration, providing a higher load that the infant has to overcome in order to exhale. This is likely to be the reason of the higher rWOB^{up} found during HHHFNC. However, as rWOB^{up} represents less than 13% of the total WOB, this potential drawback of HHHFNC appears not to be clinically relevant.

<u>Limitations</u>

Some limitations of the study were related to the measurement techniques. First, it has been pointed out that changes in oesophageal pressure may not accurately reflect changes in pleural pressure when chest wall distortion results in an uneven distribution of pleural pressure changes. However, considering that no differences were observed in thoraco-abdominal asynchronies between the techniques, possible inaccuracies due to imperfect balloon positioning and chest wall distortion should have equally affected the measurements performed in both the ventilatory modes, allowing reliable intra-subject comparisons.

Second, measurements of lung volume changes by RIP could be critical. RIP is a reliable method of determining relative change in V_L in preterm infants (Duffty et al. 1981). Whilst calibration was made using the accepted method (Duffty et al. 1981), accuracy may be limited in spontaneously breathing preterm infants with highly variable breathing pattern.

WOB has been computed without considering the curves of chest wall relaxation and lung elastic recoil, as these curves cannot be easily assessed in spontaneously breathing infants. Therefore we based the estimate of WOB on the following hypothesis: 1) changes in total respiratory system pressure-volume curve around the operating lung volumes are mainly determined by the lung, 2) the compliance is constant over the breathing cycle, 3) end-expiratory lung volume does not change significantly during the recordings. These assumptions, however, are quite common for this kind of studies (Saslow et al. 2006).

Since during the measurements the infants' mouth was kept gently closed to achieve better stability of the airways pressure, conclusions about the working mechanisms of HHHFNC in less controlled clinical settings should be drawn carefully. A closed mouth may have reduced the efficacy of gas wash-out during HHHFNC despite generating higher P_{rp} .

The comparison between HHHFNC and nCPAP was limited to two levels of P_{rp} (2 and 4 cmH₂O) because in our study HHHFNC flow rates were limited to a maximum of 6 l/min, as commonly used in clinics, and with this setting only 5 patients on 20 developed a $P_{rp} > 6$ cmH₂O. In the clinical practice, however, higher pressure levels (5-6 cmH₂O) are commonly used during nCPAP, especially for acute disease. Therefore, further studies are required to evaluate whether HHHFNC is effective also in these patients, by means of working mechanisms other than the provision of a positive airway opening pressure.

In conclusion, when similar end-expiratory pressures are applied, in spite of the different mechanisms of pressure generation, nCPAP and HHHFNC show comparable effects in terms of breathing pattern, gas exchange, lung mechanics and work of breathing in preterm infants with RDS.

3.2 BREATHING PATTERN VARIABILITY AS MARKER OF ADEQUATE RESPIRATORY SUPPORT

Respiratory system includes many non-linear feedback mechanisms and a network of subsystems that are interacting with each other (vagally mediated peripheral stretch and chemoreceptor feedback control loops, central chemoreceptors, non-respiratory systems influences) and influence the respiratory generator within different time scales and gain. The controlled physiological parameters fluctuate within acceptable limits under non-equilibrium conditions (Frey et al. 2011). Variability analysis of these parameters provides an evaluation of the overall properties of such a complex system and is affected both by maturation and disease (Baldwin et al. 2006). Both lower and excessive variability has been associated with pathological conditions that results respectively in reduced adaptability of the system or loss of control (Frey et al. 2011).

Variability in the respiratory system has been found to be important for cell activity as it positively affects surfactant secretion (Arold et al. 2009; Arold et al. 2003); be related to the maturation of the breathing control in infants (U Frey et al. 1998; Engoren et al. 2009); be correlated with weaning success (Wysocki et al. 2006; Kaczmarek et al. 2013; Engoren 1998) and be modified by RDS and assisted ventilation in newborns (Emeriaud et al. 2010). Therefore a ventilation strategy that can preserve the natural variability could be of major importance. Although Nasal Continuous Positive Airways Pressure (nCPAP) is a common modality of support for preterm infants, the influence of different nCPAP levels on the control of breathing is still not known.

The application of pressure might modify EELV, lung mechanics, the input to the neural centres that arrives from the stress receptor, gas concentrations and consequently the variability of the breathing pattern. Goldman et al. showed that the application of a pressure at the airways opening modify the multifractal properties of the intra breath interval in children (Goldman et al. 2008). Preterm babies could be more sensible to pressure levels as they have strong reflexes that play a large role in the control of breathing, immature control of breathing and they have to actively elevate EELV above the resting volume of the respiratory system.

Variability analysis can be applied to different signals related to breathing but care should be taken in choosing the measurement technique as the set up can influence the results. Optoelectronic plethysmography (OEP) permits to measure variation of lung volume and has the advantages not to be subject to drift, to provide different breathing pattern indices including EELV without assuming only two degree of freedom for the highly deformable infant chest wall or requiring a subject specific and difficult calibration. Moreover it has been shown that it permits accurate measurements in infants (Dellaca et al. 2010).

Different methods have been proposed to study variability of the breathing pattern (Bravi et al. 2011). Up to now just some of them (autocorrelation function, entropy metrics, techniques to quantify fractal properties, etc...) have been applied to the study of the breathing pattern (BP) of newborns. Between these techniques the detrended fluctuation analysis (DFA) has the advantages of non-requiring the stationarity of the data and distinguishing between intrinsic fluctuation generated by a complex system and those caused by external stimuli acting on the system.

The aim of the present study is to applied DFA to characterize the variability in the BP of preterm newborns during spontaneous breathing (CPAP = 0 cmH_2O , nCPAPO) on the first day of life and how this is influenced when a high nCPAP level (10 cmH₂O, nCPAP10) or a CPAP level optimized for maximum oxygen saturation (SpO₂) (nCPAPO₂) is applied.

3.2.1 OPTOELECTRONIC PLETHYSMOGRAPHY

24 reflective markers were placed on the skin of the infants using bi-adhesive hypoallergenic tape. The three-dimensional position of each marker was measured by an automatic

optoelectronic motion analyser (OEP System, BTS, Milano, Italy) at a frequency rate of 60Hz using six specially-designed infrared video cameras provided with infrared flashing light-emitting diodes. Cameras were positioned so that each marker was simultaneously seen by at least two cameras (Figure 3-5), in order to reconstruct their three-dimensional position and displacement during respiration by stereo-photogrammetric methods and that operative calibrated volume was large enough to include the whole trunk of the infant lying on the bed (Dellaca et al. 2010).



Figure 3-5. Measurement set-up for the application of OEP to newborns in supine position. V_{RC} ribcage volume; V_{AB} abdominal volume; V_{CW} chest wall volume. From (Dellaca et al. 2010).

