Tidal breathing parameters in extremely premature infants. Feasibility and clinical utility of electromagnetic inductance plethysmography

Mariann Haavik Lysfjord Bentsen

Thesis for the Degree of Philosophiae Doctor (PhD)
University of Bergen, Norway
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Name: Mariann Haavik Lysfjord Bentsen

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«Medicine is a science of uncertainty and an art of probability.»

William Osler

CONTENT

1. PREFACE

1.1 Scientific environment

This thesis emerges from the PhD program at the Department of Clinical Science, Faculty of Medicine and Dentistry at the University of Bergen. The present work was carried out within the frames of the 'West Paed Research'-group at the Department of Paediatrics at Haukeland University Hospital, and partly also at the Department of Paediatrics at Stavanger University Hospital.

The major funding institution was the Western Norway Regional Health Authority, Haukeland University Hospital. The vests used in studies 1-3 was sponsored by the manufacturer (VoluSense, Bergen, Norway), but the rest of the equipment used was owned by Haukeland University Hospital.

My supervisors have been Professor Thomas Halvorsen, Professor Trond Markestad and Professor Knut Øymar, all paediatricians.

Morten Eriksen, who is the inventor of the EIP method, has provided helpful technical assistance and expert advice regarding technical issues.

1.2 Acknowledgements

As a young paediatrician specializing in neonatology and signaling interest in doing some research, I was asked in 2010 by my two main supervisors, Trond Markestad and Thomas Halvorsen, to participate in 'BabyPEP' - a large prospective follow-up study on infants born extremely premature that they had visions and plans of starting. This became my entry into clinical research, and since then I have worked as the 'managing director' of the 'BabyPEP' project. I am truly grateful for having been given this opportunity – it has given me insight into the incredibly exciting world of research, and this PhD-thesis is a spin-off of the 'BabyPEP' project.

First, I am grateful to all the infants and parents participating in the studies comprising this thesis. Without their patience and cooperation this work would have been impossible.

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1.3 Summary of thesis

Background: Extremely preterm (EP) birth is associated with adverse lung growth, due to disturbance of programmed and fine-tuned sequences of normal intrauterine development, as well as the trauma inflicted by life-saving treatments. Moreover, EP-born neonates are probably born with variable susceptibilities or predispositions for lung injury. The pulmonary consequences of EP birth are heterogeneous and insufficiently mapped and understood. Particularly, our understanding of the detailed lung mechanics in EP-born infants is limited, largely due to the fact that lung function measurements in neonates and infants are complex and not readily available. Electromagnetic inductance plethysmography (EIP) is a novel non-invasive system that provides tidal breathing parameters in the form of tidal flow volume loops.

With these studies, we aimed to: (1) Study feasibility and validity of the EIP method in preterm and term-born infants, (2) use the EIP method to compare lung function in EP and term-born infants at term-equivalent age, and test if lung function measurements obtained at this stage in EP-born infants were associated with respiratory health during their first year of life, and (3) study if tidal breathing parameters, obtained from a mechanical ventilator during the few first hours of life in EP-born neonates, could predict bronchopulmonary dysplasia (BPD).

Methods: In study #1, we tested repeatability of the EIP method in a total of 30 preterm born infants who were at various stages of their development. A nurse experienced with the system measured all patients before and after meals, and these measurements were repeated by nurses new to the system. In study #2, the EIP-method was validated against a 'gold standard method' (mask-based ultrasonic flowmeter) in 30 infants at postmenstrual ages between 36 to 43 weeks and weights from 2.3 to 4.8 kg, applying both methods simultaneously. In study #3, within a population-based design, 52 EP-born infants recruited at Haukeland and Stavanger University Hospitals had their lung function measured at term-equivalent age using the EIP method, and the results were compared with 45 term-born controls. The EP-born infants were followed during their first year of life, including clinical examinations and questionnaires addressing respiratory symptoms and treatments. In study #4, using custom made

software, we calculated tidal breathing parameters from the flow signals of 33 ventilator treated EP born neonates during their first hours of life, and the data were compared between those who later developed moderate/severe versus no/mild BPD.

Results: We found in studies #1 and #2 that the EIP method was well tolerated by the infants and also feasible in a busy NICU setting. The repeatability was better for term-born than preterm-born infants, but comparable to what has been reported for "mask-based" infant spirometry. The experience of the personnel and the relationship to meals influenced the reproducibility. Tidal breathing parameters obtained by the EIP method corresponded well with those obtained by the "mask-based" system when applied simultaneously in infants at term-equivalent age. In study #3, we found that lung function measured by EIP was strikingly abnormal in EP compared to healthy term-born infants at term-equivalent age, characterized by obstructive pulmonary abnormalities, and by increased tidal volumes and minute ventilation. Abnormalities were most pronounced for the group of infants with BPD, but significantly present also in infants without BPD. The tidal breathing parameter TEF_{50}/PTEF predicted respiratory morbidity in the first year of life in infants born EP. In study #4, we found that flow data easily obtained from a ventilator during the first 48 hours of life could be used to compute breathing parameters that discriminated between neonates who went on to develop the severe forms of BPD and those who did not.

Conclusions and implications: EIP emerges as a feasible method of infant lung function testing. Its major strengths are the relative simplicity by which it can be put to use, and the avoidance of a facemask. This thesis has demonstrated that EIP can provide tidal breathing measurements that can aid the characterization of lung disease after EP birth, and that tidal breathing parameters obtained shortly after birth may identify neonates with predisposition for a difficult clinical respiratory course. If subsequent evaluations were to confirm the findings of this thesis, EIP and tidal breathing parameters have the potential of early recognition of neonates at risk of the severe forms of lung disease of prematurity and of later respiratory morbidity, which may pave the way for lifelong targeted surveillance and guidance of clinical management and intervention studies.

1.4 List of papers

I. Bentsen MH, Haaland ØA, Lønning LB, Gudmundsdottir HK, Markestad T, Halvorsen T. A new non-invasive method of infant spirometry demonstrates a level of repeatability that is comparable to traditional methods.
Acta Paediatrica 2015 Nov;104(11):1130-7.
doi: 10.1111/apa.13155. PMID: 26287280

II. Bentsen MH, Eriksen M, Olsen MS, Markestad T, Halvorsen T. Electromagnetic inductance plethysmography is well suited to measure tidal breathing in infants.
ERJ Open Research. 2016;2(4):00062-02016.
doi:10.1183/23120541.00062-2016. PMID: 28053968

III. Bentsen MH, Markestad T, Øymar K, Halvorsen T. Lung function at term in extremely preterm-born infants: a regional prospective cohort study
BMJ Open. 2017 Oct 25;7(10):e016868.
doi: 10.1136/bmjopen-2017-016868. PMID: 29074512

IV. Bentsen MH, Markestad T, Halvorsen T. Ventilator flow data predict bronchopulmonary dysplasia in extremely premature neonates.
ERJ Open Research. 2018 Mar 13;4(1).
doi: 10.1183/23120541.00099-2017. PMID:29546045

1.5. Abbreviations

AGA	Appropriate for gestational age
ATS	American Thoracic Society
AUC	Area under the curve
BPD	Bronchopulmonary dysplasia
BW	Birth weight
CI	Confidence Interval
COPD	Chronic obstructive pulmonary disease
CPAP	Continuous positive airways pressure
CR	Coefficient of repeatability
CV	Coefficient of variation
EIP	Electromagnetic inductance plethysmography
EP	Extremely premature
ERS	European Respiratory Society
FiO_2	Fraction of inspired oxygen
FRC	Functional residual capacity
FVg	Flow volume center of gravity
GA	Gestational age
HFNC	High flow nasal cannulae
ICC	Intraclass correlation coefficient
ISAAC	International Study of Asthma and Allergy in Childhood
IUGR	Intrauterine Growth Restriction
LoA	Limits of agreement
NICU	Neonatal Intensive Care Unit
NPV	Negative predictive value

pCO_2	Partial pressure of carbon dioxide
PEEP	Positive end expiratory pressure
PFT	Pulmonary function test
PIP	Peak inspiratory pressure
pO_2	Partial pressure of oxygen
PMA	Postmenstrual age
PPV	Positive predictive value
PTEF	Peak tidal expiratory flow
RDS	Respiratory distress syndrome
ROC	Receiver-operator characteristic
RR	Respiratory rate
SaO_2	Saturation of oxygen
SD	Standard deviation
SGA	Small for gestational age
TBM	Tidal Breathing Measurement
Te	Expiratory time
TEF_{50}/PTEF	Tidal flow at 50% expired volume as a percent of PTEF
TEF_{75}/PTEF	Tidal flow at 75% expired volume as a percent of PTEF
TFV-loop	Tidal Flow Volume loop
Ti	Inspiratory time
Tptef/Te	Time to peak tidal expiratory flow as a ratio of the expiratory time
USFM	Ultrasonic flow meter
VSP	VoluSense Pediatrics
Vt	Tidal volume
V'E	Minute ventilation

2. GENERAL INTRODUCTION

2.1 The human respiratory system – anatomy and function

The human respiratory system is divided into an upper and lower respiratory tract. The upper tract includes the nose, nasal cavities, sinuses, pharynx and larynx above the vocal folds. The lower tract includes the lower part of the larynx, the trachea, bronchi and lungs.

During breathing, the thoracic cavity expands, and air is sucked in through the nose and/or the mouth, via the pharynx and through the larynx, which is the narrowest part of the airways, and down through the trachea to the carina where the trachea branches into the right and left primary (main) bronchi. Each of these bronchi branches into secondary (lobar) bronchi that branch into tertiary (segmental) bronchi that branch into smaller airways called bronchioles that eventually connect with tiny specialized structures called alveoli where the air passage ends and gas exchange occurs. Altogether there are 23 generations of airway divisions in the human lungs, and at each division one airway branches into two or more smaller airways[1,2].

The human respiratory system can also be divided into a conducting zone and a respiratory zone, based on whether its role is to transport gases or to exchange them with the gases of the blood circulation (Fig. 1). The conducting zone includes the nose, pharynx, larynx, trachea, bronchi, and terminal bronchioles. It constitutes the anatomical dead space of the respiratory system, i.e. the volume of air in this zone does not participate in gas exchange. The conducting zone warms and moistens the air that is inhaled, and provides a major defense role by filtering the air through mucus and cilia. The conducting zone represents the 1$^{st.}$ through the 16$^{th.}$ division of the respiratory tract. The respiratory zone includes the respiratory bronchioles, alveolar ducts and alveoli. In a fully developed lung, the respiratory bronchioles and the alveolar ducts are responsible for about 10% of all gas exchange in the respiratory system, while the alveoli are responsible for the rest. The respiratory zone represents the 17$^{th.}$ through the 23$^{rd.}$ division of the respiratory tract (Fig. 1)[1-3].

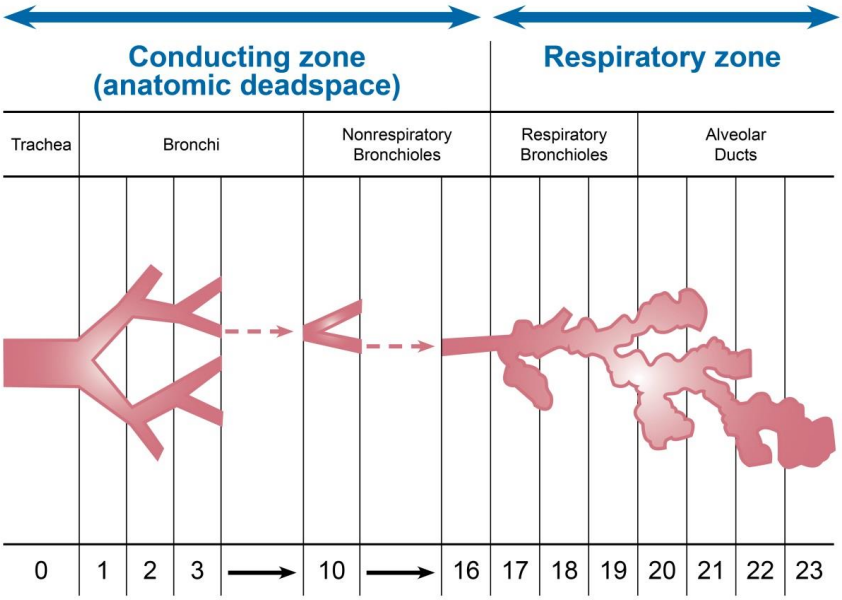
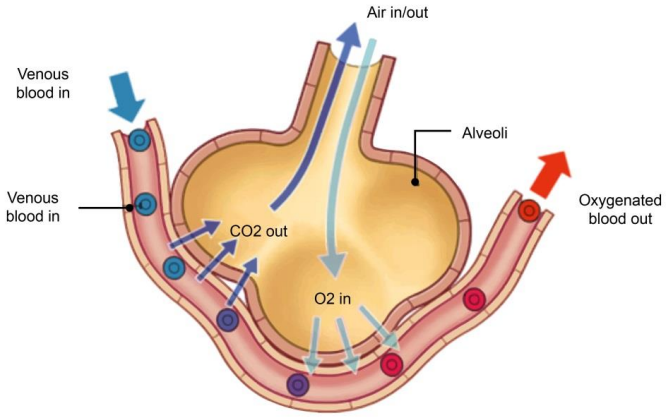


Figure 1. The conducting and respiratory units of the human lung. Modified from Koeppen & Stanton: Berne and Levy Physiology, 6th Edition

The two main functions of the respiratory system are ventilation and gas exchange. ***Ventilation*** is the movement of air into and out of the lungs. It has two phases: 1) inspiration, the process of moving air into the lungs, and 2) expiration, the process of moving air out of the lungs. Ventilation is achieved by the action of the respiratory muscles, i.e. the diaphragm, intercostal and accessory respiratory muscles, all acting on the thoracic cage. The respiratory muscles are mainly active during inspiration. Expiration is usually passive or silent, except at high ventilation rates. Ventilation is essential for respiration to occur.

Gas exchange is the process of providing oxygen to the systemic arterial blood and to remove carbon dioxide from the venous blood. The gas concentration gradients across the alveoli and the lung capillaries facilitate the exchange of oxygen and carbon dioxide through the process of diffusion (Fig. 2).

Figure 2. Gas exchange of the alveoli
Modified from slowlorisresource.weebly.com



The lungs are located in the thoracic cavity and separated from the stomach and intestines by the diaphragm, the main muscle of respiration, formed like a bell-shaped sheet at the base of the lungs. The lungs are protected from trauma by the rib cage, and are enclosed by the pleura, a two-layered serous membrane that folds in on itself. The inner (visceral) layer of the pleura covers the surface of the lungs, and the outer (parietal) pleura faces the inner surface of the thoracic cage. In this way, the lungs follow the movements of the thoracic cage. The folding of the pleural layers creates a cavity, or rather a potential space as the layers are in close proximity to each other, which contains pleural fluid. The pleural fluid decreases the amount of friction between the inner side of the thoracic cage and the lungs during breathing. The pressure inside the pleural cavity (also called intrathoracic pressure) is negative, which is an important characteristic, keeping the lungs open[4].

As already pointed out, the lung alveoli, shaped as tiny hollow cavities, represent the ends of the branching respiratory tree. Each alveolus is wrapped in a fine mesh of capillaries, and the alveolar and capillary walls collectively constitute the membrane over which gas exchange takes place (Fig. 2). The inside of the alveoli is covered by a

liquid film layer with water molecules that pull on each other, the overall effect being an inward directed pressure pulling also on the alveolar walls. This surface tension of the alveoli is the primary factor causing the elastic recoil pressure of the lungs, in addition to elastin in the elastic fibers of the interstitial connective tissue. The inward directed elastic recoil pressure of the lungs increases when the lungs are stretched by inhalation. However, two factors prevent the lungs from collapsing: surfactant and the intrapleural pressure.

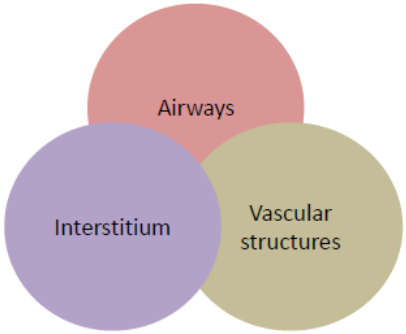
Surfactant is a surface-active lipoprotein complex produced by type II alveolar cells, one of the cell types of the alveolar epithelium. The proteins and lipids that constitute surfactant both have a hydrophilic region and a hydrophobic region. By attaching the hydrophilic region to the liquid film of the alveoli, the hydrophobic tails will face towards the air, thereby reducing the surface tension of the alveoli and thus reducing the size of the elastic recoil pressure of the lungs. Surfactant also stabilizes the surface area, resulting in a more regular rate of shrinking[5]. The intrapleural pressure is a consequence of a dynamic balance between the inward recoil pressure of the lungs and the outward recoil pressure set up by the chest wall[4]. This outward working chest wall pressure is a mechanical consequence of the structure of the thoracic cage.

During inspiration, the diaphragm contracts and moves downwards, and the ribs of the thoracic cage moves upwards. This increases the volume and the negative pressure inside the thoracic cage and of the intrapleural cavity, expanding the lungs with its bronchi, whereupon air is being sucked in through the bronchial tree and the upper respiratory tract. During expiration, the diaphragm and the intercostal muscles relaxes, and the inward directed recoil pressure of the lungs takes over as the driving force, causing the alveoli to deflate.

Functional Residual Capacity (FRC) is the volume of air present in the lungs at the end of passive expiration. At FRC, the opposing inward recoil pressure of the lungs and the outward recoil pressure of the chest wall are in equilibrium and there is no activity of the diaphragm or other respiratory muscles.

Anatomically and metaphorically, the lung can be seen as a distended spring, attached to the inside of the thoracic cage. The airways, paralleled by vasculature, constitute the framework for the spring, and the interstitium, consisting of the alveoli, supportive tissue and immune-active cells, ties the framework together. Thus, there is a functional interdependence within the lung architecture, with the parenchyma tethering the airways and serving as a framework for the gas-exchanging units (Fig. 3).

Figure 3. The interdependent structures of the respiratory system



Airways: the trachea (extrapulmonary), bronchi, bronchioles, and terminal bronchioles. **Interstitium:** the supportive tissue, alveoli, and immune-active cells. **Vascular structures :** the bronchial and pulmonary arteries and veins.

2.1.1 Some important concepts of normal human respiratory physiology

Lung compliance is a measure of the distensibility of the lung, i.e. its ability to stretch and expand by pressure changes. It is defined as change in volume divided by change in pressure[6]. The elastic recoil pressure of the lungs is inversely related to lung compliance.

Resistance is defined as driving pressure divided by flow[6]. Resistance of the respiratory system reflects the sum of the resistance of the airways, lung tissue and chest wall. Inspiratory airway resistance is mainly influenced by the diameter/size of the upper respiratory tract, while expiratory airway resistance is mainly influenced by the diameter/size of the lower respiratory tract.

Anatomic and physiologic dead space: Dead space is the volume of a breath that does not participate in gas exchange. Anatomic dead space is the volume of gas within the conducting zone, i.e. the nose, pharynx, larynx, trachea, the bronchi, bronchioles and terminal bronchioles (Fig. 1). Alveolar dead space is the volume of gas within un-perfused or relatively under-perfused alveoli, and thus gas not participating in gas exchange. It is usually negligible in healthy persons. Physiologic or total dead space is the sum of anatomic dead space and alveolar dead space[7].

Work of breathing is the energy used to inhale and exhale air. Inhalation is an active process requiring work. Some of this work consists of overcoming airway resistance to flow, which is modulated by the size of the laryngeal inlet and the tone of the smooth muscles in the conducting airways. However, most of the work is used to deform elastic tissues, which depend on the elastic recoil pressure of the alveoli and the property of the elastic fibers in the connective tissue of the lungs (lung compliance), as well as the lung volume level at which the tidal volume shifts occur[8]. The energy used during inspiration to stretch elastic fibers is stored as potential energy, which is recovered during the passive process of exhalation. Tidal exhalation does not require active muscle contractions, and the required energy to overcome airway resistance (mostly in the lower respiratory tract), is provided by the stored elastic energy.

2.1.2 Regulation of ventilation/control of breathing

Ventilation of the lungs is regulated by the overall target of maintaining blood gas homeostasis[9]. Sensors for the partial pressure of carbon dioxide (pCO_2) and pH of the arterial blood are located on the anterior and lateral surfaces of the medulla oblongata. Sensors of the partial pressure of oxygen (pO2) in the arterial blood are located in the carotid and aortic bodies positioned on the common carotid artery and the aortic arch, respectively. Information from these chemoreceptors or sensors is transferred along nerves to the four respiratory centers in the brain stem; two located in the medulla oblongata and two in the pons. From the respiratory centers, the respiratory muscles (in particular the diaphragm) are periodically activated to cause air to move in and out of the lungs. Under most conditions, the pCO_2 controls ventilation. Blood levels of

oxygen become important in hypoxia. Levels of carbon dioxide rise in the blood when the metabolic use of oxygen and the production of carbon dioxide increase, for example during exercise. The carbon dioxide in the blood is transported largely as bicarbonate ($HCO3^-$) ions, by conversion first to carbonic acid (H_2CO_3), and then to hydrogen ions ($H^{+)}$ and $HCO3^-$. Build-up of carbon dioxide therefore causes an equivalent build-up of the dissociated hydrogen ions, which, by definition, decreases the pH of the blood. The pH sensors in the brain stem immediately sense this fall in pH, causing the respiratory center to increase the rate and depth of breathing. The consequence is that the carbon dioxide does not change when going from rest to exercise.

Mechanoreceptors located in the airways and lungs also play a role in the regulation of ventilation, and are responsible for a variety of reflex responses, including the Hering-Breuer reflex that terminates inspiration to avoid over-inflation of the lungs.

Ventilation is normally unconscious and automatic, but can be affected by free will or emotional state (via input from the limbic system). Voluntary or conscious control of respiration is provided via the cerebral cortex; however chemoreceptor reflexes can override it.

2.1.3 The distinctive features of infant respiratory physiology

Newborns and infants are less able to cope with respiratory stress compared to adults. They are therefore more prone to severe manifestations of respiratory diseases. There are a number of reasons for this[10]:

- Their oxygen consumption per body weight is 2-3 times higher than in adults.
- Infants have fewer alveoli and by that a smaller surface area available for gas exchange.
- The central control of breathing is immature. After birth, the peripheral chemoreceptors must adapt from intrauterine to extrauterine arterial oxygen tensions, and this postnatal adaptation requires time. Responses to hypercapnia and hypoxia are therefore decreased, and this explains the irregular breathing pattern of

newborns, i.e. a high breath-to-breath variability, periodic breathing and a high prevalence of apneas. This is even more pronounced in infants born prematurely, as much of the maturation of control of breathing occurs during the last weeks of gestation.

- Newborns and infants are obligate nose breathers due to the configuration of the upper airways (i.e. a relatively large epiglottis and tongue), and the airway resistance of the nasal passages is high.
- The resistance of the lower respiratory tract is higher in infants due to the small airway size. Minor absolute changes of the airway radius create large relative increases in resistance to airflow as the resistance increases by the fourth power of radius (Poiseuille's law).
- The elastic tissue in the septa of the alveoli that surround the conducting airways provide an elastic recoil that enables the airways to remain open. As newborns and infants have relatively few alveoli, this airway support is small and the recoil pressure of the lung correspondingly low, and the airways are thus vulnerable to collapse.
- The larynx, trachea, bronchi and chest wall are all soft and have higher compliance in infants than in older children or adults. This makes the airways of infants more susceptible to compressive forces and airway collapse. The outward recoil pressure of the chest wall is low, and since the balance of this pressure and the inward recoil pressure of the lungs determines the static resting volume of the lungs (FRC), infants reach this equilibrium at a relatively lower lung volume than older children and adults. To increase FRC and prevent airway closure, infants have to adopt a breathing strategy that dynamically elevates their end-expiratory lung volume. This is achieved by several mechanisms: 1) high respiratory rate with insufficient time to exhale to the elastic equilibrium volume, 2) laryngeal adduction during expiration, and 3) maintenance of some diaphragmatic muscle tone during expiration. These complex physiological mechanisms might vary slightly over time, causing variations also of the end-expiratory lung volume.

- The diaphragm acts less efficiently due to the highly compliant chest wall, lower number of fatigue-resistant type I muscle fibers and a more horizontal insertion at the rib cage.

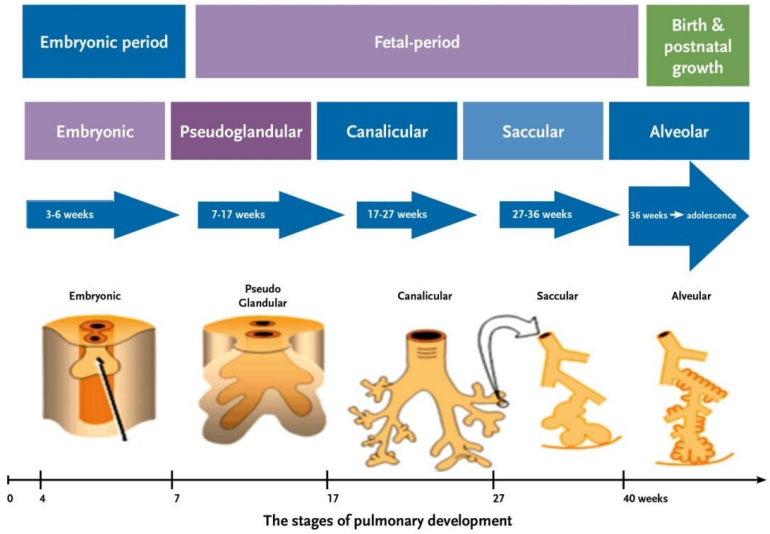
2.2 Development of the respiratory system

2.2.1 Fetal lung development

Lung development in humans starts as a primitive lung bud in early embryonic life and continues into postnatal life up to young adulthood. Each step of the development is a thoroughly regulated process governed by genetic, hormonal and environmental factors. The structural and vascular development are closely related and progress simultaneously.

Human lung development is typically divided into five stages: embryonic, pseudoglandular, canalicular, saccular and alveolar stages (Fig. 4). The stages overlap, and the gestational age when transition occurs from one step to the next is not definite[2,3].

Figure 4. The different stages of lung development
Modified from Centre de Référencé de Maladies Respiratoires Rares 2015



The ***embryonic stage*** begins at around 3-4 weeks of embryonic life. During this phase, what will become the larynx and the trachea form as an outgrowing bud from the ventral wall of the primitive foregut, the laryngotracheal groove. The bud expands and divides into the right and left main bronchi and subsequently into lobar and segmental bronchi. A vessel plexus develops as a bud from the 6^{th} aortic arch and grow caudally by vasculogenesis with the lung buds.

The ***pseudoglandular stage*** lasts from approximately gestational week 7 to 17. This phase is characterized by progressive branching into terminal bronchioles, i.e. completion of the conducting airways. Connective tissue, cartilage, smooth muscle and submucosal glands begin to form around the larger airways. The airway epithelium starts to differentiate, and pulmonary arteries and veins are formed.

The ***canalicular stage*** takes place between week 17 and week 27 of gestation. During this stage the acinar structures comprising the respiratory bronchioles, alveolar ducts and primitive alveoli are formed and vascularized. There is growth of the capillary network and a decrease of interstitial tissue. Cuboidal cells lining the airways differentiate to type II pneumocytes, and type I pneumocytes starts differentiating from type II cells. Type I pneumocytes are very thin, membrane-like cells that provide an air-blood interface that makes gas exchange possible. Lamellar bodies associated with surfactant storage starts to appear in type II alveolar cells. Surfactant protein is detectable by week 24 of gestation, but surfactant deficiency leading to respiratory distress syndrome is inevitable if premature birth occurs at this stage.

The ***saccular stage*** lasts from gestational week 28 to 36. During this stage the alveolar ducts dilate and form "saccules" and thinning of the airway walls, generating increased surface area for gas exchange. There is also a further differentiation of type II pneumocytes to type I pneumocytes.

The ***alveolar stage*** begins at approximately week 36 of gestation and continues postnatally up to the age of at least 2 years, probably longer. During this stage the saccules are multiplied and divided into alveoli by double capillary walled septa.

2.2.2 Postnatal lung growth

At birth only about 15-30% of alveoli have formed, approximately 50-150 million in absolute numbers. In the postnatal period, alveolar septation and multiplication continues at least up to the age of 2 – 3 years, probably longer[11]. At that time the double capillary network of the alveoli gradually merges to a single capillary layer, and new alveolar septa cannot longer be formed. However, the alveolar size and surface increases until after adolescence, most likely until about 18 – 22 years of age. The end result is a lung with a tremendously large surface area for gas exchange that is approximately 50-100 m^2 and with a thickness 1/50 of paper[12]. For all ages, boys have a larger number of alveoli than girls[13].

2.3 The lungs at birth

During fetal life, the placenta provides fetal gas exchange. The lungs are functionally inactive and filled with fluid excreted by epithelial cells lining the airways, and perfused by only about 10% of the cardiac output. For the lungs to obtain their function as a gas exchanging organ, a number of changes must take place. The first breath of air marks the beginning of individual life and induces at least three important physiological changes; 1) formation of a stable alveolar gas volume, i.e. the functional residual capacity (FRC), 2) removal of liquid from the fetal lungs, and 3) increase in pulmonary blood flow[5].

Formation of FRC. As the term newborn exits the birth canal, the activity of the nervous system and the respiratory muscles are triggered. This causes the newborn to take his or her first breath to inflate the lungs with air and go on breathing spontaneously. In a normal term-born infant, the alveoli are lined by surfactant. As explained previously, surfactant prevents the alveoli from collapsing under the inward pressure generated by their surfaces, and instead allows them to expand. This surface film of surfactant in the alveoli is formed when air first enters the lung. During the first breath, an opening pressure of approximately 18 cm H_2O is required before any air can enter the lungs. The lungs then inflate readily to their full volume with only a minor change of pressure[5]. Because of surfactant, the second and subsequent inflations of the

lungs are different, as no opening pressure is now required. The lungs can inflate to 80% of their volume at a pressure that was insufficient to open them the first time. Thus, in the lungs of a normal term-born infant, a relatively stable alveolar volume is formed after the first inflation at a level corresponding to the FRC, which, as explained earlier, is the lung volume that balances the inward recoil pressure of the lung and the outward recoil pressure of the thoracic cage. Each succeeding tidal breath exchanges about 30% of this FRC for fresh air[14], while oxygen and carbon dioxide exchange continuously between the alveolar gas and the capillary blood flow passing by. Surfactant also tends to stabilize the distribution of air in the lungs. Because of the relationship between pressure and radius in the La Place equation, smaller alveoli would develop a higher inward pressure than larger alveoli, and thus small alveoli would tend to empty into large alveoli. However, as an alveolus decreases in size, the surfactant film will become more compressed and its surface tension fall. This tends to equalize the pressures in alveoli of different sizes and consequently also equalize their size.

Removal of liquid. The factors involved in removal of lung liquid at birth are still unclear. Some liquid is squeezed out through the mouth during the second stage of labor, or drained by gravity. The remaining fluid must be cleared when breathing starts. The liquid is usually assumed to be absorbed directly into the blood. However no change that would facilitate such a transfer is known to occur, like a fall in pulmonary capillary pressure. On the contrary, the pulmonary vasodilation at the onset of breathing causes a rise in capillary pressure. Studies have shown that a large increase in lymph flow from the lungs accompanies the onset of ventilation in mature lambs. It seems likely that some of the lung liquid is cleared by this route. The mechanism pictured is that air entering the alveoli displaces liquid into the inter-alveolar spaces of the lung where lymphatic capillaries are plentiful.

Increase in pulmonary blood flow. Gas exchange in the lungs also depends on the pulmonary blood flow. In the adult, pulmonary blood flow equals right ventricular output. In contrast, during fetal life, most of the inflow from the great veins bypasses

the lungs. About half the flow from the inferior vena cava passes directly through the foramen ovale to the left atrium, the left ventricle, and the aorta. The remaining inferior and superior vena caval flow enter the right atrium, right ventricle, and pulmonary artery. From the pulmonary artery most of the flow goes to the aorta through the ductus arteriosus, and only a small fraction passes through the lungs. This fetal blood flow distribution depends on the vascular resistance in the lungs being higher than the systemic vascular resistance. At the start of ventilation there is a decrease in pulmonary vascular resistance that causes a redistribution of cardiac output and an increase in blood flow to the lungs. This reduction in vascular resistance is in large part due to an increase in pO_2 and a decrease in pCO_2 which has relaxing effects on the muscular tone of the pulmonary vessels.

2.4 Preterm birth

Preterm birth is defined as birth that occurs before 37 completed weeks, or 259 days, of pregnancy[15]. Different degrees of prematurity are defined by gestational age (GA), calculated from the first day of the mother's last menstrual period[16] (Table 1).

Table 1: Classification of prematurity based upon gestational age

	Gestational age, weeks
Term	37 – 42
Preterm	< 37
Moderate-to-late preterm	32 – 36
Very preterm (VP)	28 – 31
Extremely preterm (EP)	< 28

Birth weights by percentile for the appropriate GA have been provided[17,18], and newborns may also be classified by weight according to GA. No consensus on definitions has been established, but the most commonly used by clinicians are[19]:

a) Small for GA (SGA): BW < 10 percentile according to GA
b) Appropriate for GA (AGA): BW 10 – 90 percentile according to GA
c) Large for GA (LGA): BW > 90 percentile according to GA

Intra uterine growth restriction (IUGR) complicates as many as 10-25% of preterm births; the proportion increasing with decreasing GA[20,21]. Premature neonates with IUGR are at increased risk for death and complications related to prematurity compared with (AGA) control infants[22,23]. It is important to realize that IUGR may be present also in infants born AGA but who have failed to reach their full growth potential, and that infants born SGA who are 'meant to be small' might not be IUGR.

2.5 Respiratory consequences of preterm birth

The lungs as breathing organs are unnecessary for intrauterine existence. However, they must be developed to such an extent that they are immediately capable of gas exchange after birth. Preterm birth in the second trimester poses a great challenge. Gas exchange will have to take place in developmentally small and immature lungs that are at least partly unable to produce surfactant. In addition, further lung development has to take place in a NICU environment that is totally different from the wet, protective and relatively hypoxic state inside the uterus. Although advances in perinatal medicine during the last decades have improved survival of preterm neonates, premature birth in the second trimester is probably still the most important factor for adverse lung growth, due to disturbance of the programmed and fine-tuned sequences of normal development as well as the trauma inflicted by life-saving treatment measures, such as oxygen and positive pressure ventilation. This results in immediate as well as long-term consequences for respiratory health.

2.5.1 Respiratory Distress Syndrome

Respiratory failure known as respiratory distress syndrome (RDS) is caused by lack of surfactant combined with both structural and functional immaturity of gas-exchanging units[24]. RDS was previously labelled hyaline membrane disease due to the characteristic histopathology with hyaline membranes, which are mainly a blood transudate composed of cellular debris, fibrin, red blood cells, neutrophils and macrophages lining the collapsed alveoli. RDS begins relatively shortly after birth and

is characterized by fast breathing, chest wall retractions, expiratory grunting, nasal flaring, low oxygen saturation and respiratory acidosis. In the immature lungs without surfactant and high alveolar surface tensions, ventilation requires large pressures to open the primitive alveoli with each new breath. This leads to a low FRC, and thus promotes atelectasis that further decreases lung compliance. This in turn contributes to vicious circles with ventilation-perfusion mismatch, under-ventilation and a rise in pulmonary vascular resistance, increasing right-to-left shunting, i.e., driving the circulation towards a fetal flow distribution[5].

Numerous studies have explored the importance of surfactant replacement in preterm neonates to prevent and treat RDS. This can be obtained by either antenatal corticosteroid therapy, as it promotes lung maturation and increases endogenous surfactant production[25], and/or by exogenous surfactant administration after birth[26,27]. Whether exogenous surfactant should be administered as an early prophylactic or delayed selective treatment remains a matter of debate[28].

2.5.2 Ventilatory- and respiratory support

Clinical interventions and treatments are essential for EP-born neonates (Table 1) to survive, but at the same time they are potentially harmful, in particular assisted ventilation and oxygen supplementation.

Lung injury caused by assisted ventilation is primarily due to stretching and over-distension of fragile airways in poorly compliant lungs[29,30]. Although there are several different mechanical ventilation modalities, including pressure control ventilation, volume control, and high-frequency oscillation, the optimal mode of ventilation to reduce mortality and later lung disease remains unclear in patients with RDS.

The newest guidelines from both the American Academy of Pediatrics (AAP), the American Heart Association (AHA), the International Liaison Committee on Resuscitation (ILCOR), and the European Consensus Guidelines, state that nasal continuous positive airway pressure (CPAP) should be the initial preferred intervention in preterm infants at risk of - or with established - RDS without

respiratory failure [31-34]. Patients with neonatal RDS who have respiratory failure despite CPAP treatment (defined as pH below 7.25 or who require oxygen supplementation ≥ 40%) should be intubated and administrated surfactant. Several systematic reviews have shown that CPAP is more effective with lower mortality and reduced risk of bronchopulmonary dysplasia compared with intubation with or without surfactant administration[35-37]. This is supported also by follow-up studies that have shown less respiratory morbidity and no difference in death or neurodevelopmental outcome [38,39]. Early administration of caffeine therapy to increase respiratory drive may enhance the use of CPAP, as apnea of prematurity occurs in essentially all infants with a gestational age less than 28 weeks. Although the use of CPAP has increased over the last decades, studies have shown no improvement in pulmonary function at mid-childhood[40]. However, interpretation of these results must take into consideration other temporal factors including the marked decrease in the use of postnatal steroids (their use reduce the risk of lung injury but increases the risk of brain injury) and the reduced mortality rate after the millennium shift, which may have increased the number of survivors at risk for lung injury. Heated, humidified high-flow nasal cannulas (HFNC) are increasingly being used to provide positive end-expiratory pressure (PEEP) instead of traditional CPAP devices. However, research results so far report a higher failure rate when HFNC has been used as the primary therapy for neonatal RDS compared with CPAP [41]. Nasal intermittent positive pressure ventilation (NIPPV) augments CPAP by delivering ventilator breaths via nasal prongs or a nasal mask. That is, it requires the use of a ventilator, but avoids the trauma of placing an endotracheal tube. Limited evidence of moderate quality implies that early use of NIPPV is superior to CPAP as initial non-invasive respiratory support treatment of preterm infants with RDS[42]. However, further data are needed to confirm this.