Volume computation

Total V_{cw} was determined by approximating chest wall surface by 34 triangles connecting the markers and computing the volume enclosed by all these triangles. The posterior surface of the chest wall (hidden in supine position) was defined by nine "virtual" markers assumed to lie on the supporting plane Figure 3-6.



Figure 3-6. Left panel: markers' positioning and chest wall compartments for newborns in the supine position. Right panel: three dimensional models of the rib cage and of the abdomen. Continuous lines: triangles connecting real markers (solid circles); dashed lines: triangles connecting "virtual" markers (open circles). From (Dellaca et al. 2010). The link between the desiderated volume and the surface that limits the volume is given by Gauss's Theorem. Gauss's Theorem asserts that the volume integral of the divergence of F over a region of space V and the surface integral of F over the boundary of V (S), are related by:

$$\int_{V} \nabla \vec{F} dV = \int_{S} \vec{F} \circ \vec{n} dS \tag{3.1}$$

where n is the normal unit vector at the different points of S, and ∇ is the divergence operator. The operative procedure to compute the volume is composed of different steps.

- Triangulation of the whole trunk surface S. The surface S that encloses the total chest wall volume V, is approximated by some triangular plain patches. Each patch has three markers as vertexes and each vertex B_{ij} (with j = 1,2,3) has coordinates x_{ij} , y_{ij} , z_{ij} .

- Choice of the arbitrary flow vector F in the volume V. The general expression of this vector is:

$$F = x_F i + y_F j + z_F k$$

For our purpose it is convenient to choose F with unit divergence.

- Selection of the vector n normal to the surface. Because of the discretization of the surface S with plain triangles, this vector of unit module and orthogonal in every point to the surface, can be represented by the normal unit vector n_i of the i^{th} triangle.

- Computation of the total volume V. In this case, the surface is approximated by points, so it is difficult to treat the continuous form of the divergence expressions. Passing from continuous to discrete form, the Gauss's equation becomes:

$$\sum_{i=1}^{N} \vec{F} \circ \vec{n}_i A_i = V \tag{3.2}$$

where N is the number of triangles chosen to have a good triangulation of the surface and A_i is the area of the ith triangle, and \mathbf{n}_i is the normal unit vector of the ith triangle.

3.2.2 DETRENDED FLUCTUATION ANALYSIS (DFA)

Detrended fluctuation analysis was introduced by Peng et al. in 1994 (Peng et al. 1994) to quantify long ranges scale-invariant (fractal) correlation in non-stationary time series, distinguishing between fluctuation cause by external stimuli acting on the system and by the intrinsic complex nonlinear dynamics of the system itself. The hypothesis is that only the fluctuations arising from the dynamics of the complex, multiple-component system show long-range correlations while the effect of fluctuations driven by uncorrelated stimuli cause a local effect having characteristic time scales and can be removed by the detrending process.

Several steps are involved in this analysis: i) the original time series x(i) is integrated. The obtained series y(k) represents an evaluation of the trends as if the value of the series (x(i)) remains lower than the mean (\bar{x}) , y(k) decrease as k increase while if it remains higher than the mean y(k) increase as k increase.

$$y(k) = \sum_{i=1}^{k} (x(i) - \bar{x})$$
(3.3)

ii) The series y(k) of length N is divided into equal non-overlapping windows of length n. Each window is detrended by removing the regression line fitting the data $(y_n(k))$. The root-mean-square fluctuation of the integrated and detrended time series of y(k) is computed as follows:

$$F(n) = \sqrt{\frac{1}{N} \sum_{k=1}^{N} (y(k) - y_n(k))^2}$$
(3.4)

iii) This computation is repeated for different values of n. Typically F(n) increases with the window size n. A linear relationship on a double log graph indicates the presence of scaling behaviour and fluctuations scale according as a power of the window length. Under such conditions, the fluctuations can be characterized by the slope of the line relating log(F(n)) to log(n) usually called α or the scaling exponent.

For a random process, α is 0.5. α is between 0 and 0.5 for an anti-correlated signal in which large fluctuations are likely to be followed by small fluctuations while α is higher than 0.5 for a positively correlated signal in which large fluctuations are likely to be followed by large fluctuations. $\alpha = 1$ corresponds to 1/f noise and $\alpha = 1.5$ corresponds to Brownian noise.

The advantage of this technique is that is does not require the stationarity of the data, a condition not satisfied by biological signals. A disadvantage of this method is the assumption that the same scaling pattern is present throughout the signal. To address this issue multifractal analysis has been proposed but requires a longer time series, difficult to obtain when dealing with biological signals.

3.2.3 PRELIMINARY MEASUREMENTS AND CONSIDERATIONS

OEP is not affected by trend and it is stable with humidity and temperature (Dellacà et al. 2001). However this is not enough to guarantee that the acquisition system and algorithms do not introduce long range correlations that are not due to the infants' breathing pattern.

To address this issue we used the OEP to track the position of a single marker placed on the piston of a servocontrolled linear motor. We acquired the movement of the piston while it was driven by a correlated and by an uncorrelated signals. The correlated signal was obtained as the sequence of 1445 sinusoids, the periods of which were set equal to the inter-breath intervals got from the volume signal of a representative infant. The uncorrelated signal was obtained by shuffling (randomising) the sequence of the sinusoids included in the correlated signal.

From the 3D position of the marker acquired by OEP, we extracted the time series of periods and DFA analysis was performed. The value of α obtained for the correlated sequence (0.718±0.034; r2=0.99) was the same of coherent with the one of the infant and α of the uncorrelated sequence (0.518±0.040; r2=0.98) was coherent with white noise. Therefore we concluded that the acquisition system does not introduce a long range correlation.

In order to check the effect of the volume computation algorithm we compared α obtained from the volume trace with α obtained from the changes in position of a single marker (marker number 17 on the model – see previous paragraph) in the studied infants. We found no statistical differences.

Therefore we concluded that if a long term correlation is detected it is due to the characteristic of the breathing pattern of the infants.

3.2.4 STUDY PROTOCOL

Preterm infants requiring nCPAP on the first day of life were recruited. Measurements were performed while nCPAP was stepwise increased (0–4-8-10 cmH₂O) and then decreased (8-6-4-2 cmH₂O) with each step lasting for 20 min.