Immature lungs are insufficient gas exchanging organs, and most EP-born neonates are therefore in need of supplemental oxygen. In utero, the arterial oxygen tension in the healthy fetus is about 4.7 kPa, and the arterial oxygen saturation (SaO_2) is 80-90%[43]. Room air, which has an oxygen tension of 11-13 kPa, is therefore relatively hyperoxic compared to the arterial oxygen tension in utero. High concentrations of inspired oxygen can damage the lungs, although the exact level or duration of exposure that is

harmful or unsafe is unknown. Overproduction of reactive oxygen species causes cellular damage by directly damaging DNA and proteins, and by activating lipid peroxidation and inflammation. Because of a poorly developed antioxidant system, immature neonates are particularly vulnerable to such damage[44]. There are also studies that suggest genetic variation in antioxidant defenses[45]. The risk of lung injury in EP-born infants increases with increasing accumulation of supplemental oxygen during the first two weeks after birth[46]. A large number of studies indicate that even low levels of supplemental oxygen can be harmful, and current practice is therefore to use the lowest possible fraction of inspired oxygen to maintain adequate SaO_2, which by most are considered to be between 90 and 95 percent[47-49]. Data comparing high and low target oxygen saturation levels demonstrate that values above 95 percent and below 89 percent are associated with poorer outcome in very preterm infants[49].

2.5.3 Bronchopulmonary dysplasia

Bronchopulmonary dysplasia (BPD), also referred to as chronic lung disease of prematurity (CLD), is the most common complication of prematurity, and leads to short and long-term morbidity and mortality. It is associated with brain damage, poor neurodevelopmental outcome and chronic respiratory conditions such as an asthma-like condition and pulmonary hypertension[50-53]. The disorder was first described by Northway et al. in 1967 as a condition resulting from effects of oxygen and mechanical ventilation in premature infants with severe RDS[54]. It was initially defined based on both radiographic and clinical criteria, but since then, the definition has continuously evolved due to changes in the population at risk (more survivors at earlier gestational ages) and improved neonatal management (antenatal steroids, surfactant and less aggressive mechanical ventilation) that have altered its pathology and clinical course. What is now reckoned as ‘old’ BPD was characterized by major pathological changes related to oxygen toxicity and barotrauma caused by simple ventilators applying high pressures to the lungs and airways of moderately preterm infants[54]. Post-mortem histological findings were diffuse airway damage with

inflammation, extensive parenchymal fibrosis, smooth muscle hypertrophy and atelectasis.

Treatment with surfactant from the early 1990s and more gentle and modern ventilation therapy altered the patterns of lung injury in preterm born infants, leading instead to 'new BPD' - what we nowadays simply refer to as BPD. New BPD is most prominent in the smallest and most immature infants who do not necessarily have RDS at birth. Instead, new BPD is mainly a developmental disorder. Exposure to even minimal traumas at a very early and vulnerable developmental stage leads to dysregulation of normal lung development [1,51,55,56]. Decreased alveolar septation leads to fewer and larger alveoli (alveolar hypoplasia) and reduced surface area available for gas exchange. Pulmonary microvasculature is also less and abnormally developed, with abnormal distribution and thickening of the muscle layer of the pulmonary arterioles and capillaries, resulting in increased pulmonary resistance. Elastic tissue formation is increased, and there is thickening of the pulmonary interstitium due to interstitial cell proliferation. In comparison with "old" BPD, there is less airway injury and inflammation, and fibrosis is a less prominent feature[57-60]. However, in some surfactant-treated infants who develop severe BPD, fibrosis, bronchial smooth muscle hypertrophy, and interstitial edema ('old' BPD) may be superimposed on the characteristic reduced numbers of alveoli and capillaries ('new'BPD). As regards histopathological findings, however, it must be taken into consideration that most biopsies come from animals or from subjects who subsequently died[29,58,59].

The currently applied definition of BPD was adopted on a consensus conference of the United States National Institute of Child Health and Human Development (NICHD) in 2001. According to the NICHD criteria, a diagnosis of BPD should be given to premature infants who remain dependent on oxygen supplementation for 28 postnatal days. The severity of BPD is further defined by the need for oxygen supplementation at specific time-points. Infants born at GA < 32 weeks are assessed at 36 weeks GA or at discharge, whatever comes first. A diagnosis of mild BPD is assigned if the infant is breathing room air at that stage, moderate BPD is assigned if the infant is in need of an $FiO_2 < 0.30$, and severe BPD is assigned to infants in need of an $FiO_2 \geq 0.30$, or if

positive pressure ventilation is required[50]. For infants born ≥ 32 weeks, the assessment of severity is made at 56 postnatal days or at discharge, whatever comes first. The NICHD criteria also proposed to standardize the use of supplemental oxygen by using a physiologic test to confirm the need for oxygen supplementation, although a specific test was not defined. Studies have shown that the NICHD criteria more accurately predict later pulmonary and neurodevelopmental outcomes in preterm infants than previous definitions[61]. However, it is increasingly recognized that also the current definition of BPD is a relatively poor measure of neonatal lung injury and predictor of long term respiratory outcomes[62-67]. This disparity might not be surprising, considering that BPD primarily reflects the ability of the immature lungs to perform gas exchange whereas most follow-up studies focus on bronchial disorders and flow characteristics.

Pathophysiology of BPD. The pathophysiology of BPD is incompletely understood. The etiology is multifactorial and is most likely due to the fact that immature lungs in a vulnerable developmental phase with surfactant deficiency and immature defense mechanisms are forced to develop in a harmful environment, cut off from important hormones and growth factors that promote normal lung growth and mediate repair. Antenatal and/or postnatal factors such as infections and IUGR, malnutrition, life-saving neonatal interventions such as oxygen supplementation and assisted ventilation, and the fact that gas exchange now has to take place in developmentally fetal lungs, contribute to the onset and perpetuation of inflammatory responses[50,68-70]. Studies of bronchoalveolar fluid from preterm-born neonates developing BPD show that there is an influx of neutrophils into the lung followed by macrophage recruitment[71]. The characteristic cytokine profile includes elevated levels of interleukin (IL-) 8, IL-1β, IL-6, tumor necrosis factor (TNF)-α, monocyte chemo-attractant proteins, MCP-1, MCP-2 and MCP-3, as well as macrophage inflammatory proteins, MIP-1a and MIP-1b and decreased expression of IL-10, implying a disturbed balance of pro-and anti-inflammatory factors[68]. Increased amounts of transforming growth factor (TGF)-β1 and dysregulation of transcription factors such as nuclear factor (NF)-kB[68] suggest recruitment and trans-endothelial migration of inflammatory cells that is associated with release of enzymes with tissue-damaging effects (proteases), cellular apoptosis, and dysregulation of central signaling pathways. Inflammation is also associated with

down-regulation of growth factors that induce alveolarization and angiogenesis; PDGF (platelet derived growth factor), FGF (fibroblast growth factor family), HIF 1α (hypoxia-inducible factor 1α) and VEGF (vascular endothelial growth factor). Our understanding of molecular pathways involved in BPD development and their critical interplay are insufficiently understood, and current knowledge is largely based on animal studies. There is an urgent need to identify the underlying disease signaling pathways in *human* lung cells in order to improve current, insufficient therapeutic strategies and to tailor personalized medicine on the basis of innate predisposition to lung disease.

Incidence of BPD. The incidence of BPD increases with decreasing GA and BW. Infants with BW <1250 g and GA < 30 weeks account for 97 percent of BPD cases[72]. The rate of BPD varies among institutions, which may reflect neonatal risk factors, care practices (e.g. target levels for acceptable oxygen saturation), and differences in the clinical definitions of BPD (e.g. based upon physiologic testing or not). Of infants born in the National Institute of Child Health and Human Development Neonatal Research (NICHD) network in the USA in 2003 – 2007 (GA of 22 to 28 weeks and birthweights of 401 to 1500 g), the overall incidence of BPD (mild, moderate or severe) was 68% [73]. The incidence of BPD increased with the degree of prematurity, and for infants born after 23 gestational weeks the incidence was 100%, while it was 61% for infants born after 27 weeks. In a nationwide study in Norway of EP-born infants born in 1999 and 2000, the incidence of moderate and/or severe BPD was 67% at GA 23-25 weeks and 37% at GA 26-30 weeks[74], and higher in males[75].

It is unclear whether or not the incidence of BPD is changing. In the above study from the NICHD neonatal network, the incidence of BPD did not change over the five years study period[73]. An unchanged BPD incidence was also found in the EPICure cohorts (UK) born in 1995 and 2006[76], however another study reported an increase in moderate/severe BPD from 48% to 58% from 2001 to 2006 for infants born at GA 23-29 weeks. The increase could not be explained by survival differences, but decreased use of surfactant and more non-invasive ventilation strategies may have contributed[77].

Prediction of BPD. As discussed above, the risk of BPD is linked to low GA, low birth weight and male gender [51,78,79]. Beyond that, at the time of birth it can be difficult to predict which EP-born neonates will go on to develop significant BPD. The role of antenatal events and/or exposures has been increasingly focused, and neonates are probably born with variable innate susceptibilities or predispositions. However, strategies to identify those at risk have so far not been very successful. Studies examining bronchoalveolar lavage/tracheal aspirates and blood collected shortly after birth from EP-born patients, have revealed differences in cytokine- and growth factor patterns between patients who develop moderate-severe BPD and those who develop no or only mild BPD[68,80]. These studies support assumptions of an innate predisposition for the disease, and genetic factors probably play a significant role in the development of BPD[81]. Twin studies suggest that heritability of BPD is > 50%[82,83]. However, different strategies to identify heritable factors, including genome-wide association studies (GWAS)[84] and identification of frequency differences in single-nucleotide polymorphisms (SNPs) in candidate genes[85] have not identified loci or pathways accounting for this heritability. Most likely, differences in *gene expression* in the lungs explain the differences. So far, however, no studies have been able to prove such a hypothesis because gene expression / epigenetic studies are difficult to conduct since they require lung biopsies. Gene expression of for example white blood cells cannot be assumed to completely reflect gene expression in the lungs[86], and there is no data on relationships between gene expression in circulating cells and lung tissue.

Lack of reproducible, genetic, biochemical and physiological biomarkers limits the ability to identify an impending BPD for early intervention and reliably predict the long-term outcomes[67]. There is therefore an urgent need to unravel what predicts and causes progression of lung disease in EP-born neonates in order to provide targeted management for those at particular risk and to facilitate customized prevention and early therapeutic measures.

Clinical- and radiographic findings in infants diagnosed with BPD. Most infants with BPD are tachypneic or have other clinical findings including retractions, rales, and wheezes. The chest radiographic findings in infants with BPD vary from clear

lung fields to a diffuse haziness, reflecting atelectasis, inflammation, and pulmonary congestions/edema. In severe cases there will be cystic changes.

2.5.4 Long-term respiratory consequences of preterm birth

Most infants with BPD have a mild or moderate disease, and although slow to heal, their lungs in later childhood approach normal function[64,87]. A sub-population has chronic lung problems, such as impairment in lung function, premature lung aging with early onset emphysema[88] and pulmonary hypertension[89]. Besides this, severe BPD is an independent risk factor of adverse neurodevelopmental and psychosocial outcome, adding to high socioeconomic burden[90].

Childhood: In infancy and early childhood, recurrent wheezing is common. During their first year of life, up to 50% of EP-born infants are readmitted to hospital due to respiratory problems and/or infections[66,91-93]. Those with moderate and severe BPD are those most affected[94]. Rates of wheezing illnesses and hospital readmissions due to respiratory symptoms decline after the first year of life[92,95]. Still, EP-born children have more coughing, wheezing and asthma-like symptoms during school age. Pulmonary function tests show lower forced expiratory flows and volumes and increased residual volumes, illustrating their airway obstruction and pulmonary hyperinflation, especially in EP-born children with moderate or severe BPD[64,96].

Adolescence and early adulthood: Compared to term-born adolescents and young adults, forced expiratory flows and volumes remain lower and residual volumes higher in those who are born EP[53,97,98]. EP-born adolescents and young adults more often report asthma-like symptoms, wheezing and coughing, and they have increased risk of hospitalization that persist into early adulthood, although the data are not consistant[97].

Adulthood: Few studies have examined long-term pulmonary outcomes beyond their twenties for people born EP in the 'Surfactant era'. Valuable information from studies on cohorts born in the 1980's and later will presumably be available in the years to come. Development of chronic obstructive pulmonary disease (COPD) is a feared scenario, at least in subgroups of adults born EP, when taken into account a normal age-related decline adding to an already significant airway obstruction[53,99].

2.6 Infant pulmonary function tests

Pulmonary function tests (PFTs) is a common term for a battery of studies or procedures performed using standardized equipment to measure lung function. PFTs are an important supplement to patient history, physical examinations, various image examinations and arterial blood gas analyses. If investigations beyond these are necessary, invasive testing such as bronchoscopy and open-lung biopsy are used.

Objective assessment of lung function is important for several reasons. It can increase basic knowledge about lung growth, development and function, help diagnose lung impairment or physiological processes, quantify severity of lung disease and predict prognosis. It can also be helpful to evaluate therapy.

Children aged about 5-6 years or older are usually able to perform PFTs via an active exhalation. In younger children, however, it has been a challenge to assess lung function. There are several reasons for this. Neonates, infants and toddlers are unable to cooperate and therefore difficult to study. In addition, most methods are time-consuming and disturb the infants, which means that they can only be used for brief periods and are not really suitable for clinical settings other than in dedicated specialist laboratories. Several of the tests are intrusive, in the sense that they involve the use of a face mask, which may alter the pattern of breathing, and the added dead space will influence the parameters measured[100-104]. The tests usually require neonates and infants to be asleep; therefore sedation is often required. Besides, lung mechanics in neonates and infants may change rapidly, and disease or illness state often makes testing impossible.

In addition, information attained from PFTs of neonates and infants may be limited by lack of appropriate reference data that are necessary to separate the effects of lung disease from those of normal physiological variabilities, growth and development.

PFTs can roughly be divided into those that evaluate different aspects of lung ventilation, i.e. lung volumes and lung mechanics, and those that evaluate gas exchange. Commercially available PFTs in neonates and infants are occlusion

techniques, tidal forced expirations, whole-body plethysmography, inert gas washout tests and tidal breathing measurements.

2.6.1 Whole-body plethysmography

Whole-body plethysmography is the most accurate way to measure exact lung volumes[105]. However, the only static lung volume that can be readily assessed by this method in non-co-operative neonates and infants is the FRC, i.e. the volume of gas remaining in the lungs after tidal expiration (as explained previously). Due to the relative complexity and large size of the equipment it is practically infeasible in a NICU bedside-setting. The technique involves the use of a facemask and a pneumotach that will affect the breathing pattern of the infants, and consequently the accuracies of the results. In addition, sedation is most often required.

2.6.2 Inert gas washout tests (single- or multiple-breath washout techniques)

Washout recording systems determine inspired and expired inert gas volumes, by continuously measuring inert gas concentrations synchronized with respiratory flow. Single- or multiple-breath washout tests (SBW and MBW, respectively) can measure the FRC of the lungs, as well as the gas-mixing efficiency, described as the Lung Clearance Index (LCI)[106]. LCI is defined as the cumulative expired volume at the point where end-tidal inert gas concentration falls below 1/40th of the original concentration, divided by FRC[107]. When lung tissue and airways are normal, inhaled gas is distributed evenly throughout the lung and the mixing and turnover of alveolar gas is relatively rapid. When airway obstruction is present, gas distribution tends to become more uneven and the mixing and turnover takes longer. The LCI is a way to measure these ventilation inhomogeneities. It is basically a description of how much ventilation is required to completely clear the FRC. The method is relatively time-consuming, partly because both the wash-in and the wash-out periods require at least 5 minutes each, but also because the LCI test should be performed several times in order to ensure repeatable results. In addition, testing neonates and infants with this method requires a facemask.

2.6.3 Occlusion techniques

The single and multiple occlusion techniques are the two most common methods for measuring passive respiratory mechanics. For these techniques, the Hering-Breuer reflex (i.e. vagus initiated inhibition of the central inspiratory drive and thus inhibition of inspiration and initiation of expiration) must be triggered to obtain relaxation of the respiratory system. The equipment needed must measure flow, volume and airway opening pressure accurately, and involves the use of a facemask. Usually sedation is required. Indices of respiratory mechanics that are measured include compliance, resistance and the expiratory time constant of the respiratory system, which is the product of the respiratory system compliance and resistance[108].

2.6.4 Tidal forced expirations

The tidal volume rapid thoraco-abdominal compression technique (RTC) can be used to assess partial forced expiratory flow-volume curves and measure peak expiratory flow (PEF) and the maximal flow at functional residual capacity ($V'_{max, FRC}$). The equipment needed include a facemask and a squeeze jacket, and sedation is required[109].

2.6.5 Tidal breathing measurements (TBMs)

Tidal breathing refers to inhalation and exhalation during restful breathing. As regulation of breathing is controlled by complex physiological functions, TBMs probably represent information about both airway mechanics as well as control of breathing.

Tidal breathing lung function measurements were first reported by Bouhuys in 1957, who observed that the shape of the tidal breathing loops differed between subjects with and without airway obstruction[110]. Greater interest for tidal breathing measurements did not occur until the 1990s, when the necessary equipment became commercially available. Early studies demonstrated that TBMs could be performed in both awake and sleeping neonates and young children[111,112]. Measurements were performed

primarily for research purposes, and this raised the issue of standardization of measurement procedures. A standardization document discussing TBMs was published in 2000 constituting one of a series of six documents produced by the European Respiratory Society/American Thoracic Society Task Force on standards for infant respiratory function testing[113]. Details of equipment, measurement conditions and data acquisition can be found in this series of papers.

***Measuring devices*.** Methods used to measure tidal breathing can be divided into two groups:

1. Those that respond to *flow at the airway opening (mouth/nose)*
2. Those that sense *chest wall movement*

When measuring flow as a function of time, the flow signals are converted into volume signals by mathematical integration. When measuring volume as a function of time, the volume signals are converted into flow signals by numerical differentiation.

Flow measurements at the airway opening can be performed either by a pneumotachograph or an ultrasonic flowmeter attached to a facemask, and the method is considered to be the gold standard method of TBMs. The facemask must be well fit and placed firmly over the infant's mouth and nose, ensuring a good seal. The dead-space as well as the resistance of the equipment must be kept to a minimum[113]. The infants usually have to sleep to accept the mask, and sedation is sometimes needed. Due to the dead-space in the system (facemask and flow head), this method is usually not approved for children < 2 kg.

Respiratory inductance plethysmography (RIP) is a rather simple technique that has been available for decades. It consists of two sinusoidally arranged wire coils enclosed in soft elastic bands. The transducer bands are placed around the thorax and the abdomen and connected to an oscillator and a computer. A low-voltage oscillatory current passes through the elastic coils. The cross-sectional area of the rib cage and abdomen circumscribed by the bands increases and decreases as the infants breaths. This alters the self-inductance of the coils and the frequency of their oscillation. The

increase and decrease in cross-sectional area is proportional to lung volume changes, and the electrical signal changes are used to create volume vs. time waves and tidal breathing parameters[113,114]. RIP has been used to assess breathing asynchrony, detect airway obstruction and monitor sleep apnea[115-117]. It has also been used to evaluate the effect of non-invasive ventilation support[118,119]. However, the clinical use of RIP has been limited by complex calibration procedures, which is necessary to assess accurate tidal volumes. This includes a period of breathing through a calibrated flow sensor, such as a pneumotach, to scale the final RIP signal to known reference volumes[114].

Optoelectronic plethysmography (OEP) estimates chest wall volume by measuring the three-dimensional position of several reflective markers placed on the infant's thorax by an automatic motion analyzer that gets input signals from several cameras[120]. The method requires no calibration. However; the complexity of its bedside setup limits its routine application[114,120].

Electrical impedance tomography (EIT) is a type of radiation-free medical imaging that can detect regional changes in lung volumes. Surface electrodes are placed on the thorax, and from these electrodes the electrical conductivity (i.e. the inverse of airway resistance), permittivity, and impedance of the lungs are derived by advanced data acquisition techniques and sophisticated reconstruction algorithms[114,121]. Clinical studies have shown EIT to be useful in several clinical areas including evaluation of distribution of ventilation during endotracheal suctioning[122], body positioning[123], surfactant therapy[124] and prior to extubation[125]. Some of the limitations of use are the lack of standardized measurement protocols and appropriate reference values, rather low resolution of the generated images and a somewhat difficult placement of the electrodes[114].

Electromagnetic inductance plethysmography (EIP) is a relatively new method of measuring tidal breathing. The EIP system basically consists of an electromagnet and a patient vest encircling the torso in order to quantify chest and abdominal changes. The method requires no sedation, and allows continuous and prolonged recording of respiratory data without using a facemask. EIP can be seen as an extension of RIP. However, in contrast to RIP, where clinical use has been limited by complex

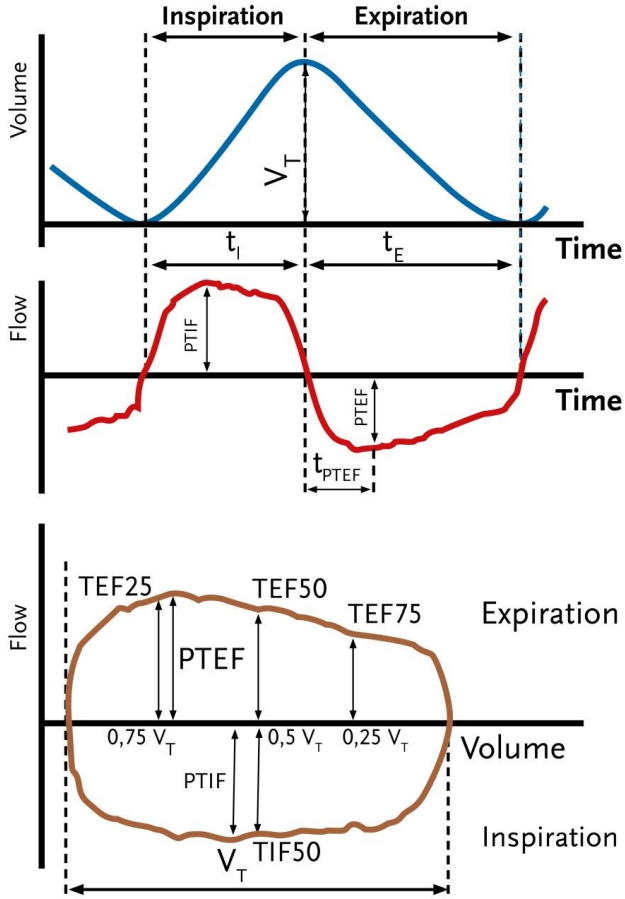
calibration procedures, EIP allows for simple and patient-independent calibration. The method will be described in details later in this thesis, in the Methods section.

Measured Indices. The most common indices derived from flow-time, volume-time and flow-volume curves during tidal breathing that are used to describe respiratory function are: (Fig. 5)

- ***V_T, tidal volume***; the volume change (in ml) from the peak of a tidal volume waveform to the next trough or vice versa.
- ***RR, respiratory rate or frequency***; the number of breaths that occur per minute
- ***V'E, minute ventilation***; the product of the respiratory rate and tidal volume (in ml/min)
- ***Ti, inspiratory time***; the time (in seconds) from the trough of a tidal volume waveform to its peak. Taken from a flow waveform, it is the time (in seconds) from the point of zero flow at the end of the expiratory pause of the preceding breath to the next point of zero flow at the end of inspiration.
- ***Te, expiratory time***; the time (in seconds) from the peak of a tidal volume waveform to the trough of the next breath. Taken from a flow waveform, it is the time (in seconds) from the point of zero flow at the end of inspiration to the next point of zero flow at the end of expiration. There is usually a brief pause before the onset of inspiration, but in infants the expiratory pause in negligible and is usually added to the expiratory time. Expiratory time also defines the duration of apneic events or pauses.
- ***Ti/Te***; the ratio of the inspiratory time and the expiratory time (in percent).
- ***PTIF***; the peak tidal inspiratory flow (in ml/s)
- ***PTEF***; the peak tidal expiratory flow (in ml/s)
- ***Vptef***; the volume (in ml) expired at peak tidal expiratory flow
- ***Tptef***; time (in seconds) from the onset of expiration to peak expiratory flow
- ***Tptef/Te***; the ratio of the time to peak expiratory flow and the expiratory time (in percent).

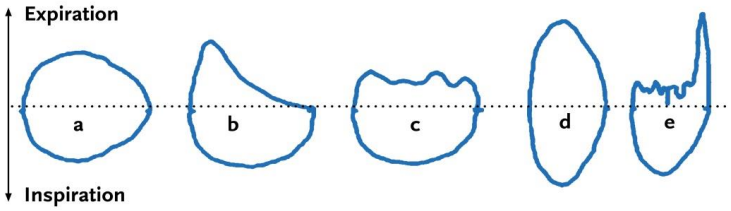
- ***TEF_{25}/PTEF***; the flow at 25% expired volume as a percent of PTEF, read from the flow-volume loop.
- ***TEF_{50}/PTEF***; the flow at 50% expired volume as a percent of PTEF, read from the flow-volume loop.
- ***TEF_{75}/PTEF***; the flow at 75% expired volume as a percent of PTEF, read from the flow-volume loop.

Figure 5. Commonly measured tidal breathing parameters
Modified from Schmalisch et al., 2005[126]



Shape of flow-volume loops. Useful information about breathing dynamics can be obtained simply by observing the shapes or patterns of tidal flow-volume loops/curves. There are clearly differences between the shapes of flow-volume loops observed in some infants with respiratory disease compared with those from healthy infants. For example, in infants with airway obstruction, the expiratory limb is concave relative to the volume axis (Fig. 6).

Figure 6. Different tidal flow-volume curves.



Modified from Stick in Infant Respiratory Function Testing, 1996. a. Normal tidal flow-volume loop. b. Concave expiratory limb. This can be observed when there is small airway obstruction. c. Laryngeal braking. d. Raised respiratory rate (as seen in infants with low respiratory system compliance). e. Expiratory grunting

2.6.6 Lung function findings in infants diagnosed with BPD

Studies examining lung function in preterm-born infants at term-equivalent age and during infancy are scarce. This is, as discussed above, mainly due to the fact that most measurement techniques suitable at that age are complex and time-consuming, require sedation or involve the application of a facemask that adds dead space and alters the breathing pattern of the infant [100-104]. This is a sad situation because such measurements have the potential to give important insight on the type and degree of lung injury present in the individual EP-born infant and perhaps enhance our ability to more accurately predict his or hers later respiratory health. Lung function

measurements at this age can also be helpful to objectively assess interventions aimed at early prevention and treatment of lung disease in these vulnerable infants. Some few studies of EP-born infants at term-equivalent age have shown lower lung volumes (lower FRC) and increased ventilation inhomogeneity in infants with BPD[65,127-129]. However, other studies have shown no clear differences, so the results are conflicting[130]. Results are also conflicting when it comes to measurements of tidal volume (Vt), as some studies show lower tidal volume per kilo bodyweight (Vt/kg) in infants with BPD compared to non-BPD infants[127], other studies show higher Vt/kg[131], and some studies show no difference between the groups[126,130]. The results conflict possibly due to differences in sedation and measurement techniques. Findings are more unambiguous as regards lung compliance, which seems to be lower in BPD infants[65,127], and airway resistance, which seems to be higher[127,132]. Studies also generally agree that infants with BPD have increased respiratory rate (RR) and increased small-airway obstruction as measured by increased Tptef/Te (the ratio of the time to peak expiratory flow and the expiratory time) and illustrated by typical "scooped-out" obstructive expiratory limbs of their flow-volume loops [126,127,130,131].

We clearly need more knowledge on lung function in EP-born children, measured at term-equivalent age when most EP-born infants are discharged from hospital. Preferably, this knowledge should be obtained by non-invasive methods, independent of sedation, reflecting the natural breathing patterns of the infants.

The importance of detecting reduced lung function early in life cannot be understated, as lung function in infancy seems to track through life[133].

2.7 Gaps in knowledge

Pulmonary consequences of EP birth are heterogeneous and insufficiently mapped and understood. We lack reliable clinical methods that, during the early postnatal period, reflect the extent of lung injury and thus the risk of future lung disease, in the individual EP-born neonate. Such methods are needed in order to improve our understanding of the life-long lung disease process, and thus provide a basis for

prevention and targeted early management in neonates at particular risk. Accurate predictive measures for later (post NICU discharge) respiratory health are needed, to improve medical care for individuals at high risk of severe respiratory morbidity. Our understanding of the detailed lung mechanics in infants surviving EP birth is limited, largely due to the fact that lung function measurements in neonates and infants are complex and not readily available. This situation hampers our approach to medical care and also complicates lifelong tracking of lung function. We need reliable, accurate, non-invasive and easy-to-use methods for measuring lung function in neonates and infants, reflecting their natural breathing pattern, with no disturbances from sedation or a face mask.

3. AIMS OT THE THESIS

A. Study feasibility and validity of electromagnetic inductance plethysmography (EIP) in preterm and term born infants.
   1. Investigate how well EIP is tolerated by infants.
   2. Investigate if EIP can be performed within a busy NICU environment.
   3. Estimate the consistency of repeated measurements, i.e. examine the repeatability, reproducibility and reliability of the method in preterm and term-born infants.
   4. Estimate the accuracy of EIP compared to a validated system measuring airflow via a facemask using an ultrasonic flowmeter (USFM).

B. Study the effect on tidal breathing of applying a facemask.

C. Compare lung function in term-born and EP-born infants at approximately term-equivalent age applying EIP, to obtain objective measures of the lung injury of the EP-born infants at this stage.

D. Study the predictive ability of EIP lung function measurements at term age for later respiratory health in EP-born infants.

E. Examine whether tidal breathing parameters, obtained from flow data from a mechanical ventilator during the first hours of life in EP-born neonates, could predict later BPD.

The main hypotheses of this thesis were the following:

1. The EIP method is well tolerated by infants, term-born and preterm-born.
2. The repeatability of the EIP method is as good as those reported for traditional infant spirometry.
3. The repeatability of the EIP method is as good for preterm as for term-born infants.
4. Repeatability of the EIP method in neonates depends on the experience of the person who performs the measurement and on the relationship to meals.
5. The results of EIP measurements are independent on who is selecting the breathing traces included in computerized analysis.

6. The same lung function variables obtained with the EIP method (VoluSense Pediatrics) and with a system that measure airflow via a facemask using an ultrasonic flowmeter (Exhalyzer D) are within 15% of each other.
7. Application of a facemask will affect the measurement result when performing tidal breathing measurements in infants.
8. The lung function at term-equivalent age of infants born EP, with or without BPD, will different from the lung function of term-born infants.
9. Tidal breathing parameters at term-equivalent age can predict pulmonary health in infants born EP.
10. Early lung mechanics, as expressed by tidal breathing parameters, reflect a susceptibility for later development of BPD in EP-born neonates.

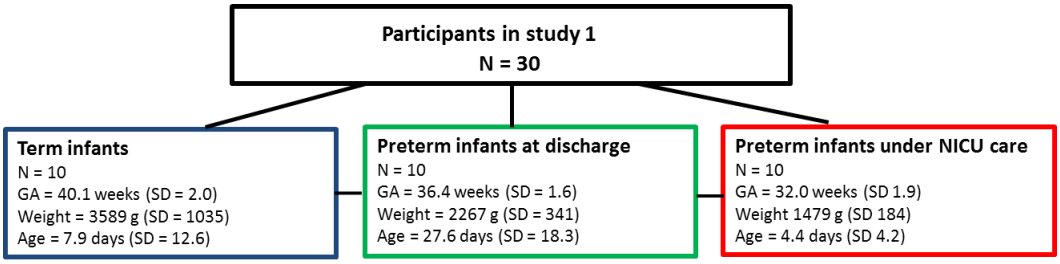
4. METHODS

4.1 Subjects and study design

The first two studies in this thesis (studies #1 and #2) were methodological studies validating the EIP method. The participants in these studies, as well as the term-born control subjects in study #3, were recruited based on convenience sampling from the intensive care unit (NICU) (studies #1 and #2) and the maternity clinic at Haukeland University Hospital (studies #2 and #3). Subjects recruited from the maternity clinic were studied either just before discharge, or when returning to the outpatient clinic for routine metabolic screening or weight measurement. None of the participants had respiratory symptoms at the time of the measurements. Exclusion criteria were significant perinatal disease, malformations or syndromes.

In study #1, we recruited a total of 30 patients from 3 different patient-groups based on current gestational age and weight, aiming to examine if the groups differed in terms of repeatability and how well the method was tolerated (Fig. 7). All measurements in this study were performed in 2011.

Figure 7. Participants in study #1.



GA = gestational age. GA, weight and age stated are at the time of measurement. SD = standard deviation. NICU = neonatal intensive care unit.

In study #2, we recruited term-born infants with weight ≥ 2 kg, because the mask-based ultrasonic flowmeter set-up that was used as the reference method (Exhalyzer D) is not approved for children < 2 kg. Only healthy children were recruited, as

simultaneous measurements with two methods were expected to be somewhat cumbersome and time consuming. The measurements in study #2 were performed in the period from September 2015 to November 2015.

In study #3, the term-born control participants were born in the period November 2015 through March 2016 and were recruited and measured on days when our study nurse had the opportunity to do the measurements at the maternity clinic.

In studies #3 and #4, the preterm born participants were part of a larger prospective population-based cohort study called 'Project extreme prematurity' ('BabyPEP') that has been ongoing since December 2010 in the two only tertiary hospitals within Western Norway Regional Health Authority that care for EP-born infants (Haukeland and Stavanger University Hospital). Women with threatening preterm delivery before 28 weeks' gestation are invited, and their children included if born before 28 weeks' GA. Detailed prenatal, perinatal and neonatal history, examinations and treatments are consecutively recorded in a database during the hospital stay.

Study # 3: In 'BabyPEP', at discharge or at term age, whatever comes first, the lung function is assessed by EIP. Subjects included in 'BabyPEP' who had their lung function measured by EIP at discharge/term age when we had the EIP method available (i.e. FloRight in the period from 2011–2013 and VSP in 2015–September 2016), constitute the preterm born participants in study #3.

Study #4: Since 2014, flow data have been logged from the ventilator for the neonates who receive conventional ventilator support during their first 48 hours of life, and these subjects (if born before August 2016) constitute the preterm-born participants in study #4.

4.2. Measurement equipment and testing conditions

4.2.1 Electromagnetic inductance plethysmography (studies #1, #2 and #3)

As described previously, electromagnetic inductance plethysmography (EIP) is a relatively new method for infant PFT, measuring tidal breathing. It consists of an

electromagnet and a patient vest encircling the torso in order to quantify chest and abdominal changes. The method requires no sedation or facemask, and allows continuous and prolonged recording of respiratory data.

FloRight™, developed by the Norwegian company VoluSense, was made available for research in 2003 and was the first prototype to use the EIP principle. The FloRight vest is a soft, circular cotton vest, covering the infant from the armpits to the groin, with two thin metal wire coils sewn into the fabric in separate multi-turn helixes, the upper covering the chest and the lower the abdomen, enabling separate analyses and detection of thoraco-abdominal asynchrony. A weak alternating electric current of 100 mA is passed through the coils, producing a magnetic field around the infant's torso proportional in size to the volume covered by the coils, which expands and decreases in accordance with the infant's breathing movements. The volume signals are converted into flow signals by numerical differentiation, and flow-volume loops and tidal breathing parameters are calculated. All calculations and storage of raw data are performed at a rate of 100 Hz. The volume variations of the torso are assumed to be caused by variations in the volume of air in the lungs and thus to reflect bronchial airflow.

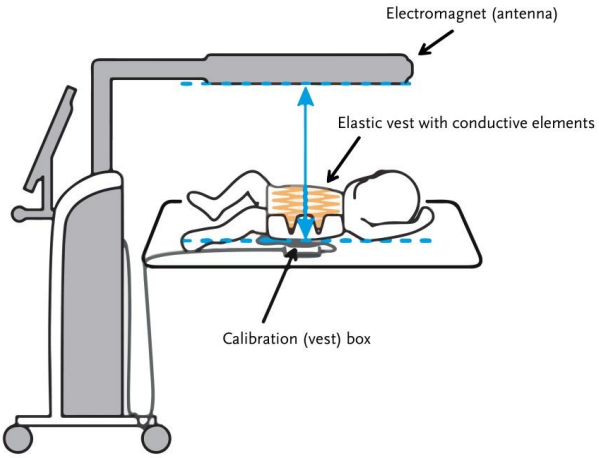
In 2015, VoluSense released an updated version of their method called VoluSense Pediatrics™ (VSP) (Fig. 8). The following changes were made from the FloRight to the VSP system:

- The VSP vests are made from a thinner and more flexible fabric than the FloRight vests, making it easier to enclose the skin tightly without creating wrinkles.
- The VSP vests are no longer circular, making them easier to dress as they do not require being pulled over the head (as for FloRight).
- As VSP vests are no longer circular, the wires are attached in a zig-zag pattern instead of a multi-turn helix (as in FloRight) in order to produce a voltage proportional to the instantaneous average cross-sectional area of the torso covered by the conductive elements (according to Faradays law of induction).
- The VSP vests are no longer energized to produce the magnetic field, but instead used for recording an induced voltage originating from the field produced by the

electromagnet antenna above the bed. This can mathematically be shown to give the same overall performance as in FloRight, with the added advantage of exposing the patient to far lower magnetic field strengths.

- The apparatus is smaller in size and easier to maneuver.
- The computational methods are unchanged, but the development tools are updated to harmonize with advances in computer technology.
- The hardware is re-designed to adapt to the change in configuration of the magnetic field.

Figure 8. Schematic drawing of the VoluSense Pediatrics measurement system.