Chest wall volume changes assessed by optoelectronic plethysmography (OEP), SpO_2 , transcutaneous arterial partial pressure of oxygen ($PtrO_2$) and carbon dioxyde ($PtrCO_2$) were measured at each step during the trial.
Once volume traces have been computed (see paragraph 3.2.1), end-inspiratory and endexpiratory points were identified automatically and manually check for artefacts or errors before the computation of time series of breathing pattern parameters. BP parameters were computed breath by breath over approximately the last 10 min of each step. The statistical properties of respiratory rate (RR), expiratory time (Te), total and compartmental (pulmonary rib cage and abdomen) tidal volume (V_T) and end-expiratory lung volume (EELV) time series were quantified in term of the coefficient of variation (CV) and the long-range correlation exponent α obtained by DFA. CV was computing each 10 breathes and then the obtained values are mediated to obtain one value for each condition. The scaling exponent α , its standard deviation and the rsquare of the linear fitting were computed for each period of interest. To ascertain that the correlations detected in breath-to-breath fluctuations of the considered parameters are really due to the correlated order of the data points, we repeat the analysis on surrogate data with the same statistical properties of the time series but with a random time sequence. In order to obtain such surrogate data we randomized (shuffled) the order of the original breath-to-breath time series. Such a rearrangement of the data results in an uncorrelated time series and hence in a scaling exponent of the shuffled time series close to 0.5. If the value of α of the original time series is significantly different from that after shuffling, it is related to the existence of longrange correlations.

 $nCPAP_{o2}$ was defined on the decreasing nCPAP trial as the nCPAP at which the ratio between PaO_2 and the fraction of inspired oxygen (FiO₂) was maximized.

Statistical analysis

Data was tested for normality using the Kolmogorov-Smirnov test and expressed as mean±standard deviation. Significance of difference between CPAP levels was tested by oneway ANOVA for repeated measures. Multiple comparisons after ANOVA was performed by Holm-Sidak test. Wilcoxon Signed Rank Test was used to test differences between chest wall compartments. Differences were considered statistically significant for p<0.05. Spearman correlation was performed to test whether there was a statistical dependence between parameters.

3.2.5 RESULTS

7 unsedated preterm infants (GA = 31 ± 3 wk; PNA = 12 ± 7 h; BW = 1443 ± 598 g) were studied on the first day of life. Patients' characteristics and ventilatory settings are reported in Table 3-3 and in Table 3-4.

Infant	GA (wk)	BW (g)	PNA (h)	Gender
1	35.4	2030	13.58	М
2	29.1	1210	5.33	Μ
3	30.2	1170	11.23	F
4	26.4	760	22.03	F
5	30.4	1030	9.80	Μ
6	36.3	2460	20.93	F
7	29.6	1445	4.02	F

Table 3-3. Patients' demographic data.

Infant	nCPAP (cmH ₂ O)	FiO ₂	PtcO ₂ (mmHg)	PtcCO ₂ (mmHg)	Silverman
1	5	31	69	48	4
2	5	21	86	36	1
3	4	21	81	50	2
4	5	21	59	41	1
5	5	26	60	53	2
6	5	21	45	31	4
7	5	30	45	59	4

Table 3-4. Patients' characteristics and ventilatory settings.

In average, 593±372 breaths per step were analysed. Correlation between GA and the scaling exponent of respiratory rate (RR), tidal volume (V_T) and end-expiratory lung volume during spontaneous breathing are shown in Figure 3-7. We found a statistically significant correlation only between GA and α RR (r=0.93; p<10^-6) and α Te (p=0.75, p=0.04). PtcO₂/FiO₂ correlated with α EELV (p=-0.78, p=0.02). No significant differences between chest wall compartments were identified.



Figure 3-7. Correlation between GA and the scaling exponent of respiratory rate (RR), tidal volume (V_T) and endexpiratory lung volume before the application of nCPAP.

Figure 3-8 shows the scaling exponent for EELV, V_T , RR and gas exchanging as function of nCPAP. All BP parameters exhibited long-range correlations defined as α >0.5 at all the level of applied nCPAP and without it. Long-range correlations were stronger for EELV than for V_T and RR.



Table 3-5 compares BP parameters and gas exchange parameters obtained without nCPAP (nCPAP₀), at the maximum level of nCPAP applied (nCPAP₁₀), and at nCPAP₀₂. EELV exhibited strong long-range correlations and decreased CV compared to V_T and RR. PtcO₂/FiO₂ at CPAP₀₂ was higher than at CPAP₀ but not than at CPAP₁₀. We found a statistically significant difference among CPAP₀, CPAP₀₂ and CPAP₁₀ in CV_{RR}. CV_{EELV} and α_{EELV} were lower at CPAP₀₂ than at CPAP₀ and CPAP₁₀.

Table 3-5.	Comparison	between BF	oparameters
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	CPAP ₀	CPAP ₀₂	CPAP ₁₀
PaO ₂ /FiO ₂ (mmHg)	2.32±0.99	3.16±0.61 *	3.04±0.72
PaCO ₂ (mmHg)	45.29±7.24	46.17±8.78	48.36±7.45
RR (breath/min)	72±28	76±24	73±24
αRR	0.82±0.14 °°°	0.79±0.16	0.74±0.08
CV _{RR}	0.29±0.14 °°	0.14±0.08 ***	0.21±0.10 ***
V _⊤ (ml)	7.6±4.8	6.2±3.0	6.0±3.6
αTV	0.79±0.14 °°°	0.79±0.13	0.81±0.16
$CV_{V^{T}}$	0.50±0.33 °°° ^	0.17±0.07	0.09±0.03
αEELV	1.39±0.18	1.13±0.17 ** §§	1.36±0.12
CV _{EELV}	0.0024±0.0009	0.0068±0.0044 **	0.0031±0.0015 **
Te (s)	0.74±0.50	0.59±0.25	0.62±0.28

Comparison between nCPAP level: *p<0.05; ** p<0.01;*** p<0.001 with CPAP₀; §§ p<0.01 with CPAP₁₀. Comparison between parameters: ° with EELV; ^ with RR.

3.2.6 Discussion

The main findings of this study were: i) the existence of long term correlation for RR, V_T and EELV series ii) α_{EELV} was greater than α_{RR} and α_{VT} at all the applied pressures; iii) the application of a level of nCPAP that maximises PtcO₂/FiO₂ maintains the long term correlation in RR and V_T while results in a lower α_{EELV} compared to the absence of nCPAP and to the highest nCPAP applied in this study.

Comparison with other studies

Our results are consistent with the one of Frey et al. (U Frey et al. 1998). They report power law behaviour in intra-breath interval (IBI) in term and preterm infants with the slope of the probability density function of the IBI that presents a tendency to increase with PNA. Similarly we found that α_{RR} is statistically different from 0.5 and increases with GA (in our study PNA=GA+1day). This increase in long term correlation could be the results of a higher complexity and integration capability of the neural control system related to maturation.

DFA has been applied before to the study of the respiratory control of infants (Baldwin et al. 2004; Cernelc et al. 2002). Cernelc et al. studied the long term correlation of V_T , end-tidal O_2 and CO_2 concentration in term infants. They found a higher α_{VT} than the one we found (0.86±0.28 vs 0.79±0.14) and this could be linked to the different GA of the population of this study. They also found that the long-range correlations for end expiratory O_2 are stronger than those for V_T and CO_2 . As it is know that ventilation has a greater impact on CO_2 concentration and lung recruitment has a greater influence on O_2 exchange, we can speculate that the differences in long term correlation between O_2 and CO_2 are partially linked to the stronger correlations we observed in EELV in respect to V_T time series.

This is the first study that analyses long term correlation in EELV in infants. One study analyzed the short term variability of EELV by autocorrelation analysis in 18 preterm infants using inductance plethysmography during the first 10 days of life (Emeriaud et al. 2010). Autocorrelation resulted markedly prolonged in patients with RDS or ventilatory support, with a higher number of breath lags with significant autocorrelation and higher autocorrelation coefficients. They suggest that the surfactant deficiency makes the control of EELV more difficult in preterm infants and results in a more prolonged 'short-term memory' of EELV in premature infants with respiratory distress with or with-out ventilatory support.

Finally Goldman et al. (Goldman et al. 2008) show how the application of a pressure at the airways opening modifies the multifractal properties of the intra breath interval in anaesthetised children. Similarly to our results, they found differences between an intermediate pressure level versus the absence of pressure or the application of a high pressure. They report that the application of an intermediate pressure seems to reset the system to a stochastic, less complex behavior while basal and high pressure would amplify the gain of non-random, correlated processes. They state that the collapse of this deterministic structure into stochastic randomness suggests that the respiratory system dynamically modulates the amount of correlated variability in the face of external perturbations under operating conditions not far from equilibrium.

EELV control in infants

We found that EELV presents more long term correlation than RR and V_T . This suggests the presence of different mechanisms of control for this parameter or a different relative contribution on variability of the control of breathing and lung mechanics. In adults, EELV results from a mechanical equilibrium between the elastic recoil of lung and chest wall. Therefore this could suggest a greater contribution of the properties of the lung tissues on EELV variability than on RR.

However the situation is different for infants. In newborns, the resting volume of the respiratory system is low because of the increased tendency of the lung to collapse and the decreased rigidity of the chest wall due to its high cartilage content and poorly developed musculature. Therefore EELV is maintained above the resting volume by active mechanisms.

These mechanisms involve the persistence of tonic diaphragmatic activity during expiration (Emeriaud et al. 2006), a flow-braking action of the laryngeal adductor muscles to increase resistance and therefore the expiratory flow (Kosch et al. 1988), and a high respiratory rate with relatively short Te that does not let enough time to the respiratory system to reach its resting volume (Kosch et al. 1984). The presence of these mechanisms suggest that a great contribution to EELV variability is determined by the control of breathing and that the variability of EELV could be related in part to variability of the diaphragmatic activity, and variability of Te.

Effects of nCPAP on breathing pattern variability

The result of this preliminary study, shows that the level of the applied pressure has an influence on α_{EELV} but not on α_{VT} and α_{RR} . Further studies are needed to understand the underlying mechanisms. We can speculate that the application of a pressure modifies lung mechanics. These changes influence the input to the brain from the mechanoreceptors that sense the mechanical condition of the respiratory system. Variations in the long term correlation with pressure are probably linked to the answer of the neural control system to the mechanical condition of the lung as suggested by Goldman et al.

Limitation of the study

The first limit of this study is the small number of patients. However we are presently studying more newborns to strengthen our results. Sleep stage were not monitored, however Larsen et al. (Larsen et al. 2004) report no difference in long term correlation between sleep states. In order to permit OEP measurements the infants were removed from their nest and positioned on a flat surface (in order to allow the cameras to see the marker). This could have influenced the measurements. However, as the position of the infants was the same in all the step of the trial, it does not influence the comparison between different pressure levels. Another limitation of this study is the lack of a measure of lung mechanics or WOB, as they could have helped in the interpretation of the results. However these measurements could have had an impact on the measured variability due to the need of the oesophageal balloon.

The comparison of EELV between different levels nCPAP has been prevented by changes in position of the infant in respect to the nine "virtual" markers assumed to lie on the supporting plane (see paragraph 3.2.1) between the different registrations. However this has an impact only on the mean EELV and not on its variability. Finally, it is possible that the time at each nCPAP level could have been not enough to see changes in variability of RR and V_T. However longer time without nCPAP or at high nCPAP values could have been dangerous for the infants.

In conclusion, the application of an optimized nCPAP reduces α_{EELV} compared to both zero and maximal clinical level of nCPAP. Even though the underlying physiological mechanisms are unknown, these results suggest that further studies on variability analysis could help tailoring the optimal nCPAP level in preterm infants.

4 RESUSCITATION AT BIRTH

Crescent attention is focused on the identification of resuscitation strategies at birth that can optimise FRC establishment and realize a protective ventilation from the beginning of air breathing.

In the transition from foetal life to air breathing, rapid clearance of foetal lung fluid and recruitment of alveoli are the critical steps for the survival of the newborn. Transpulmonary pressure plays a determinant role in this process (Hooper et al. 2007) and the application of a pressure to the airway opening helps preterm infants to remove the fluid and open closed alveoli. As once the alveoli are recruited less pressure is required to keep them open and to ventilate the lung, the application of recruitment manoeuvres at birth has the advantage to permit the onset of ventilation in the context of a more recruited lung, with a lower requirement of pressure, a more homogeneous distribution of lung volume and less stress applied to the tissue. On the other hand, at birth a surfactant-deficient lung can be injured even by few inflations with volumes that are probably harmless in other circumstances (Björklund et al. 1997; Wada et al. 1997). Therefore designing a protective strategy to recruit the lung without causing injury could have a great impact on the clinical outcome of the newborns.

Animal studies have shown that the application of a sustained lung inflation (SLI, see paragraph 1.2.3) followed by PEEP improves lung volume recruitment with respect to the conventional treatment consisting in intermittent application of pressure (te Pas, Siew, Wallace, Kitchen, Fouras, R. a Lewis, et al. 2009). The advantage of SLI could be linked to the application of pressure for a duration of time appropriate for the air/liquid interface to move into the distal airways. In fact, because of viscoelastance and other time-dependant phenomena, the tendency of previously collapsed lung units to open depends both on the applied transpulmonary pressure and duration of its application.

Despite these encouraging results on animal studies, efficacy and safety of such manoeuvres in infants are presently still under investigation while the optimal duration and level of initial inflation and subsequent level of PEEP remains unclear (Lista et al. 2012). The lack of knowledge of which is the best technique to recruit the lung (type of the manoeuvre, time and pressure settings) and its probable dependence on the specific characteristics of each lung has slow down the diffusion of SLI in clinical practice.