Modified from the VoluSense user manual. The infant is wearing an elastic vest with two conductive elements attached to the outside in a wide zig-zag pattern. The vest is wrapped around the infant's torso and fastened with Velcro to provide a snug fit without wrinkles. An electromagnet positioned above the cot at a precise height and parallel to the infant's spine, generates a weak magnetic field that alternate with a frequency of ≈ 100 kHz. The magnetic field induces a voltage in the metal wires of the vest that is proportional to the instantaneous average cross-sectional area of the torso covered by the wires, and increases and decreases proportionally to the infant's breathing movements. The system is initially calibrated against a calibration coil with a known area (A_C) positioned at the side of the infant in the cot to establish the relation between the induced voltage (U_C) and this area. During measurements, the thoracic or abdominal volumes are calculated as: $V = \frac{U_M A_C W_Z}{U_C}$, where U_M is the voltage that is measured, and W_Z is the width of the zig-zag pattern of the conducting loop in the vest[134].

4.2.2 Ultrasonic flowmeter (study #2)

In study #2, an ultrasonic flowmeter (USFM), the Exhalyzer D™ (EcoMedics AG, Duernten, Switzerland) with a flow head suitable for infants > 2 kg body weight and a flow-range of ± 0.5 l/s was used as the reference method to evaluate the accuracy of VSP. The system was calibrated according to the instructions from the manufacturer with a 10 ml volumetric syringe (Hans Rudolph, Inc., Shawnee, Kansas, US). A neonatal facemask with soft edges allowing a good seal was applied. According to the manufacturer the total dead space of the system (including the mask) was 9 ml. Measurements were performed according to the latest ERS/ATS standards of infant lung function testing [135].

4.2.3 Logging of ventilator flow data (study #4)

In study #4, ventilator flow data were logged using a custom-made software called MedLink 4.4 (Nortis Ingenieurbüro, Nürnberg, Germany), provided by the Dräger company (Dräger, Lübeck, Germany). The software was installed on a PC connected to the ventilator by a Medicus cable, and raw bi-directional flow data with a frequency of 50 Hz were logged. Flow volume curves were constructed using another custom-made software package (VoluSense, Bergen, Norway). These flow volume traces were then analyzed using the VSP analyzer software (VoluSense, Bergen, Norway). The mode of mechanical ventilation was "assist control with volume guarantee" (AC/VG) during all data acquisitions. All neonates had uncuffed endotracheal tubes of sizes 2.5 mm (n = 31) or 3.0 mm (n = 2). The individual tube size was decided at the discretion of the attending neonatologist. All neonates were sedated and pain relieved with morphine in doses between 10-15 ug/kg/h, they were all clinically stable at the time of data acquisition, and laryngeal air leaks calculated by the ventilator were less than 15%.

4.2.4 Performing the measurements

All measurements using the EIP system were performed with the infants in a supine position in a cot, quietly awake or asleep. No sedation was used, but some of the infants were given oral sucrose for relaxation. The correct-sized vests were selected according to the infants' armpit-hip length, and was put on and tightened to provide an adequate fit, but without constricting the torso or inhibiting the infants' breathing. In study #1, the vests were put on over a thin layer of clothing, in studies #2 and #3 the infants wore no clothes or diapers underneath. The antenna/electromagnet was positioned straight above the cot at the height specified by the manufacturer. Before any measurements were carried out, the system was calibrated by placing a calibration box/coil with known area/volume next to the child.

To assess the repeatability and reproducibility of the EIP method in study #1, two different nurses, Nurse A and Nurse B, carried out altogether four measurement sessions 30 minutes before a planned meal, each lasting about five minutes, in the following order: A1 before, B1 before, A2 before and B2 before. Approximately 10-15 minutes after a meal, measurements were repeated twice by Nurse A; measurements A3 after and A4 after. The vests were loosened and tightened between each of the measurements, but not removed completely from the baby. Seven nurses took part in study #1. They all received basic training in the equipment, including the manufacturer's operating instructions. Nurse A had independent experience in performing the test before the formal study was initiated, and she was the same for all the infants and all measurements. Nurse B varied and was one of six remaining nurses. However, Nurse B was always the same for the same infant, meaning that each infant was measured by the same two nurses. During all measurements in study #1, the infants' oxygen saturation and heart rate were monitored by pulse oximetry and the respiratory rate by the FloRight system. The infants' behaviour, their facial expression and breathing patterns, were subjectively observed and recorded by the examining nurse.

Based on the results and experiences from study #1, all measurements in study #3 were carried out approximately midway between two meals and by the same three

experienced persons; Merete Susan Olsen (pediatric nurse) and Mariann Bentsen (neonatologist) at Haukeland University Hospital, and Eli Sanne (pediatric nurse) at Stavanger University Hospital.

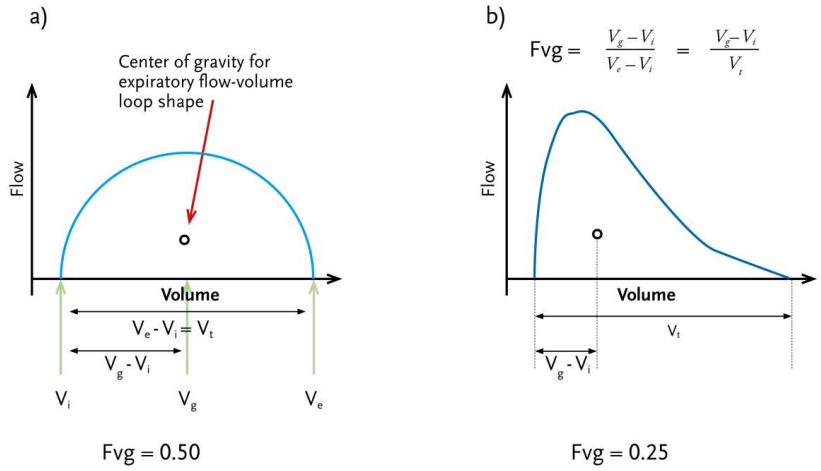
In study #2, all measurements were carried out at Haukeland University Hospital by Merete Susan Olsen and Mariann Bentsen. Because validation of VSP was based on *simultaneous* measurements with VSP and USFM (Exhalyzer D), implying that the infants had to accept a facemask, all measurements were carried out right after a meal to facilitate sleep. Once the infants fell asleep, data were collected with VSP alone for 2-3 minutes. The VSP measurement was continued while the USFM neonatal facemask was placed gently, but firmly over the infant's mouth and nose, ensuring a good seal. Once the facemask was in place, a marker was inserted in the VSP recording. The USFM measurement was started about thirty seconds after the facemask was put on, to allow adaptation to the facemask. Simultaneous recording using both devices was then performed for about 1 minute before the mask was removed. New markers were inserted in the VSP recording as the USFM measurement was started and stopped.

4.2.5 Data selection and analysis

All measurement traces were inspected visually, and stable breaths were selected for calculation of different tidal breathing parameters based on the following three criteria: (1) no obvious artifacts, (2) no sighs and (3) no obvious changes in the depth of breathing or baseline. In addition to common tidal breathing parameters all previously described in the Introduction section, a new variable; the Flow Volume Center of Gravity (FVg) was calculated. The FVg is a dimensionless variable that has been developed by the manufacturer of the VoluSense Pediatric system (Volusense AS, Norway). FVg is the expiratory flow centroid, i.e. the dimensionless value given by the position of the center of gravity of the expiratory flow-volume loop along the volume axis, where the start of expiration is defined as zero and the end as one. A symmetrical, normal curve will give an FVg of 0.5. Airway obstruction prolongs the end-expiratory phase and shifts this point to the left and gives values below 0.5, as

illustrated in Figure 9. Calculation of the expiratory flow centroid has been found to provide a good description of the tidal expiratory waveform[136] and of changes in the tidal expiratory waveform seen in airflow obstruction[131,136,137].

Figure 9: Flow-volume gravity midpoint, FVg.



Modified from Bentsen et al., Acta Paediatr. 2015. FVg is a dimensionless value given by the position of the centre of gravity of the expiratory flow-volume curve along the volume axis, using a linear scale where zero is the volume at start of expiration and one is the volume at end of expiration. FVg will therefore have a value between zero and one. a) A symmetrical (normal) curve will give an FVg of 0.5. b) Airway obstruction will shift the curve to the left and produce values for FVg below 0.5. Vg; volume of center of gravity, Vi; inspiratory volume, Ve; expiratory volume, Vt; tidal volume.

In study #1, two raters, both physicians working in the NICU, independently selected breathing segments for computerized analysis, making it possible to assess interrater and intrasession repeatability.

In study #2, markers in the VSP recording enabled visual identification of the corresponding respiratory cycles from the VSP and the USFM recording. This allowed for comparison of the same flow, volume and timing parameters from each device. Segments with movement artefacts, apneas or sighs were removed from both recordings before the breathing segments were analysed using the software provided by the respective manufacturers.

Effect of the facemask was assessed by comparing the breathing parameters obtained from the VSP before versus after putting on the Exhalyzer D mask.

4.3 Questionnaires

In BabyPEP, all study participants are followed-up at 3, 6 and 12 months' corrected age, i.e. age calculated from the expected term birth. This includes clinical examinations and parental questionnaires addressing various problems, including respiratory symptoms and treatments which are based on the ISAAC core questions on asthma (see Appendix).

4.4 Definitions

The clinical usefulness of a test method depends on the repeatability, reproducibility and reliability of the method[138-141]. *Repeatability* refers to the variations that occur when test and retest measurements are performed by the same person under identical conditions, and it provides an estimate of the minimum level of agreement between replicated measurements. *Reproducibility* is the degree of agreement between measurements performed by different examiners or under different conditions, as in our study #1, before and after a meal. *Reliability* is how much of the variations in the measurements are due to the sum of inborn within-subject variation and measurement errors as opposed to true differences in lung function mechanics. If reliability is high, the variability caused by measurement errors and inborn within-subject variations are low compared to the variations caused by subjects being truly different. Hence, subjects can be well distinguished from each other.

In study #3, BPD was simply defined as being dependent on oxygen supplementation at 36 weeks' GA. Oxygen supplementation was provided according to similar algorithms in the two participating NICUs, and usually by low-flow nasal cannulas guided by pulse oximetry targeting 90-95% saturation at 36 weeks' GA. Respiratory morbidity during the first year of life was defined by need for hospital readmission because of respiratory symptoms and/or parental report of treatment with inhaled asthma medications.

In study #4, BPD was defined according to the National Institute of Child Health and Human Development (NICHD) and National Heart, Lung, and Blood Institute (NHLBI) Workshop criteria from 2000, and the infants were retrospectively divided into two groups; no or mild BPD and moderate or severe BPD[50].

4.5 Statistical methods

All statistical calculations were performed using SPSS version 22 (IBM SPSS Statistics, Armonk, NY, USA) and MedCalc version 13.1 (MedCalc Software, Mariakerke, Belgium).

Data were reported as means with 95% confidence intervals (CIs), ranges or standard deviations (SDs) for continuous data, or counts with percentages for categorical data. Normal distribution was assessed by descriptive statistics, histograms and Q-Q plots.

To determine repeatability and reproducibility, we calculated the coefficient of repeatability (CR), defined as 1.96 standard deviations (SD) of the mean pairwise differences between measurements and CR%, which is CR as a percentage of the pairwise mean[141]. We also calculated the coefficient of variation (CV), defined as the SD of all measurements divided by their mean. Respectively, paired samples t-tests and F-tests for the equality of variances were applied to assess whether the mean pairwise differences of the various measurement sessions or their CRs differed significantly. To examine reliability, we calculated the intraclass correlation coefficient (ICC), defined as follows:

$$\mathrm{ICC} = \frac{(\text{SD between subjects})^2}{(\text{SD between subjects})^2 + (\text{SD within subjects})^2}$$

Reliability was considered good if the ICC values were ≥ 0.75 and adequate or fair to good if 0.4 < ICC < 0.75[142]. One-way analysis of variance (ANOVA) was used to estimate between-subject and within-subject SDs.

Bland-Altman plots were constructed to visualize agreement between lung function measurements with the EIP and the USFM method. We computed 95% limits of agreement for each breathing parameter (average difference ± 1.96 standard deviation of the difference), which tells us how far apart measurements by the two methods are likely to be for most individuals[140,141]. One-sample t-tests were used to assess if the mean difference between data obtained with the two methods differed significantly from zero, indicating the presence of a consistent bias. To evaluate the existence of proportional bias, i.e. that the methods did not agree equally through the range of measurements, the difference between the methods was regressed on the average of the two methods.

To evaluate the effect of the facemask, we compared the means of the different breathing parameters from the VSP recording with and without facemask using paired sample t-tests.

Independent samples t-tests or Mann-Whitney U-tests were used as appropriate to make group comparisons between different groups of EP-born infants, between EP-born infants and term-born infants and between neonates later developing different degrees of BPD. Categorical data were compared using the chi-square test. Group differences in terms of GA at birth, gender and body weight at the time of measurement were adjusted for in multiple regression analyses.

Receiver-operator characteristic (ROC) analyses were used to assess the ability of different tidal breathing parameters and selected clinical variables to discriminate between groups of EP-born infants, and to predict the respiratory morbidity of the infants during their first year of life. ROC analyses were also used to predict BPD

severity in EP-born neonates from tidal breathing parameters obtained from ventilator data during the first hours of life.

To create the best possible prediction models for later respiratory morbidity (study #3) and for BPD severity (study #4), we estimated compound models using the prognostic score (predicted probabilities) from multiple logistic regression incorporating different parameters. These scores were subsequently used as the test-variable in the ROC analyses.

4.5.1 Power calculations

To carry out *a priori* power analyses was challenging when planning studies #1 and #4, as we could not know what to expect as regards mean values or distributions of the tidal breathing parameters in these groups of infants.

When planning study #3, that is the study comparing lung function of EP-born and term-born infants, power calculations were done based on the tidal breathing parameter Vt/kg, with data distribution obtained from paper #1. We calculated that 44 infants were needed in each group to provide a power of 80% to show a 20% difference (0.6 SDs) between the groups.

To estimate the number of participants needed in study #2, the Bootstrap resampling method was used after the inclusion of 18 cases[143]. We estimated that the reliability of the 95% limits of agreement would not improve by including > 30 participants in the study.

4.6 Ethics

The Regional Committee on Medical Research Ethics of the Western Norway Health Authority approved all studies (ID 2009/1771 and ID 2010/496). Before commencing on study #1, we had an in depth discussion regarding potential dangers and pitfalls involved when applying the EIP method to preterm born neonates, especially regarding the possible impact of the vest on the infant breathing and any harmful

effects of the magnetic field. Based on the fact that the magnetic field strength in FloRight was approximately as small as 10 μT, and on the technical report from the manufacturer explaining how the pressure inside the vest varied with the tightening, we concluded that the risk scenario was very small and therefore tolerable. All parents were extensively orally informed, and additionally, informed, written consent was obtained from the parents of all participants.

4.7 Problems along the way

The first commercially available version of the EIP system (FloRight) was on the marked when the first studies of this thesis was commenced in 2011, and thus put into use in 'Baby-PEP' when working on study #1. The manufacturer (VoluSense) introduced their upgraded version (VSP) in April 2014, and we started to use this version in the 'BabyPEP'project in May 2014. In march 2015 the VoluSense company discovered that a large part of their vests had a construction defect (inaccurate connection of conductive elements), making all our study measurements collected in the period May 2014 to March 2015 worthless. The problems with the vests were solved by VoluSense during summer 2015, and in fall 2015 we were ready to start all over again. However, due to these problems, and due to the upgrading that had been performed, it became clear that the new VSP version had to be validated against a 'gold standard method' before we could proceed with further studies as well as using the system in clinics, explaining the origins of study #2 where VSP and USFM data are compared.

5. RESULTS

This section presents results from the four studies that constitute the fundament for this thesis, and some supplementary results (unpublished data) intended to clarify the reported results.

5.1 Study #1

The FloRight system did not disturb the infants or interfere with their respiratory behavior. There were no changes in respiratory rate, heart rate or oxygen saturation and no subjective changes in facial expressions, breathing patterns or other alterations in behavior while wearing the vests.

For the parameters Vt and Tptef/Te we found that the repeatability and reproducibility was universally relatively poor. The repeatability of the experienced nurse was better before meal than after meal and better than for the inexperienced nurses, although significantly so only for Vt. For the experienced nurse the CR% and CV% repeatability before meal was 37.5 and 14.1 for Vt, and 61.6 and 22.9 for Tptef/Te, respectively. The repeatability tended to be better for term-born than for smaller premature infants; however, the differences were not statistically significant. For the inexperienced nurses the CR% and CV% repeatability before meal was 79.4 and 28.0 for Vt, and 76.0 and 26.7 for Tptef/Te, respectively. CR% reproducibility for different nurses was 46.2 for Vt and 56.8 for Tptef/Te, while CR% reproducibility for Vt and Tptef before vs. after meal was 81.1 and 78.8, respectively.

The parameter FVg showed better repeatability and reproducibility. For FVg the repeatability of the experienced nurse was similar before and after meal (CR% 13.3 vs. CR% 13.8), and similar to the unexperienced nurses (CR% 13.3 vs. CR% 17.3). As for Vt and Tptef/Te the repeatability tended to be better for term-born than smaller premature infants; however, not statistically significant. FVg CR% interexaminer reproducibility was 13.4 and CR% reproducibility before vs. after meal was 17.0.

Interrater repeatability was good (low CR%) for all parameters, suggesting that *who* selected the breathing segments for computerized analysis had little influence on the results.

The reliability for Vt was good (ICC > 0.75) for term infants and preterm infants under NICU care and adequate or fair to good (0.4 < ICC < 0.75) for preterm infants at discharge. The opposite pattern was found for Tptef/Te and FVg, where the reliability was good for preterm infants at discharge and adequate or fair too good for term infants and preterm infants receiving NICU care.

5.2 Study #2

Thirty infants with gestational ages ranging between 36 and 43 weeks and a weight range of 2.3 kg to 4.8 kg were included in the study, and their average age was 13 days (95% CI: 3.4, 22.8 days) when the examinations were performed. Successful simultaneous measurements (EIP and USFM/ExhalyzerD) were obtained for all participants; in 14 cases at the first attempt, in 13 cases at the second attempt and in 3 cases at the third attempt. The most common cause of unsuccessful measurements was babies waking up disapproving the application of the facemask. Other causes were movement artefacts in the VSP recordings or obvious mask leakage in the USFM recording.

Tidal volumes (Vt/kg) showed a non-consistent and non-significant mean difference (VSP minus USFM) of -0.15 ml/kg, with 95% limits of agreement within ± 0.93 ml/kg. This corresponds to a difference of -3.0% and limits of agreement within ± 16.0%. Mean minute ventilation (V'E) was 3.8% lower for VSP, and the difference was consistent based on a one sample t-test. Measured respiratory rates (RR) differed less than 1%. Peak tidal expiratory flow (PTEF) was higher when measured by VSP than by USFM; on average the difference was +5.0%. The difference was consistent, but with a relatively large standard error and wide limits of agreements. Time to peak tidal expiratory flow as a ratio of total expiratory time (Tptef/Te) differed on average -5.4% (VSP minus USFM), with limits of agreement ± 18.0%. Regression analysis

revealed a small proportional bias of the mean difference (p = 0.0008, regression slope = 0.51 and $r^2 = 0.33$). The ratio of inspiratory to expiratory time (Ti/Te) and the ratio of tidal expiratory flow at 50% of expired volume to peak tidal expiratory flow (TEF_{50}/PTEF) both corresponded well with small mean differences; -3.1% and -3.0% respectively (VSP minus USFM), and relatively narrow limits of agreement. A small proportional bias was found for both parameters (p = 0.005, regression slope = 0.23, $R^2 = 0.25$ and p = 0.034, regression slope = 0.38, $R^2 = 0.15$, respectively).