The possibility of measuring lung condition during the manoeuvre and the availability of tools to customise it would lower the probability of injure the lung, favour the diffusion of recruitement manoeuvre at birth and permit the realization of a more protective ventilation strategy. It has been suggest that a lung volume targeted strategy could realise a more protective strategy than the application of fixed time/pressure SLI (te Pas, Siew, Wallace, Kitchen, Fouras, R. A. Lewis, et al. 2009; Tingay et al. 2013).

However a strategy considering lung mechanics could have the additional advantage to be sensible to overdistantion. In particular, measurement of Zin by FOT has been proved to provide

information on lung recruitment/distention (Dellaca et al. 2009) and has been successfully applied to the study of lung mechanics in ventilated preterm newborns (Dellacà, Veneroni, et al. 2013). In order to test the utility of FOT during resuscitation at birth, Zin measurements were tested in a preterm lamb at birth. Different strategies can be implemented on the bases of Zin and their evaluation on animal models could permit the identification of the manoeuvre that maximises the final recruitment and minimised the stress applied to the tissue. Therefore, an intelligent system that can be used for resuscitation at birth in animal studies is under development (see Future perspectives) in order to permit real time Zin measurements during resuscitation and to automatically adapt the pressure and the duration of the recruitment manoeuvre according to the chosen strategy.

However, even if the optimal recruitment strategy is known, it cannot be applied in infants unless a FOT set up suitable for measurements during the critical moment of resuscitation at birth is available. Therefore we developed a set-up for the assessment of Zin during SLI based on a neonatal mechanical ventilator, in which we slightly modified the software in order to generate the forced oscillations and export pressure and flow data for impedance computation.

In order to test the feasibility of Zin measurements during recruitment manoeuvres, the effects of leakage and how to minimize them, the developed set up was used for a preliminary study on babies admit to NICU for RDS.

4.1 SLI AT BIRTH

The effects of pressure and time settings during SLI are still under investigation. Te Pas et al. studied the effect of the duration of SLI (te Pas, Siew, Wallace, Kitchen, Fouras, R. A. Lewis, et al. 2009) and its combination with PEEP. However they suggest that a lung volume targeted strategy could realise a more protective approach than the application of fixed time/pressure SLI. The same suggestion has been made also by Tingay et al. They measured lung volume by EIT during SLI at birth and found that the time required to stabilise lung volume during a SLI exhibited a considerable variability among preterm lambs (Tingay et al. 2013).

However a strategy considering lung mechanics could have the additional advantage to be sensible to overdistantion. In order to verify if FOT measurements are possible at birth when the lung still contains foetal fluid, we measured Zin in a preterm lamb (GA=127d; BW=3200g) immediately after birth.

The lamb was connected to SLE500 ventilator. Xrs was measured at 5Hz using the square waveform of HFOV modality and computing Zin in the frequency domain as in (Dellacà, Zannin, et al. 2013). SLI was applied for 30s with a pressure setting of 20 cmH₂O and followed by PEEP of 8 cmH₂O and volume targeted ventilation.

Figure 4-1 reports Xrs data at the beginning of the manoeuvre. In the upper panel pressure trace is report. 20 cmH₂O were applied immediately at birth after intubation and FOT measurement begins after some seconds at this pressure. Xrs starts from very low values and increases quickly as the liquid is removed from the lung. Steps in Xrs values are observed in correspondence of spontaneous breathes. Figure 4-2 shows Xrs during the first 10 min of life of the studied lambs. A dashed line marks the end of SLI and the onset of VTV.



Figure 4-1. Xrs measurement during SLI in a preterm lamb. Grey arrows indicates spontaneous breathes.



Figure 4-2. Xrs as function of time. Dashed line marked the end of SLI and the onset of volume target ventilation.

Different strategies can be developed on the bases of these measurements. For example the distending pressure can be maintained until Xrs stops increasing or the pressure level can be control to avoid that Xrs goes below a threshold. The implementation of these strategies in a set up will permit the comparison of them in animal studies leading to further insides in the mechanisms of recruitment/distention and moving the first steps toward the definition of a protective recruitment maneuver (see future perspectives).

4.2 REAL TIME LUNG MECHANICS DURING SLI

The diffusion in clinical practice of SLI is limited by concerns about inducing lung damage and by the absence of consensus about the optimal duration and magnitude of initial inflations, and subsequent level of PEEP (Richmond et al. 2010; Perlman et al. 2010).

Even if animal studies are fundamental to understand the mechanisms and the role of pressure and duration in the establishment of FRC, they are not enough to identify the best strategy for treating newborns. Moreover, aiming to a customization of the treatment that will support the diffusion of SLI in clinical practice with a decreased risk of lung damage, a tool able to monitor lung function during this manoeuvre has to be available.

4.2.1 INTEGRATION OF FOT IN A MECHANICAL VENTILATOR

Starting from the right ventilator would make it possible to integrate FOT set up into it without adding any hardware part but only modifying the software. In collaboration with Acutronic Medical System (Switzerland) we developed a set up that exploits FabianHFOV device for FOT measurements. The advantages of this ventilator include flexibility, easiness of adding new functionalities and pressure and flow measured at the mouth of the patients with enough accuracy. In order to limit the changes of the ventilator software in this first phase we decided to connect a tablet to the ventilator and implement part of the functionalities in an Android application.

In particular, tasks performed by the ventilator are:

1) the generation of a sinusoidal stimulus superimposed it on the chosen ventilator modality,

flow and pressure measurements;

while, the ones implemented in the Android App are:

- 1) Zrs computation,
- 2) user interface for FOT measurements,
- 3) data recording.

The communication between the two devices is realized through serial RS232 communication. The ventilator sends pressure and flow samples to the tablet and receives commands from it.



Figure 4-3. Schematics of the set up and functions performs by each device.

VENTILATORS' SOFTWARE CHANGES

The main changes needed in the ventilator software were three:

- 1) Implementation of serial communication to receive commands and send data to the tablet.
- 2) Generation of the sinusoidal stimulus. This was implemented by over-imposing a sinusoidal oscillation on the control signal of the PEEP valve. No feedback was implemented to control the amplitude of the oscillation but the quality of the oscillation was verified during different ventilation settings.

3) Improving the resolution around zero of the flow signal. Flow at the mouth is measured by a hot-wire anemometer. With this type of flowmeter it is difficult to determine the flow direction when the flow is closed to zero. In order to avoid jitters in zero crossing detection, when the flow is below a fixed threshold it is set to zero. This threshold was lowered until it permits accurate Zin computation without interfering with flow zero crossing detection.

COMMUNICATION PROTOCOL

A RS232 protocol is used for data communication (baud-rate of 115200; 8 data bits, 1 stop bit, no parity bit and XON/XOFF flow control). Possible commands and data format are described in Table 4-1.

command	meaning	details
М	get continuous monitor data	data packages of 40 samples in the following format:
		<pre># pressione1 flow1 pressione2 flow2 pressione20 flow20</pre>
Q	quit continuous monitor data sending	
В	get data messured by the ventilator	V _T ; RR; Minute Ventilation; PEEP; Pmax; Pmean;
		FiO2; %leak; Cdyn; C20/C; R
R	get ventilator settings	Ventilator mode ; FiO ₂ ; PIP; PEEP/CPAP;
		Inspiratory time and flow; Expiratory time and flow
a[value]	set the stimulus amplitude	- a0: off
		- a1: 2,5 mbar
		- a2: 5 mbar
		- a3: 7,5 mbar
f[value]	set the stimulus frequency	- f0: 5 Hz
		- f1: 10 Hz
		- f2: 15 Hz

Table 4-1 Comunication protocol between the ventilator and the tablet.

ANDROID APPLICATION

While changes into ventilator software were done in order to leave some flexibility in the stimulus frequency and amplitude for resource purposes, this android application was thought to be used in clinical practice also during resuscitation at birth when the doctor does not have time to select software options. For this reason the only operation required to the user is the pressure of the start button and the user does not have the possibility to choose anything as the frequency and the amplitude are set to 5Hz and 5cmH₂O by the program.

The developed application is a simple application consisting of two Android Activities (*MainActivity* and *ReadingActivity*) (Figure 4-4). An Activity is a component that provides a screen with which users can interact in order to do something. An application usually consists of multiple activities that are bound to each other. Typically, one activity is specified as the "main" activity, which is presented to the user when launching the application for the first time.

When the RS232 cable of the ventilator is plugged into the tablet, the application starts automatically. The first screen visualized by the operator is the one related to the *MainActivity*. It displays if the ventilator is correctly connected or not and waits for the operator to press the start button.

When start button is pressed:

- i) 'M' is sent to the ventilator to start the continuous data sending
- ii) 'f0' and 'a2' are send to the ventilator to start 5Hz oscillation generation with a peak to peak of $5cmH_2O$

iii) The readingActivity is started.

The tasks associated with the *ReadingActivity* are:

- i) Handle data communication with the ventilator. Data are read from the serial buffer and unbundled. Commands are send to the output buffer.
- ii) Compute Zin. (See following paragraph)
- iii) Data visualization. Pressure, Flow, Rrs and Xrs are visualized in real time. In order to not overload the program the visualized signals are downsampled at 20Hz.
- iv) Data storage. The data coming from the ventilator are written in a file. The name of the file results from date and time in which the start button was pressed. Each 10min the current file is closed and a new one with the same name plus an underscore and a progressive number is open. In this way the operator does not have to choose the file name and files are not too heavy.
- v) Adjust the amplitude of the stimulus. As a result of the open loop control of the PEEP valve, when PEEP is increased above 15cmH₂O, the amplitude of the sinusoidal stimulus is appreciably increased. This is not an issue during ventilation as so high PEEP values are not used. However during resuscitation maneuvers employing high constant pressure it is necessary to compensate for this amplitude increment setting the sinusoidal amplitude to 2.5cmH₂O (command 'a1'). Therefore PEEP is continuously computed and the sinusoidal amplitude is corrected every time PEEP becomes higher or lower of 15cmH₂O.



Figure 4-4. Schematics of the Android App.

IMPEDANCE COMPUTATION

As for studies during invasive ventilation, we choose a sinusoidal stimulus for tracking changes over time maximizing the signal to noise ratio (SNR). The chosen amplitude of the signal is approximately of $5\text{cmH}_2\text{O}$, slightly higher than for the previous studies in order to obtain detectable flow signal even in presence of the very high resistance as we expect when the lung is full of liquid. As during this procedure there are no mechanical breathes but only the spontaneous activity of the infants (that has lower components ad high frequencies) we chose a stimulation frequency of 5Hz in order to maximize the sensitivity (see paragraph 2.5.2).

Pre-processing of the acquired signals and Zin computation has been implemented in the Android application and performed in real time during the manoeuvre. Within breath input impedance has been estimated by using the least mean square based approach, as it required less computational time than Zin computation in the frequency domain. Then a correction was applied to compensate for the delay between pressure and flow samples due to the different principle of functioning of the pressure and flow sensor and data elaboration into the ventilator. We quantified the delay between flow and pressure samples comparing them with synchronous flow and pressure samples obtained by the standard FOT set up on the same test-lung. We also verify that this temporal delay was not frequency and amplitude dependent by repeating the measurements changing the stimulus frequency and the test-lung. In particular, the use of test-lung with different mechanical properties permitted to obtain different flow amplitude with the same driving pressure to test if there is an effect of the flow amplitude to the delay.

4.2.2 IN VITRO VALIDATION

Flow and pressure are measured by FabianHFOV. This guarantees that the set-up is compliant with the international standards of infant lung function testing in terms of dead space, resistance, monitoring of leaks and correction of drifts, linearity and frequency response of the sensors (U. Frey et al. 2000). However, small asynchronies between the flow and pressure signals can impact on Zin measure.

Therefore, a bench model of the infant lung (as the one described in 2.2.3), different from the one used to compute the delay between pressure and flow samples, was used to test the frequency response and the accuracy of the measurement set-up vs a traditional loudspeaker-based set up.

Results of the comparison between the measurements are shown in Table 4-2

	5 Hz	7 Hz	9 Hz	11 Hz	13 Hz	15 Hz
R (cmH ₂ O*s/I)	54.7	55.9	57.1	58.3	59.5	59.8
err	5.1	3.1	1.7	1.4	0.9	1.1
err %	9.2	5.5	3.1	2.5	1.6	1.8
X (cmH₂O*s/I)	-94.2	-64.4	-46.5	-34.3	-25.2	-17.8
err	1.8	0.9	0.8	1.7	1.7	1.9
err %	2.0	1.4	1.9	5.4	7.3	11.4

 Table 4-2. Resistance (R) and reactance (X) measured at different frequencies by the developed set up and percentage differences with respect to the reference.

The difference between the measurements performed on the bench model using our set-up and a standard loudspeaker-based one was below 10% for Rrs and below 12% for Xrs in the whole range of frequencies of interest (5-15 Hz). Moreover the higher percentage error in Xrs at 13Hz and 15Hz are due to low Xrs values at these frequencies and correspond to a low absolute error (< 2cmH₂O). After that we also tested that the performance of the measurement system was not affected by the pressures generated by the ventilator. In particular the percentage difference computed at 5Hz between measurements at a mean pressure of 5cmH₂O and at 20cmH₂O were 2 and 1% for Rrs and Xrs respectively. Therefore this set up permits accurate Zin measurement.

In conclusion the developed set up: 1) it permits accurate Zin measurements in ventilated infants without disconnected the patient from the ventilator, adding dead space to the circuit or modifying the ventilator circuits 2) It does not require a trained user and can be easily used in

the clinical practice. The disadvantage is that it is ventilator dependent and can be used only if the babies are ventilated with this machine.