Application of the facemask significantly increased the tidal volume (mean increase 1.5 ml/kg, 95% CI: 1.3, 1.8) and the minute ventilation (mean increase 83.0 ml/kg/min, 95% CI: 57.0, 109.0). PTEF, Ti/Te and TEF_{50}/PTEF were also significantly increased, with average mean differences of 15.5 ml/s, 10.4% and 2.0%, respectively. No difference was found for RR or Tptef/Te.

5.3 Study #3

All EP-born infants born during the time periods when the EIP method were available (FloRight or VSP) from 2011 and up to September 2016, and who survived to discharge, were included; 41 at Haukeland University Hospital and 11 at Stavanger University Hospital. FloRight was used in 33 (63%), and VSP in the remaining 19 EP-born infants and in all the 45 term-born controls. Thirty-two (62%) of the 52 EP-born infants had BPD.

Comparisons of tidal breathing parameters between groups (Table 2):

- **All EP-born vs. term-born control infants.** Vt/kg, RR, V'E/kg and PTEF/kg were higher while the variables describing airway obstruction (TEF_{50}/PTEF, TEF_{75}/PTEF, Tptef/Te and FVg) were all lower (i.e. indicating more airway obstruction) in the EP-born compared to the term-born control group. Mean GA and body weight at the time of lung function assessment and the proportion of males were lower in the EP-born than the term-born group, and thus adjusted for in the regression analyses.

- **Non-BPD vs. term-born control infants.** As for the complete EP-born group, Vt/kg, RR, V'E/kg and PTEF/kg were significantly higher and TEF_{75}/PTEF and FVg were significantly lower in the non-BPD group when compared to the term-born control group; however, numerically less pronounced. TEF_{50}/PTEF and Tptef/Te did not differ significantly.

- **BPD vs. non-BPD infants.** TEF_{50}/PTEF, TEF_{75}/PTEF, Tptef/Te and FVg were all lower in the BPD-infants compared to non-BPD infants.

Table 2. Lung function at term-equivalent age in all infants

	Healthy term-born controls (n = 45)	All extremely preterm-born infants[1] (n = 52)	Preterm-born infants without BPD[1] (n = 20)	Preterm-born infants with BPD[2] (n = 32)
Male gender	24 (53.3%)	18 (34.6%) p = 0.035	8 (40%) p =0.28	10 (31.3%) p = 0.73
Gestational age at time of measurement; weeks (ranges)	40.6 (37.3 – 43.1)	38.8 (36.0 – 42.9) p < 0.001	38.7 (36.1 – 41.3) p < 0.0001	38.9 (36.0 – 42.9) p = 0.50
Postnatal age in days (ranges)	6.5 (3 – 21)	88.9 (60 – 115) p < 0.001	86.9 (62 – 103) p < 0.0001	90.2 (60 – 115) p = 0.42
Body weight at time of measurement; g (ranges)	3494 (2465 – 4700)	2768 (1900 – 3800) p < 0.001	2862 (2177 – 3800) p < 0.0001	2709 (1900 – 3500) p = 0.22
Vt (ml)/kg	4.6 (4.3, 4.9)	6.0 (5.5, 6.5) p = 0.019	5.8 (5.0, 6.6) p = 0.026	6.1 (5.3, 6.8) p = 0.63
RR (min^{-1})	58.2 (54.6, 61.9)	67.8 (63.4, 72.2) p = 0.012	71.1 (62.8, 79.4) p = 0.002	65.7 (60.7, 70.8) p = 0.23
V'E (ml)/kg	258.5 (236.4, 280.6)	394.0 (358.1, 429.8) p < 0.0001	405.0 (336.7, 473.3) p < 0.0001	387.1 (344.3, 429.9) p = 0.63
PTEF (ml/s)/kg	14.2 (12.5, 15.9)	27.5 (24.6, 30.5) p < 0.0001	25.6 (21.3, 30.0) p < 0.0001	28.7 (24.6, 32.8) p = 0.31
TEF_{50}/PTEF (%)	84.1 (82.2, 86.0)	78.2 (75.9, 80.4) p = 0.008	81.6 (78.7, 84.4) p = 0.51	76.1 (73.0, 79.1) p = 0.015
TEF_{75}/PTEF (%)	67.6 (64.5, 70.7)	53.1 (48.7, 57.5) p < 0.0001	59.9 (53.8, 66.1) p = 0.026	48.8 (43.1, 54.5) p = 0.012
Tptef/Te (%)	40.9 (37.6, 44.3)	30.1 (26.4, 33.8) p < 0.0001	35.1 (29.2, 41.1) p = 0.056	26.9 (22.2, 31.6) p = 0.029
FVg	0.48 (0.47, 0.49)	0.44 (0.43, 0.45) p < 0.0001	0.46 (0.44, 0.48) p = 0.019	0.43 (0.42, 0.45) p = 0.013

From Bentsen et al., BMJ Open 2017. [1]Significance tested versus the term-born control group and adjusted for differences in GA and BW when the measurements were obtained. [2]Significance tested versus the EP-born group without BPD. Vt/kg; tidal volume per kilogram body weight. RR; respiratory rate. V'E; minute ventilation. PTEF; peak tidal expiratory flow. TEF_{50}/PTEF; flow at 50% expired volume as a percent of PTEF. TEF_{75}/PTEF; flow at 75% expired volume as a percent of PTEF. Tptef/Te; time to peak tidal expiratory flow as a ratio of total expiratory time. FVg; expiratory flow volume loop center of gravity (dimensionless).

Prediction of later respiratory morbidity. At the time of data analysis of study #3, 35 of the 52 EP-born children had reached a corrected age of one year, and 11 (31%) of them had either been hospitalized and/or treated with asthma medication; i.e. the two variables used to define "respiratory morbidity" during the first year of life. In the group with respiratory morbidity, mean (95% CI) TEF_{50}/PTEF was found to be significantly lower; i.e. 73.5 (68.6, 78.6) vs. 79.9 (76.6, 83.1). TEF_{75}/PTEF, Tptef/Te and FVg also tended to be lower, but not significantly so. ROC analyses showed that the tidal breathing variable TEF_{50}/PTEF, but not BPD, predicted respiratory morbidity during the first year of life. The prediction model that incorporated TEF_{50}/PTEF, Vt/kg, BPD, BW z-score and gender gave an area under the curve (AUC) of 0.818 (95% CI: 0.651, 0.928), predicting respiratory morbidity in the first year of life of this group of infants with a sensitivity and specificity of 81.8% and 75.0%, respectively.

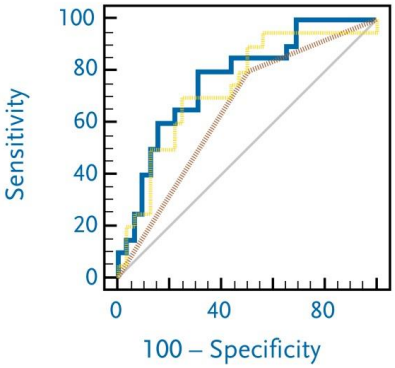
By the end of October 2017 all participants had reached a corrected age of one year. At that time, 20/52 (38%) had either been hospitalized and/or treated with asthma medication during their first year of life (Table 3). Contrasting the analyses as they were reported in paper #3, at this later stage there was a significantly higher fraction of infants with BPD in the 'respiratory morbidity group' than in the 'no respiratory morbidity group' (80% vs. 50%, p = 0.032). Nevertheless, BPD did not predict respiratory morbidity during the first year of life using ROC-analyses (Fig.10). Mean TEF_{50}/PTEF was still significantly lower in the group with respiratory morbidity during the first year of life, and at this stage also Tptef/Te was significantly lower. TEF_{50}/PTEF still predicted respiratory morbidity during the first year of life using ROC analyses. This time the prediction model incorporating TEF_{50}/PTEF, Vt/kg, BPD, BW z-score and gender gave an AUC of 0.769 (95% CI: 0.638, 0.900), predicting respiratory morbidity in the first year of life with a sensitivity and specificity of 80.0% and 68.7%, respectively (Fig. 10).

Table 3. Early clinical characteristics and lung function data obtained at near term gestational age after extremely preterm birth in children with and without respiratory morbidity during their first year of life[1].

	Respiratory morbidity (n=20)	No respiratory morbidity (n=32)	p-value
Gestational age at birth	26.0 (25.3, 26.7)	26.0 (25.6, 26.4)	0.97
Birth weight z-score	-0.19 (-0.79, 0.40)	-0.16 (-0.43, 0.11)	0.90
Male gender	7 (35%)	11 (34%)	0.94
Pat. chest X-ray at term	6/16 (38%)	15/26 (58%)	0.16
Diagnosis of BPD	16/20 (80%)	16/32 (50%)	0.032
Vt/kg (ml/kg)	6.0 (5.1, 6.9)	6.0 (5.3, 6.7)	0.96
RR (min^{-1})	68.1 (61.6, 74.6)	67.6 (61.5, 73.7)	0.92
V'E/kg (ml/kg/min)	394.4 (338.4, 450.4)	393.7 (344.7, 442.8)	0.99
PTEF/kg (ml/s/kg)	28.1 (23.2, 33.0)	27.2 (23.3, 31.1)	0.77
Tptef/Te (%)	25.4 (21.2, 29.7)	33.0 (27.6, 38.3)	0.046
TEF_{50}/PTEF (%)	74.7 (70.9, 78.5)	80.4 (77.7, 83.0)	0.012
TEF_{75}/PTEF (%)	48.1 (42.3, 53.9)	56.2 (50.1, 62.3)	0.07
FVg	0.43 (0.41, 0.45)	0.45 (0.44, 0.47)	0.062

The table reveals unpublished data. [1]Defined by the need for hospital readmission because of respiratory symptoms and/or a parental report of treatment with inhaled asthma medications. Data are means with 95% confidence intervals or numbers (percent in brackets). [2]Independent sample t-tests or the chi-square test.

Figure 10. Prediction of respiratory morbidity during the first year of life of extremely preterm-born individuals.



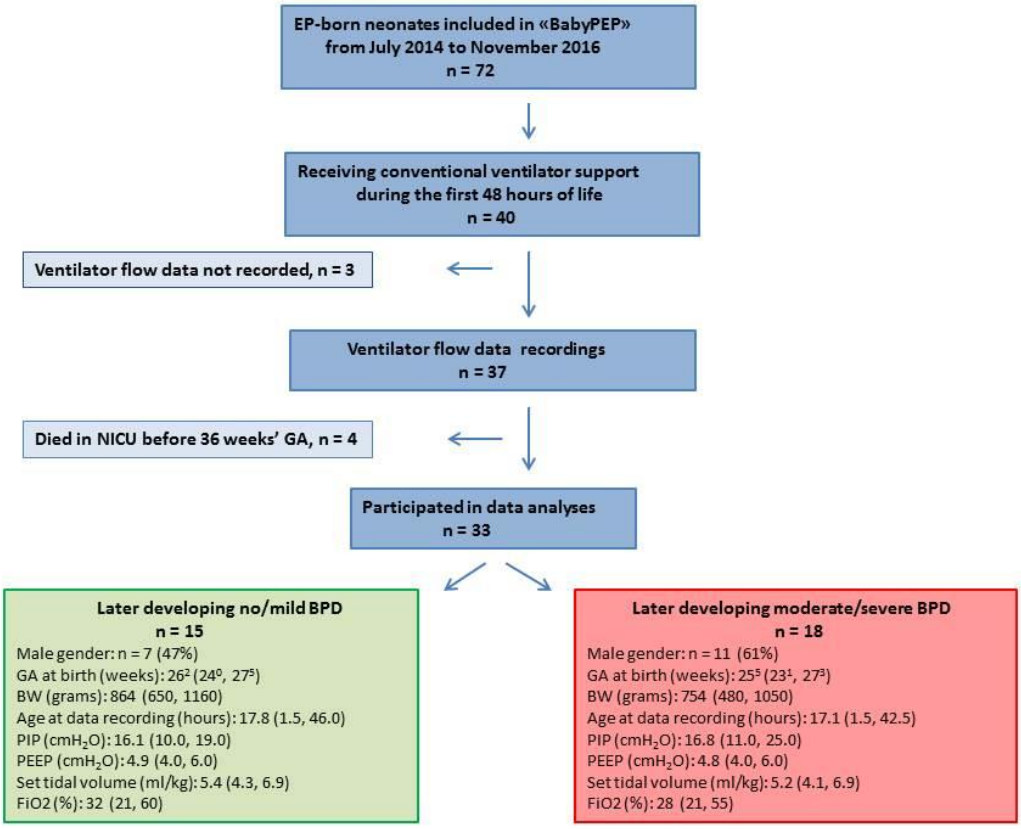
		AUC with 95% CI
	Compound model	0.769 (0.638, 0.900)
	BPD	0.650 (0.498, 0.802)
	TEF_{50}/PTEF	0.678 (0.528, 0.829)

The figure reveals unpublished data. Receiver-operator characteristic (ROC) curves comparing the ability of TEF_{50}/PTEF, BPD and additionally a compound model incorporating TEF_{50}/PTEF, Vt/kg, BPD, birth weight z-score and gender, used to predict development of respiratory distress requiring readmission or treatment with asthma medication during the first year of life of extremely preterm-born individuals (n = 52). The compound model achieved the best sensitivity and specificity; i.e. respectively 80.0% and 68.7% at a cut-off value of 0.36. AUC; area under the ROC curve. If the 95% confidence interval (95% CI) of the AUC includes 0.5 (no discrimination) the parameter does not predict later respiratory distress. The optimal cut-off point is where the sensitivity and specificity are maximal. This value corresponds with the point on the ROC curve farthest from the diagonal line.

5.4 Study # 4

Ventilator flow data were collected for 33 EP-born patients. Recruitment of the participants and their clinical characteristics and perinatal data is depicted in Figure 11. There were more boys, and also a tendency for lower GA, BW and BW z-score in the preterm-born neonates who developed moderate/severe BPD; however, differences were not statistically significant.

Figure 11. Recruitment of subjects for ventilator flow data analysis and their perinatal characteristics.

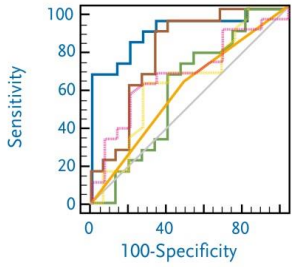


PIP: peak ventilator inspiratory pressure. PEEP: ventilator expiration pressure. FiO_2: fraction of inspired oxygen. Data are presented as means with 95% confidence intervals.

The moderate/severe BPD group had higher numerical values for all the expiratory breathing parameters; i.e. suggesting less obstruction, than the no/mild BPD group; however, the difference was only significant for TEF_{50}/PTEF, mean (95% CI) values being respectively 85.1 (81.8, 88.4) vs. 71.8 (62.9, 80.6) ($p = 0.007$). This finding remained highly significant also after performing a Bonferroni adjustment (adjusted p-value of 0.01).

TEF_{50}/PTEF also significantly predicted moderate/severe BPD using ROC-analyses (Fig. 12), in contrast to other neonatal variables such as GA, BW and BW z-score. A compound model incorporating TEF_{50}/PTEF, birth weight z-score and gender predicted moderate/severe BPD with good accuracy (AUC 0.893; 95% CI: 0.735, 0.973). The optimal cut-off point for this combined model was 0.785, meaning that a prognostic score > 0.785 predicted BPD with sensitivity and specificity of 66.7% and 100%, respectively, corresponding to positive (PPV) and negative (NPV) predictive values of 100% and 71.4% in this study.

Figure 12. Prediction of development of BPD in extremely preterm-born neonates



	AUC with 95% cl	Optimal cut-off	Sensivity	Specificity
Compound model	0.893 (0.735, 0.973)	0.785	66.7	100
TEF_{50}/PTEF	0.774 (0.596, 0.901)	77.5	88.9	66.7
FVg	0.626 (0.441, 0.787)			
Tptef/Te	0.576 (0.392, 0.745)			
BW z-score	0.667 (0.482, 0.820)			
Gender	0.572 (0.389, 0.742)			

Modified from Bentsen et al., ERJ OR 2018. Receiver-operator characteristic (ROC) curves comparing the ability of different expiratory breathing parameters and perinatal variables (birth weight z-score and gender) to predict development of BPD (n= 33). The Compound model incorporates TEF50/PTEF, birth weight z-score and gender. AUC; area under the ROC curve. If the 95% confidence interval (95% CI) of the AUC includes 0.5 (no discrimination) the parameter does not predict BPD. The optimal cut-off point is where the sensitivity and specificity are maximal.

6. DISCUSSION

The principal findings of the studies comprising this thesis are that lung function measurements by EIP could feasibly be performed in a busy NICU setting and that the method was well tolerated by infants. The repeatability of the method, represented by FloRight, was similar to what has been reported for traditional infant spirometry. The repeatability was better for term-born than preterm-born infants, and the experience of the personnel and the relationship to meals influenced the reproducibility of the measurement results. The accuracy of EIP, represented by VSP, was similar to that obtained by a mask-based system with a USFM when tested in infants at term-equivalent age. Application of a facemask influenced several of the tidal breathing parameters, confirming that a facemask affects the natural way of breathing in infants. Lung function, as measured by EIP, was strikingly abnormal in EP compared to healthy term-born infants at term-equivalent age, characterized by primarily obstructive pulmonary abnormalities, as well as increased tidal volumes and minute ventilation. The abnormalities were most pronounced for the group of infants with BPD, but significantly present also in the group without this diagnosis. The tidal breathing parameter TEF_{50}/PTEF predicted respiratory morbidity in the first year of life in infants born EP. Flow data easily obtained from a ventilator during the first 48 hours of life could be used to compute breathing parameters that discriminated between neonates who went on to develop the severe forms of BPD and those who did not, the former group showing a less obstructive airflow pattern. A model combining the parameter TEF_{50}/PTEF with birth weight z-score and gender predicted moderate/severe BPD with good accuracy.

6.1. Methodological considerations

The purpose of clinical medical research is to generate knowledge that is based on the population sample under study, but is applicable also to other and comparable groups. The study population should therefore be an unbiased sample of the larger population the study aims to describe. A bias is a systematic error which distorts study findings. It

is caused by faults or defects in study design, data collection or analysis. Valid generalizations cannot be made from biased or unrepresentative samples.

6.1.1 Subjects and study design

The study populations in studies #1 and #2 were recruited based on convenience sampling. A convenience sample is a type of non-probability sampling method where the sample is taken from a group of people easy to contact or reach. The only criterion is whether the participants agree to participate. With non-probability samples, it is difficult to know whether the sample represents the population well or not.