4.2.3 PRELIMINARY MEASUREMENTS IN NEWBORNS

Thanks to the collaboration with Acutronic Medical System we developed and validate in vitro a set up that allows the application of FOT in preterm newborns without requiring the presence of technical staff. It satisfies the requirements to be used during the critical moment of resuscitation at birth as: i) ,employing a NICU ventilator, guarantees the safety of the newborns during the manoeuvre and the possibility to support the respiration with mechanical breathes if needed; ii) minimises the tasks required to the user for measuring Zin.

However before using it in studies at birth, preliminary measurements in a less critical contest are required to assess the feasibility of Zin measurements with this set up in a clinical environment. In particular, as the connection with the infants is through a facial mask, leakages are possible especially when high pressure is delivered. Understanding the entity of these leakages, their impact on the measured Zin and how to minimize them are issues to be address.

Therefore the aim of this preliminary study was to test the feasibility of Zin measurements in preterm newborns with this set up during SLI.

STUDY PROTOCOL

All measurements were performed in the Neonatal Intensive Care Unit of Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, University of Milan. The study was approved by the local Ethics Committee and informed parental consent was obtained for each infant. Infants admitted to NICU and treated with nCPAP for RDS with no other clinical complication were enrolled.

A detailed description of the measurement set-up is reported in paragraph 0. Oxygen saturation and heart rate where monitored for all the time of the study.

Zin values vs time were provided by the Android application. Before and after SLI values of Rrs and Xrs at end–expiration were obtained as the average values of 3 breaths free from artefacts (manually selected). Values of of Rrs and Xrs during SLI were considered at end–expiration when a spontaneous breathing activity was detected also during the manoeuvre. 2-3 breathes at the beginning of the manoeuvre and in 2-3 breathes in the last part of the manoeuvre were averaged to obtained two data points. When no spontaneous breathing activity was present during SLI, the mean and standard deviation of Zin were computed in two windows selected respectively in the first and last part of the manoeuvre.

RESULTS

Four babies (GA=32±2 wk; BW=1229±139 g;PNA=2 2 wk) have been studied. Patients' characteristics are reported in Table 4-3.

Infant	GA (wk)	BW (g)	PNA (wk)
1	30	1300	4
2	30	1020	4
3	34	1285	1
4	33	1310	1

Table 4-3. Patients' characteristics.

Experimental traces are shown in Figure 4-5. Values of Zin during the first breathes were discharged as the presence of leakage results in artificially lower values of Rrs and higher values of Xrs.



Figure 4-5. Experimental traces. Dashed lines underline end-expiration during spontaneous breathing.

Figure 4-6 shows the Zin data obtained before, during and after SLI in the studied subjects. In the upper part of the figure, both on the left and on the right side, a schematic representation of the change in pressure occurring during SLI is reported as reference. In general both Rrs and Xrs decrease during the manoeuvre. Xrs decreases during SLI, and increases when the pressure is removed, stabilizing to higher values than before SLI for all the infants but the last. Rrs decreases during SLI and recover the initial value after the manoeuvre in all but the first infants.



Figure 4-6. Zin value during SLI.

DISCUSSION

The main result of this study was that FOT allows monitoring of lung mechanics changes during SLI in preterm babies.

Rrs decreases during SLI, likely due to the increased airways diameter, which is in turn due to the elastic recoil of the lung tissue on the airways walls which increases with increasing lung volumes (Jordan et al. 1981). The Xrs decreases during SLI and reaches higher values after SLI than before SLI. This behaviour is consistent with tissue distension of the already open alveoli and recruitment of collapsed or fluid-filled alveoli during SLI. During SLI new alveolar units are opened but this does not result in an increase of Xrs during the manoeuvre as it is masked by distention of the opened alveoli. When inflation stops, the recruited alveoli may remain open as the closing pressure of an alveolus is lower than its opening pressure, while the compliance of the previously distended alveoli increases again.

We verified also that accurate positioning of the facial mask permits to minimize the leakage and obtain accurate measurements. The presence of significant leakage may results in artificially lower values of Rrs and higher values of Xrs. However leakages are easily detected by computed the mean flow and the corresponding data can be discharged. Moreover as the stimulus is at 5Hz it is less affected by leakage than lower frequency making FOT measurement relatively robust to small leakage.

In conclusion, Zin can be monitor easily with the developed set up during SLI. These open the way to future studies at birth that could provide further insides in the mechanism involved in establishment of FRC and useful information to customize recruitment manoeuvre in order to avoid excessive stress to the tissue.

CONCLUSIONS

Choosing how to support ventilation in a preterm newborn is a difficult task as his lung is highly vulnerable to injury and there is little knowledge about protective ventilation strategies. Customization and continuous optimization of the treatment from the first moment of life play a key part to avoid secondary injure. In particular, it is important to: i) recruit the lung at birth without overdistending it, ii) keep it open by applying the lowest pressure that prevents derecruitment during invasive or non-invasive supports and iii) adjust the settings according to the lung function changes related to lung growth and to the course of the pathology. However in order to achieve this, measurement tools available at bedside that can monitor lung function and provide useful information for optimizing the treatment are needed.

Therefore different methods and technologies have been developed to be applied during the various procedures and types of ventilatory support. In particular, both the assessment of lung mechanics by FOT and variability analysis of the breathing pattern by DFA have been proposed in the attempt to account for: i) the different measurement constraints entailed by the different modalities of treatment and ii) the complexity of the respiratory system that includes not only the mechanical system (lung, chest wall, respiratory muscles) but also the control of breathing and shows lag, cumulative, memory and disproportionate effects in response to treatments and stimuli.

<u>Invasive ventilation</u>. For monitoring lung function during invasive ventilation measurement of input impedance by FOT has been identified as a promising tool. With an appropriate set up Zin can be easily monitored during clinical practice and animal studies have shown that it can identify an optimal mechanical PEEP in animal models of RDS. Therefore we developed and validated in vitro new set-ups and methods to permit Zin measurements in preterm newborns. In vivo measurements proved them to be able to provide accurate Zin measurement without interfering with breathing. Moreover Zin was useful to study clinical interventions that could modify EELV and its distribution like PEEP and prone positioning.

In particular we observed that different group of patients (RDS, evolving BPD and healthy lung) respond differently to PEEP. RDS infants present a similar behaviour to animal models of RDS, suggesting that a PEEP optimized on Zin bases could attenuate signs of VILI (Kostic et al. 2011). Moreover the trend of Zin with PEEP in BPD patients suggests that the role of PEEP in this population could be different and related to maintained airways patency. Even if these results are based on a small population and need confirmation, they suggest that Zin could have a role in optimize the settings also for this group of patients. Not only the changes in the relationship between Zin and PEEP with pathology but also how this relationship evolves during the first week of life of ELGAN infants has been studied. During this week the respiratory system undergoes several changes while adapting at air breathing. Our results show differences in the relationship between Xrs and PEEP in the different days and suggest that on the 1st day of life

particular care should be taken in setting PEEP as the lung is easily over-distended probably because of incomplete reabsorption of foetal fluid. Finally, we also applied Zin measurements to the study of the effect of prone positioning as a potential tool for improving respiratory support and permitting adequate gas exchange with a less aggressive mechanical ventilation. Our results suggest that prone positioning does not offer significant advantages over supine positioning in the management of intubated and mechanically ventilated infants with RDS on short term basis. In patients with evolving BPD prone positioning is associated to lower Rrs values and could have a role in the clinical management of highly obstructed patient as it reduce the time constants of the respiratory system. The data obtained from these studies have provided important information also for understanding physiological mechanisms and for tuning algorithms and the approach to Zin measurement. Finally, the results of these studies emphasize the importance of customization and continuous adaptation of the treatment to the respiratory system condition. They provide a justification for the efforts required to integrate FOT into a mechanical ventilator in order to make Zin measurement possible in clinical practice. This will open the way to clinical studies evaluating the impact of this measure on clinical outcome of these small patients.

<u>Non invasive ventilation</u>. Nowadays significant efforts are directed to the improvement and development of new modalities of non-invasive ventilation in order to overcome the limits of the present technology and to avoid intubation in the majority of the infants. However the mechanisms of functioning of the new modalities are still unclear and tools to optimise the support are still missing. We investigate the role of the generated pressure during HHHFNC. Our results show that this modality could be used also to generate a pressure with comparable effects to nCPAP. These results support a wider diffusion of this ventilation modality as it offers ease of use, better tolerance and improved feeding and bonding.

During these modalities the assessment of lung mechanics is becoming more difficult as the interfaces with infants are developed to reduce dead space and improve infants' comfort. Moreover a crucial role is played by the infant's spontaneous respiratory activity and it is more evident in respect to invasive ventilation that the mechanical condition of the lung is the results of the interaction between the respiratory support and control of breathing.

We identified the variability analysis of breathing pattern as a potentially useful tool as it permits to obtain information about control of breathing as well as mechanical properties of the thoraco-pulmonary system. Between the different method to quantify variability, detrended fluctuation analysis (DFA) has the advantage of not requiring the stationarity of the data and of distinguishing between intrinsic fluctuations generated by a complex system and those caused by external stimuli acting on the system. In a group of preterm infants treated with nCPAP in their first day of life, we found that optimising nCPAP on oxygenation bases results into a lower long term correlation of EELV with respect to the absence of nCPAP or a high nCPAP value. These preliminary results suggest that variability analysis of the breathing pattern could provide useful information for tailoring ventilatory support. Studying the variability on different time scale of different signals that can be easily obtained in clinical practice and quantified it by different metrics is a possibility worthy to explore.

<u>Resuscitation at birth.</u> Finally, input impedance by FOT can be useful to monitor lung mechanics during resuscitation and provide important information for the optimization of this procedure. A dedicated set up has been developed in order to allow the measurement in a such delicate moment without the presence of technical staff. Employing a commercial NICU ventilator, this setup guarantees the safety of the newborns during the manoeuvre and the possibility to support the respiration with mechanical breathes if needed. Only pressing of a button is required to start Zin measurements. Preliminary in vivo studies suggest that Zin can be useful to understand changes in respiratory mechanics due to this manoeuvre and to design a strategy of resuscitation at birth that maximises the final recruitment and minimizes the stress

applied to the tissue during the manoeuvre. Having this measure it will not be necessary to identify a procedure that works for all infants, but it will be possible to personalise the settings in real time based on the response of the respiratory system. Even if further studies are needed to investigate the role of these measurements in the management of preterm infants, they have the potential to be used in clinical practice providing important feedback on lung function to the clinicians.

The presented studies have shown that the developed methods permit monitoring of lung function in infants and provide information useful to improve the tailoring of the ventilatory strategy.

FUTURE PERSPECTIVES

The availability of the developed methods opens the possibility to a variety of studies. In this paragraph a summary of the studies we have already started is given.

INVASIVE VENTILATION

Variability analysis.

In collaboration with Uppsala University Children Hospital (Uppsala, Sweden) we aim to compare V_T and Edi variability in ventilated preterm newborns during PSV and NAVA. Each infant undergoes to both PSV and NAVA modality in a random order. After 30-45 min of stabilization the data are acquire for 15-20 min and variability quantified by DFA and entropy indices. Preliminary data (1 infants, GA= 27.9 wk; BW=964; PNA=10d) shows that both Edi and V_T signal present long term memory during NAVA and PSV.



NON-INVASIVE VENTILATION

Set up for Ztr measurements.

In order to make possible measurements of lung mechanics in infants treated with these modalities a new set up for measure the movement of the chest wall in a non-invasive way and that could be suitable also for used in clinical practice has been developed. This set up used in combination with a device for generating FOT stimulus permits the computation of Ztr.

It employs 6 accelerometers to measure the movements of the chest wall. The signal from the accelerometers is sampled and sent wireless to a personal computer for further elaboration and data storage. A microcontroller handles data acquisition and the communication with the computer. A schematic of the system is represented in the following figure. Next steps will include set up validation and evaluation of the information that this measure can provide together with its clinical utility.



RESUSCITATION AT BIRTH

Evaluation of Zin changes at birth during SLI.

In collaboration with the Neonatal Intensive Care Unit of Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico in Milan we aim to study how Zin changes in preterm infants in the first minute of life and compare these changes between infants treated with the standard resuscitation manoeuvre and infants treated with SLI. We have obtained the approval of the Ethical Committee and we are about to start data acquisition.

Development of a set up to study recruitment manoeuvres at birth in animal models

An intelligent system that can be used for resuscitation at birth in animal studies is been developed. The aim of this system is to permit real time Zin measurements during resuscitation and automatically adapt pressure on Zin bases. Different strategy will be implemented and compared in vivo studies in order to identify the recruitment strategy that maximise the final recruitment and minimised tissue stress during the manoeuvre.

The system is composed of:1) a blower to provide the flow for the ventilation; 2) a PEEP valve that will be controlled to generate both the pressure needed for ventilation and the sinusoidal stimulus for FOT measurements; 3) ventilation circuit; 5) an anemometer and pressure sensor to monitor flow and pressure; 4) a microcontroller based board that handle real time control of the blower and PEEP valve and the data acquisition and 5) a tablet with Android to implement the user interface.

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"what is essential is invisible to the eye". The little prince, Antoine de Saint-Exupéry