In study #1, where EIP repeatability was the issue, the study population consisted of three different sub-groups each consisting of relatively few participants (n = 10), and they were all selected from the same NICU. Thus, the risk of sample bias was present. Studies #1 and #2 were both validation studies performed to test feasibility, repeatability and accuracy of a method relative to a 'gold standard' method, and it may be argued that selection bias is less important in this context than in studies aiming to determine clinical variables. However, strictly speaking, a method is valid only in the population group where it has been tested, and the tested sample should therefore represent this population in the best possible way. To conclude on this issue, we hold that the participants of study #1 represented contemporary 'NICU dwellers' fairly well, but that more participants would have strengthened the testing of repeatability and reproducibility.

In study #3 a control group of infants was used to compare the outcome (lung function) between those exposed (EP-born infants) and not exposed (term-born infants) to extremely preterm birth. This control group was also recruited based on convenience sampling, and it was recruited from only one of the two centers participating in the study, which further constitutes a limitation. The mean birth weight of the control group was 3.5 kg, which matches the average birth weight in Norway, and 53.3% were boys, which indicate that the group constitutes a representative selection of the general newborn population. The respiratory rate of this control group

was 58.2 breaths per minute (95% CI: 54.6, 61.9), which was higher than expected based on knowledge from large population-based studies of healthy newborns [144]. We cannot fully explain this finding, and acknowledge that it questions the generalizability of the control group in our study. A possible technical cause could be that the EIP method misinterprets uneven or choppy breathing cycles, and counts some of them as two instead of one. However, this does not correspond with our findings in study #2, which showed very good agreement for RR between VSP and USFM. It is also possible that the high respiratory rate was caused by the pressure of the vest. When the VSP vests are wrapped around infants, the elastic force of the textile will cause an increased hydrostatic pressure under the vest. This might in turn negatively affect respiration. However, in practical terms, the pressure from the vest to the body is comparable to the pressure of a regular "baby-body". The manufacturer has shown that when the textile in the VSP vest is stretched as much as 15% when used in term infants, it generates a pressure of approximately 0.4 cmH_2O (VoluSense AS, unpublished data). Thus, we conclude that the control group in study #3 had surprisingly high respiratory rate for which we have no good explanation.

The control group was not matched to individual cases by gender or gestational age at the time of measurement. As matching was not used, group comparisons were done by unpaired statistical analyses. The EP-born group had lower proportion of males and lower mean body weight at the time of measurements than the term-born group. It is well known that more girls survive EP birth and that EP-born infants seldom achieve normal birth weight at term-equivalent age. These group differences were adjusted for in the regression models, but they nevertheless represent potential sources of bias.

Studies of EP-born infants should preferably be population based, and studies #3 and #4 were part of a larger prospective population-based cohort study of EP-born infants in Western Norway called 'BabyPEP'. About 95% of all EP-born infants in Western Norway have been included in 'BabyPEP' since study startup. In study #3 a potential for selection bias was introduced by differences regarding birth year between the EP and term-born infants (2011-2013 and 2015-2016 vs. 2015-2016, respectively).

In study #4 an obvious selection bias was introduced by the fact that only EP-born neonates receiving conventional ventilator support during the first 48 hours of life could be included. Thus, the results from study #4 are valid only for EP-born children who require intubation and conventional intermittent positive pressure ventilation during their first days of life.

About previous validation studies. When embarking on these studies, we were aware that data from the few clinical validation studies that had compared EIP with ultrasonic flow meters[131,145] or pneumotachographs[137] did not show unequivocal conformity. That is, Petrus et al. had found that tidal volumes were, on average, 1.3 mL/kg lower with the FloRight system[145] than with an ultrasonic flowmeter, while others had found reasonable agreement between the FloRight system and mask-based methods[131,137]. The other tidal breathing parameters seemed to correspond fairly well in all of these studies.

The somewhat conflicting tidal volume data were of course disturbing. We reasoned that although EIP might possibly underestimate tidal volumes, repeatability data would nevertheless be important in a practical context, particularly if this measurement bias was systematic and thus possible to correct for. Moreover, despite a potential discrepancy from a "gold standard" method, we believed the EIP method could still be used in various settings, including group comparisons and to measure volume changes over time; e.g. in relation to interventions. Given these reflections and the obvious advantage of getting access to an "easy-to-use" measurement system that left the test person undisturbed and potentially could be used in very small and vulnerable babies, we decided to embark on our own repeatability and method comparison studies.

6.1.2 Data collection

One objective of study #1 was to address feasibility; i.e. how well the EIP method (FloRight) was tolerated by the participating infants. This was done objectively by monitoring heart rate, oxygen saturation and respiratory rate, and subjectively by evaluation of infant behavior and facial expressions. The subjective assessments were

of course challenging to standardize; however, all nurses were experienced NICU nurses with long practice in observing newborns. The respiratory rates were monitored by the FloRight system itself, and we could therefore regrettably not directly compare the respiratory rates before versus during the measurements. Moreover, an alternative way of assessing respiratory rates would also have contributed to clarify the aforementioned issues raised by the observed high respiratory rates of the control group in study #3 (pages 71-72). Despite these limitations, we found no reasons to suspect that the application of the vest, or the measurement procedure by itself, disturbed the infants in any noticeable ways.

In study #2 we performed the breathing measurements shortly after a meal although the repeatability study (paper #1) had shown that proximity to meals affected tidal breathing and reduced repeatability. We chose this as the 'lesser of two evils' as the meal made it easier to make the infants sleep and to accept the facemask without using sedation. Since measurements with EIP and USFM were done simultaneously in study #2, the timing of testing in relationship to meals should not affect the comparisons, as this was the same in the respiratory cycles that were compared. So, although relationships to meals seem to be an important issue when it comes to the utility of tidal breathing measurements *per se*, in this particular context it was unlikely to affect the extent of agreement of the EIP and USFM principles.

Study # 3 is one of few studies comparing lung function in EP and healthy term-born infants using tidal breathing parameters obtained with a non-invasive method that does not involve sedation and/or the application of a face mask. In our opinion, the use of this method is an important strength of this study, since a face mask inevitably adds dead space and alters the breathing pattern of the baby, possibly in different ways in health and disease[100-102,104]. None of the participants in this study were sedated, and the data therefore reflect the infants' natural breathing pattern.

Unfortunately, the manufacturer upgraded the equipment during the study and we were forced to comply. We found no systematic differences between FloRight and VSP for any of the tidal breathing parameters when comparing groups of infants who presumably should be equal, suggesting that bias was not introduced (supplemental

material of paper #3). Yet, a full and adequate comparison of the two models were impossible to implement due to the fact that it is impossible to perform simultaneous measurements with two different types of vests, and besides, production of FloRight-vests had ceased before VSP was introduced.

BPD is defined by the need for supplemental oxygen at certain ages[50]. A standardized assessment of 'need for supplemental oxygen' has not yet been implemented into most NICU routines, also not in ours. Thus, differences in administration practices for oxygen supplementation between different departments constitute a well-known source of bias in all studies of this kind, including ours. Studies have shown that using a physiological test, like the room air challenge, reduces the overall rate of infants who are assigned a BPD diagnosis[146,147]. The increasing use of respiratory support strategies that administer ambient air without supplementary oxygen also confounds oxygen-based definitions of BPD[67]. Having said this, the rates of BPD reported in studies from our department does not seem to differ from what is being reported from other departments treating similar groups of preterm born children[72,73,75].
In study #3, the variable 'respiratory morbidity during the first year of life' was defined by need for hospital readmission because of respiratory symptoms, and/or parental reports of treatment with inhaled asthma medication. Both criteria reflect choices that were based on subjective assessments executed by physicians responsible for the care of these children after NICU discharge, and may constitute sources of bias. The threshold for hospital readmissions and start-up with asthma medications probably vary somewhat from doctor to doctor[148] as there are no clear criteria for either of them. However, this lack of standardization would apply similarly to all participating children, and we therefore hold that overall, the described relations between the tested potentially explanatory variables and outcome reasonably well describes the clinical situation of these children.

Nevertheless, a high level of accuracy as regards the prediction of later respiratory morbidity is difficult to achieve, as this is bound to be influenced also by other than perinatal conditions, for example exposures to passive cigarette smoking or contagions and infections induced by older siblings, for which we could not adjust our statistical

models due to few participants. Such factors constitute important sources of bias in study #3, and must be acknowledged when interpreting the results.

6.1.3 Data analysis and statistical methods

In study #1 one may question the apparent discrepancy between relatively similar mean values between the various subgroups of the study, but nevertheless relatively poor measures of repeatability. This important issue of repeatability studies has been carefully discussed in three papers that were referred to in paper #1; two by Bland and Altman and one by Bartlett and Frost[138,140,141]. Differences between group mean values provide an estimate of the average bias of one measurement relative to the other. In our study, all group mean values were close, indicating that no particular bias seemed to be introduced, e.g. by involving an inexperienced examiner or extending the time frame between two measurements or by providing a meal to the infant. This is important information of practical interest. However, we also need to know how well two repeated measurements are likely to agree *for an individual*. In that respect, the standard deviation of the differences between repeated measurements were used to construct a range within which a given percentage of repeated measurements are expected to be found with a given level of certainty; e.g. the range given by the 95% limits of agreement as defined by Bland and Altman. An equivalent measure is the coefficient of repeatability (CR) or CR% (CR as a percentage of the pairwise mean) which is defined as 1.96 standard deviations of the mean pairwise differences between repeated measurements. The CR or CR% provides (as the 95% limits of agreement) an estimate of the size of the expected test-retest measurement interval in any given individual participant. Both these aspects are important to know when putting a new method into practice; i.e., the likelihood of introducing a systematic bias and the size of the range within which two repeated measurements is likely to be found.

The coefficient of variation (CV) is sometimes used to describe test and retest variability and intra-individual variations in lung function measures[149,150]. In our view, this statistical measure is not a preferable way of expressing repeatability[139], but it was calculated to be able to compare our results with those of other studies.

When assessing the findings of study #2, one must keep in mind that it is very unlikely for two different methods or instruments, and also for repeated measurements made with the same method in the same individual, to be exactly in agreement. Therefore, one must decide if any given bias or range of repeatability in any specific context is acceptable or not. A small difference between any predicted normative value and the actually measured value may not be likely to affect patient management decisions. This is particularly so as regards measurements dealing with physiological data that to some extent must vary by nature, such as tidal breathing in infants.

The method comparison problem is discussed in depth by Bland and Altman in articles that are referred to also in paper #2[140,141]. According to Bland and Altman, the answer to how well two methods agree has two components: Firstly, the mean difference is an estimate of the average bias of one method relative to the other. In our study, for all parameters the maximum mean difference was less than 5.5%, and so we can say that the methods agreed very well - on average. Secondly, it is essential to consider how well the methods are likely to agree for a given individual, which is expressed by the limits of agreement. The limits of agreement (average difference ± 1.96 standard deviation of the difference), tell us how far apart the measurements obtained by two different methods are likely to be for most individuals. If the range covered by these limits of agreement is considered 'not clinically important', the two methods may be used interchangeably. What might be 'clinically important'; i.e. how wide limits would be inacceptable, is discretionary and must be decided based on the context in which the method is applied. To our knowledge there is no consensus on this issue as regards tidal breathing measurements. As the physiological variability of infants' breathing patterns are rather wide, strongly influencing most of the parameters of tidal breathing measurements, limits of agreement between two methods within +/- 15% should, in our opinion, be considered acceptable in a clinical context. An open discussion regarding these issues between experts in the field would certainly be welcomed.

Receiver Operating Characteristic (ROC) curve analysis is well suited to evaluate the diagnostic performance of a test[151], or the accuracy of a test to discriminate diseased

cases from normal cases[152]. ROC curves can also be used to compare the diagnostic performance of two or more diagnostic tests. In addition, ROC analysis is often used to find the optimal combination of markers or disease indicators. In disease screening or prediction, a common practice is to obtain various markers that reflect diverse aspects of the disease but that alone may not be a great diagnostic tool, and combine them to potentially help achieve better accuracy. The ROC curve is a useful tool to evaluate the combined diagnostic accuracy[151], and the area under the curve (AUC) is a measure of test accuracy. The major advantage of optimal AUC combinations is that no distributional assumptions are needed for the markers. In addition, it is flexible to incorporate linear or nonlinear combinations[153].

The ROC curve is based only on sensitivity and specificity of a test and takes no account of the prevalence of the disease that is tested for. Whereas sensitivity and specificity (and therefore the ROC curve) are independent of disease prevalence, positive and negative predictive values (PPV and NPV, respectively) are highly dependent on disease prevalence. Therefore, PPV and NPV were not calculated in paper #3, as the prevalence of respiratory morbidity during the first year of life was unknown. PPV and NPV reported in paper # 4 are based on the BPD prevalence in that study, which was 55% (18/33). The reported values for PPV and NPV are thus valid only for this study population. However based on what is reported about BPD occurrence rates in larger studies[73], we believe the reported figures for PPV and NPV are relative representative also for the general EP-born population.

All studies on which this thesis is based have relatively few participants and they should be confirmed by studies with larger number of participants and by research groups operating at other and larger institutions. Due to the relatively small number of participants, particularly in studies #1, #3 and #4, group differences that may have been truly present could have gone undetected due to lack of statistical power (type II statistical errors). Studies that test several outcome variables based on relatively few participants are also at risk of making spurious significant findings; i.e. type I statistical errors, where the null-hypothesis is incorrectly rejected and a supposed

significant effect or relationship is reported when it in fact does not exist. Bonferroni corrections were applied when appropriate in order to control for this latter possibility.

6.2 Discussion of the main results of the study

6.2.1 Validation of the EIP method

Repeatability and reproducibility

Measuring the repeatability of biological variables is challenging, as it is difficult to distinguish exactly how much of the measurement variability is due to the measurement method and how much is due to inborn within-subject variation. It is well known that neonates and infants vary their breathing pattern over time, and that this variation is influenced by factors such as GA and sleep stage[100,154]. In study #1 we found that the repeatability and reproducibility of the FloRight system was relatively poor for the parameters Vt and Tptef/Te. Although in study #1, our study group was composed of three different sub-groups of neonates with different GA and BW as well as different postnatal age and weight when the measurements were performed (all of which factors that obviously constitute sources of heterogeneity), the results were comparable to those reported by other researchers who have studied more homogenous groups[111,149,155,156]. Thus, the relatively poor repeatability reported from our study is likely to reflect variable breathing patterns of the infants and the heterogeneity of the participating study groups, more than the measurement method in itself. However, it is reasonable to assume that loosening and retightening of the vests have contributed to at least some of the variability. Additionally, the infants wore a baby-body underneath the vests during this study, a situation that may have caused a variable degree of wrinkles and thereby variable degrees of underestimation of measured volumes. One may also speculate if repeatability could have been improved if we had included more breaths to the computerized analyses. Considering the variable breathing pattern of infants, our average use of 28 breaths may have been insufficient to provide a representative characterization of their lung function. The simplicity by which the data acquisition period can be extended is one of several advantages of the EIP principle, and this must be tested in future research.

A more variable breathing pattern in neonates with lower GA is reflected in our repeatability figures, as we found poorer repeatability for the smaller premature infants. The differences were not statistically significant, but this might very well be due to the small number of participants in each sub-group (type II statistical error). Of course, it is also conceivable that a smaller torso may contribute to relatively larger measurement errors in smaller infants.

The importance of experience. Experience with the equipment is likely to influence repeatability and accuracy of most types of measurements. We found that to be true also for EIP, reflected by better repeatability for the experienced versus the unexperienced nurses when testing FloRight in study #1. Although all nurses who participated in the study reported that the FloRight system was easy to use, the accuracy of EIP measurements will depend on seemingly minor factors such that the vest fitting properly and correct placement of the antenna. It is likely that experience will improve management of a range of such procedural details, and thus explain the overall finding of better data quality obtained by the experienced nurse. The number of recordings in study #1 where the data quality was considered adequate for analysis was somewhat low (72%), but we believe that this would have improved with further experience with the equipment. As our research team has gained experience with the EIP method, we have learned that we can achieve successful measurements in almost all infants.

Standardization of measurements in relation to meal. Standardization of infant spirometry in relation to meals has been given relatively little attention in the literature. The ERS/ATS Task Force group published a series of documents in the European Respiratory Journal at the beginning of this century on standards for infant respiratory function tests. Comments on relationships between measurements and meals are scarce in those papers. However, an article by Gaultier et al. is being referred to regarding test conditions[157]. Gaultier stated the following about feeding: "*Tests tend to be more successful if the infant is fed, clean and dry. Providing the infant is feeding enterally, most workers feel that it is not necessary to fast the infant prior to testing, even when performing oesophageal manometry or partial expiratory*

flow manoeuvres. However, due consideration should be given to any relevant underlying pathology, particularly oesophageal reflux, with a suitable delay (≥ 30 min) between feeding and measurements in such cases. Although preterm infants with chronic lung disease may be particularly prone to oxygen desaturation following a feed, there is only limited evidence that feeding influences pulmonary function tests in infants."

In our opinion more attention could be drawn to the issue of relationships between meals and pulmonary function testing in infants, and we think that this is emphasized by our findings in study #1 where postprandial measurements were clearly more variable than those obtained prior to a meal. It may be that our results are particularly relevant for the EIP method due to abdominal distention and changes in torso topography after a meal. On the other hand, clinical experience suggests that infants breathe more irregularly shortly after meals. As a consequence of our results in study #1, all measurements in study #3 were made at least half an hour after meals.

About the repeatability of FVg. The FVg parameter, reflecting the shape of the TFV loops and thus potentially suitable for detecting airway obstruction, turned out to have far better repeatability than Tptef/Te. However, since FVg most often will take a value between 0.3 and 0.55, its capacity to differentiate between health and disease may be limited, which is also reflected in the relatively low ICC values for this parameter in study #1. Olden et al. showed that FVg differentiated between prematurely born infants with and without BPD[131]. This corresponds well with our own findings in study #3; however, we reached that same conclusion also for most of the applied 'obstruction parameters' such as TEF_{50}/PTEF, TEF_{75}/PTEF and Tptef/Te. Which parameter will be the better to differentiate healthy from diseased on an *individual* basis, needs to be explored. In this respect there is a need for repeatability data on *all* tidal breathing parameters, and not just the three we tested in study#1.

Ability to separate healthy from diseased – reliability. The relatively large variation in breathing patterns in infants may limit the use of tidal breathing measurements in clinical practice. However, based on the reliability results in study #1 expressed by the ICC figures, the ability of the EIP method to distinguish between subjects based on

lung function measurement can generally be considered to be adequate to good. However, these figures must be interpreted with great care, as they are based on calculations from small numbers of participants (n = 10). The ability of the EIP method to diagnose lung disorder in groups of infants is demonstrated in study #3. However, the ability to diagnose lung disease or to monitor potential effects of clinical interventions on an *individual* basis, still has to be explored. Particularly, such individual use will require development of normative data and reference equations based on healthy neonates and infants of all weight classes, weight, GA at birth, postnatal age and gender.

Agreement between VSP and a 'gold standard method' (USFM)

Our research group is the first to validate VSP, the upgraded version of the EIP method, and so these results cannot be compared to others. However, we found that the agreement between VSP and USFM was very good on average, as all breathing parameters had a maximum 5.5% mean difference, and the results were comparable with previous validation studies of FloRight[131,137]. As mentioned previously, the result of Petrus et al. differ from other validation studies of FloRight as regards tidal volumes (Vt/kg). They found that tidal volumes, on average, were 1.3 ml/kg lower using FloRight compared to an ultrasonic flowmeter and a facemask[145]. This contrasts the difference of only -0.15 ml/kg (VSP minus USFM) that was found in our study#2, and also the results of Olden et al.[131] and of Williams et al.[137] However, substantiated by these findings, and considering the fact that it is difficult to completely avoid a mask leak that will lead to the opposite volume bias, we conclude that both FloRight and VSP probably measure volumes that might be somewhat lower than the actual volumes.

As discussed above, in validation studies comparing two methods, it is also essential to consider how well the methods are likely to agree *for an individual*, which is expressed by the limits of agreement. We hypothesized that the limits of agreement (LoA) (average difference between measurements ± 1.96 standard deviation of the difference) would be within 15% of each other for all tidal breathing parameters when comparing VSP and USFM. Although very close, this was not so for the parameters

Vt/kg (16.0%), V'E/kg (17.0%) and Tptef/Te (18.0%), and the LoA for PTEF was far from what we predicted; 25.9%. Studies comparing FloRight to either an ultrasonic flowmeter or a pneumotachograph show divergent results regarding the variability between paired measurements. Compared to the results of Petrus et al. we found smaller mean differences (i.e. less average bias of one method relative to the other) but somewhat wider limits of agreement, for all breathing parameters[145]. Also compared to Olden et al. the limits of agreement for Vt and Tptef/Te were wider in our study[131]. However, compared to the validation study of Williams et al., comparing FloRight to a pneumotachograph[137], our limits of agreement were narrower. These divergent results are as expected, as the studies referred to have all used different 'gold standard methods'. It should be emphasized that in a context of measuring lung function variables in infants, no 'gold standard method' can be expected to produce 'true values'. Thus, the limits of agreement that were obtained in our case reflect how much variability that can be expected between the two applied measurement methods, not how much measurements obtained with VSP are expected to differ from some 'true value'.

It is important to note that our validation of the accuracy of VSP only applies to "healthy" individuals, as only healthy individuals were tested. Thus, strictly speaking the study cannot provide data that applies also to infants with respiratory distress. Nevertheless, we believe that it is not wrong to infer from the data presented in study#2 that VSP seems well suited for tidal breathing measurements also in respiratory distressed infants; however, this needs to be verified in future studies. In this regard it should also be specified that since the EIP method has been upgraded through VSP, a new repeatability study using the VSP equipment should be carried out. The results of our repeatability study (study #1) - strictly speaking - apply only to the FloRight version of EIP.

Possible influences on the results of agreement. There are several technical factors that can explain differences between the VSP and the USFM. The first one is the facemask. Mask leaks are difficult to avoid completely, and this will influence both flow, volume and time derived parameters, challenging comparisons being made

between data obtained with a flow-based and a plethysmographic method. A common problem during mask-based recording of respiratory waveforms is a leak around the rim of the mask. This will often be more pronounced during expiration, and especially peak expiration, since the pressure inside the mask will be elevated, and thus push the mask away from the face. During inspiration, the mask will instead be sucked towards the face, reducing or even eliminating the leak. This fits well with our results, as VSP largely estimated PTEF higher than USFM, and PTEF was the parameter with the widest LoA. Avoidance of a facemask is perhaps the major advantage of the EIP method. EIP measures the infants' natural and undisturbed way of breathing, and this opens for extended recordings in babies who are respiratory unstable or use equipment that may preclude the use of a facemask, primarily CPAP or HFNC. In accordance with previous studies, we found (study #2) that applying a facemask significantly increased the minute ventilation of the infant[101-103]. This finding is physiologically plausible and most likely explained by increased dead space ventilation induced by the facemask or by tactile stimulation of the facial skin. We also found that parameters reflecting airway obstruction, i.e. Ti/Te and TEF_{50}/PTEF, were significantly increased by the facemask. We believe that this apparent 'anti-obstructive effect' was caused by a slight positive expiratory counter pressure generated mainly by the resistance of the flowmeter. This might increase the average diameter of small and medium sized airways, and thereby cause Te to decrease and TEF_{50} to increase, which is in accordance with our findings.

The VSP recordings may have been influenced by variable use of accessory respiratory muscles incorrectly recognized as thoracic volume changes. Moreover, the VSP measurements can have been influenced by variable displacement of venous truncal blood volume into the head and limbs during respiration. VSP calculates flow from volume changes based on the assumption that all internal volume variations of the torso is caused by airflow within the respiratory system, and that all such airflow induces corresponding external volume variations of the torso. These are small, but not insignificant simplifications that can explain some of the discrepancies we found for most of the tidal breathing parameters. Precise control over these issues requires whole body plethysmography.

Software differences are also a potential source of bias that may influence the agreement between the two measurement methods. Determining the beginning and the end of inspiration and expiration is a well-known problem when it comes to computer based analysis of tidal breathing[113]. It is not possible to know what are the 'true' time points for these events (stop and start). If the USFM (Exhalyzer D) in fact interprets the start of expiration slightly later than VSP, such phase shifts can explain some of the minor differences found for Tptef/Te, Ti/Te and TEF_{50}/PTEF.

The most plausible reason for EIP measuring volumes that might be somewhat lower than the actual volumes, is the inability of the vest to capture breathing movements at the top of the thorax, above the armpit level, as well as at the back towards the underneath surface where the magnetic field fails to create voltage changes. Petrus et al. showed in their study that the surface underneath the infants had a significant influence on the difference in tidal volume between the systems, with a firm surface (cot) giving more accurate results than soft surfaces[145]. This finding seems reasonable, as more of the thoraco-abdominal breathing movements of the infants will "disappear" into a soft underneath surface compared to a firm one. At the back/underneath the baby there is no magnetic field generated by the antenna and no voltage changes are induced. Breathing movements occurring at the back will not be picked up by the EIP method which can therefore explain some of the volume discrepancies we found in our study.

Poor fitting of the vests may also explain differences in tidal volume measurements between EIP and USFM. The high sensitivity to a poor fit of the vest is perhaps the major weakness of the EIP method. The vests should be applied directly to the skin, as the fabric has to enclose the skin tightly with no wrinkles. Any body surface moving with respiration that is not sufficiently covered by the vest will lead to an underestimation of measured volumes. As a consequence, the infants must have their hips extended during the measurements, because flexion of the hips will cause folding of the fabric. Presence of e.g. surgery wounds, gastrochisis, stomas, drainage tubes and malformations of the torso that generate concave regions of the body surface will limit its clinical application. Smaller objects attached to the body surface, such as ECG

electrodes, will give a static volume contribution similar to all tissues already present inside the torso, and will not affect the measurements.

One may speculate whether thoraco-abdominal asynchrony potentially can affect the accuracy of the EIP method. However, EIP makes separate measurements of thoracic and abdominal volume variations. As long as the volumes are accurately estimated, their sum will also be accurate, regardless of the degree of asynchrony. However, if any of the measurements (thorax or abdomen) has a scaling error, then the magnitude of the overall error in the summed signal might be affected, particularly in the case of severe asynchrony. In study #2 the participants were respiratory healthy and did not have much thoraco-abdominal asynchrony, which is an indication of respiratory distress in infants. It is therefore unlikely that this has affected any of the results.

6.2.2. Characterization of lung function in EP-born infants using EIP

Tidal volumes, respiratory rates and minute ventilation

Based on autopsy findings in preterm born infants who have died from BPD[58,158], it seems reasonable to assume that gas exchange in surviving preterm born infants can be compromised or challenged by immature pulmonary gas exchanging units (alveoli) that are characterized by abnormal microvasculature and thicker membranes with less surface area. This would all contribute to higher physiological deadspace ventilation, and thus necessitate higher minute ventilation in order to maintain adequate gas exchange. In principle, minute ventilation can be increased by increasing the respiratory rate or the tidal volume or both.

In study #3 we found that both tidal volume and minute ventilation were higher in EP-born infants compared to term-born infants (6.0 ml/s vs. 4.6 ml/s and 394 ml/kg vs. 259 ml/kg, respectively). This corresponds with the findings of Olden et al., also using EIP (FloRight). They found that mean Vt/kg was 7.0 ml in prematurely born infants with BPD and 5.4 ml in healthy term-born infants[131]. Increased tidal volumes are also potentially consistent with studies using the multiple-breath washout method, reporting lower FRC in EP-born infants[65,129], as one could speculate that a lower FRC could

reflect a lower end-expiratory lung volume and thus possibly also a higher tidal volume. This line of thinking would suggest that preterm-born infants might raise their tidal volumes in order to obtain higher minute ventilation in order to maintain adequate gas exchange. However, this contrasts findings in studies where tidal breathing parameters were assessed with mask-based methods, such as the studies by Schmalisch et al.[126] and Hjalmarson et al.[127]. They report that preterm-born infants with chronic lung disease had *lower* tidal volumes compared to term-born controls, and that higher minute ventilation was instead obtained by a substantially higher respiratory rate. Again; increased tidal volumes may not be captured by mask-based measurements as the face mask alters the breathing pattern.

Lung compliance in preterm-born infants has been shown to be low in some studies[65,159]. If that is correct, and if these infants manage to adjust their breathing to what is the least energy-consuming pattern, this would lead to a breathing pattern that fits best with the findings of Schmalisch et al[126] and Hjalmarson et al.[127], as it is less energy demanding to breathe with lower tidal volumes and higher respiratory rates when compliance is low, i.e. it reduces the work of breathing. On the other hand, higher respiratory rates inevitably lead to higher physiological deadspace which in this scenario is clearly counterproductive.

To conclude on this issue, we have no *proof of concept* for any of these theories. Given the circumstantial evidence that is available, it seems logical that ventilation requirements are in fact increased in these infants, and that various infants may find various ways of solving this challenge according to what leads to the most energy efficient breathing pattern for that particular individual, be it increasing their respiratory rates or volumes – or both.

Airway obstruction

One of the most feared long-term complications of preterm birth is early onset chronic obstructive pulmonary disease or COPD[51]. It is now widely accepted that no single adult lifestyle factor can fully account for the development of COPD, and that the origins of this highly prevalent and much dreaded disease must be sought for in early life or even antenatally[160] and that early life experiences are highly important[161]. Thus,

provision of measures that can be chased longitudinally from the very first beginning of life into adulthood, and that properly describes bronchial dimensions and function by means of numerical and continuous outcome variables are of utmost importance.

We found in study #3 that the lungs of EP-born infants at term equivalent age were characterized by airway obstruction, and that although this was more pronounced in those with a neonatal diagnosis of BPD, it applied also to those who had not been assigned this diagnostic label. Our findings are consistent with most studies comparing EP-born infants at term-equivalent-age with term-born controls, especially for infants with BPD. This applies to studies using EIP as well as mask-based methods[126,130,131]. It is also consistent with long-term follow-up studies that show persistent airway obstruction in older EP-born children[64]. The question is of course; what are the mechanisms behind the observed airway obstruction? And why do those with moderate/severe BPD become the most obstructive? These issues are poorly understood, and all attempts to answer must evidently become speculations.
Lung injury in BPD is associated with altered interstitial fibrous and elastic tissue formation and thickening of the interstitium, and the complex interactions between the interdependent structures of the pulmonary system (i.e. the airways, the vascular structures and the interstitium) are difficult to separate from one another by means of physiologic testing, making it difficult to disentangle the mechanisms behind the impairments. It is also possible that airway obstruction partly has to do with a learned breathing pattern due to the nature of the chest wall. The low outward recoil of the chest wall in EP-born infants allows the lung to deflate to low lung volumes, where peripheral airway function and gas exchange are impaired. To overcome the tendency of the lung to collapse to very low volumes, the infant adopts a strategy of modulating expiratory flow to prolong the expiratory time constant and sustain an adequate resting volume or functional residual capacity (FRC)[126,130].

Is it time to expand on the diagnosis of BPD?

The results of ours as well as those of other studies addressing lung function in EP-born infants[65,162] have questioned the correctness of classifying lung disease of prematurity by the relatively simple and categorical variable BPD. A diagnosis of BPD

contains no information on lung mechanics or function, and in no way provides a comprehensive or complete description of pulmonary disease caused by prematurity. It is used as a dichotomous measure to describe a developmental disorder that, as such, it seems logical to assume is characterized by continuous and perhaps normally distributed outcome measures. In line with these arguments, lung disease of prematurity appears to represent a continuum, and classifying lung morbidity simply by the presence of BPD or not seems too imprecise, both in characterizing lung injury *per se,* and as an outcome measure for attempts to improve early neonatal care, and as a tool to predict future pulmonary health. These issues have been pointed out also by others in recent literature[64,65,162,163].

BPD is by definition a disease that primarily reflects the ability of the lungs to provide the individual with adequate gas exchange and oxygenation while breathing environmental room air without supplementation of additional oxygen. Thus, it seems reasonable to assume that BPD primarily reflects acinar and not bronchial structural and/or functional limitations. Tidal breathing measurements reflect *ventilation* and not gas exchange and as such, tidal breathing measurements are likely to be better suited than BPD to describe bronchial structure and/or function. Moreover, and contrasting BPD, tidal breathing measurements provide data that are by nature numerical and continuous, and thus likely to be well suited for testing relationships between early characteristics, exposures or events versus later outcomes relating to obstructive airway disease. Importantly, tidal breathing measurements are therefore also likely to be suitable outcome measures for interventional studies that need to be performed in high risk preterm born neonates in the years to come[164].

In conclusion, the data provided by study #3 support the notion that BPD needs to be replaced as a measure of lung disease after preterm birth, and that we need more refined, numerical and continuous variables that are targeted towards measuring the capacities of the *airway tree*, which constitutes the organ primarily involved in COPD. Tidal breathing measurements might very well provide such variables, and EIP might be a suitable way of obtaining them. This should be tested in larger studies than ours. Particularly, it should be addressed in carefully planned long-term follow-up studies, if

and to what extent neonatal tidal breathing measurements predict future ventilation incapacity and disease.

6.2.4 Prediction of later respiratory morbidity in infants born EP

In study #3 we addressed if tidal breathing parameters at term age could predict respiratory morbidity during the first year of life. This is important as we know that a large proportion of EP-born infants are susceptible of respiratory tract infections and recurrent wheezing in early childhood[93,94]. The proportion of EP-born infants readmitted to hospital or treated for asthma-like symptoms during their first year of life in this study corresponded well with data from other studies [66,91,165]. Infants with tidal breathing parameters compatible with airway obstruction were more likely to develop respiratory symptoms that were regarded to require treatment in their first year of life, significantly so for the parameters TEF_{50}/PTEF and Tptef/Te. This is in agreement with Proietti et al. who also found that reduced Tptef/Te in preterm infants assessed near term was associated with wheezing during the first year of life[91], and with Drysdale et al. who used the single-breath occlusion technique and found higher airway resistance at 36 weeks' GA in preterm-born infants who were later hospitalized because of viral lower respiratory tract infections[166].

Data from several studies have suggested a life-long tracking of lung function, also in people born EP[167-170]. If lung function measurements at term age could prove, in larger studies, to be so closely related to future lung function that they can be used as a proxy of long term pulmonary outcome in intervention studies aiming at prevention or treatment of neonatal lung disease, it would be of great value.

6.2.5 BPD prediction from ventilator flow data

In study#4 we aimed to find lung function measures that can be obtained shortly after birth, and that might reflect inborn susceptibilities for the lung injuries that are associated with prematurity. This is particularly important as it may be that preterm birth, at least partly and/or in some individuals, represents an intermediate variable for an unfavorable intrauterine environment or for disadvantageous events or series of events that occurred *before birth*, and led to preterm birth *as well* as to later obstructive

airway disorder; i.e. a classical 'set-up' where apparent associations are confused by one or more confounding factors. In this context it is pertinent to quote Wilcox who stated that "*preterm babies carry the burden of whatever pathology triggered their early birth*[171]. Obviously, BPD being a diagnosis assigned at one month´s postnatal age, could be a misleading variable if this scenario should prove important in the causal pathway or pathways that lead to lung disease after preterm birth.

Thus, the original plan for this study was to measure lung function immediately after birth in all ventilator dependent EP-born individuals by acquiring flow and volume data from the ventilator and to construct tidal breathing variables based on this information. Moreover, the EIP method was meant to be applied in all admitted infants in order to track lung function from birth and during their NICU stay until discharge, and thus produce longitudinal sets of the same tidal breathing variables, thereby providing a set-up for longitudinal tracking from birth of variables that presumably directly reflect airway obstruction. However this set-up was complicated by the previously described technical challenges and lack of functioning measurement equipment during a critical period of time of this project. Additionally, validation and use of the EIP method in the smallest EP-born neonates was considered insufficient. Thus, what we can report on at this stage, are tidal breathing variables acquired shortly after birth from ventilator data in ventilator dependent neonates, and tidal breathing variables acquired at discharge using the EIP method. Thus, we cannot provide (as planned) data from all preterm born neonates during the first few hours of life but only from those who were ventilator dependent, and we are left with a knowledge gap as regards longitudinal data covering the period between birth and discharge. These unfortunate circumstances constitute (annoying) limitation of this study.

We found in study #4 that the group of EP-born neonates who later went on to develop the more severe clinical course and who were characterized by the most prolonged need of oxygen supplementation, in fact where those whose lung function appeared least obstructive shortly after birth. This was certainly unexpected, considering the aforementioned clear tendency for more obstructive airway patterns in a similar group

of individuals at term equivalent age, and also that almost all studies that report on lung function later in life consider BPD a major risk factor for airway obstruction.

We cannot exclude that our findings might reflect flow conditions that were influenced by characteristics of the endotracheal tubes or the ventilator treatment *per se* or by leaks or secretions in the tube. As regard production of secretions, this usually increases with ventilator treatment time, and participants of this study were measured during their first few hours of life (mean age 17 hours). In our NICU, suction of the endotracheal tube is performed 'as needed'. This implies that some of the neonates had had their tube suctioned, while others had not – and for those who had, the time between suction and measurement varied. Besides, there were no clinical signs of tube obstruction in any of the participants during the time of data acquisition, and all neonates had an appropriately sized tube with minimal laryngeal air leaks. Furthermore, the obtained expiratory flow patterns do not agree with the pattern expected with tube obstructions or leaks. In conclusion, it seems reasonable to assume that mucous production, suction versus no suction, tube size or air leaks did not play a major role in explaining the findings.

Our findings were unexpected, as BPD in preterm-born infants is usually associated with an obstructive airflow pattern at term-equivalent age, and also throughout childhood, adolescence and early adulthood[64,126,131]. The study did not produce data that can contribute to a solid understanding of the physiology behind these unexpected observations, and one should be careful about speculating too much on possible explanatory models. However, it is not unreasonable to assume that those neonates who later went on to develop the more severe forms of BPD were those who were born with the most immature lungs, characterized by low compliance due to low surfactant production and less developed and stiffer airway structures[5,172]. The chest wall has a major influence on the underlying lungs, and its outward recoil is generally low in preterm born infants because of the soft rib cage and scarce intercostal muscles[172]. Perceivably, the overall effect for the most severely ill neonates could thus be an initial phase characterized by primarily high pulmonary elastic recoil pressures and low compliance, creating tidal airflow patterns that appear less obstructive. One may

further speculate that low compliance could lead to more intense early interstitial and peribronchial inflammatory responses to positive pressure ventilation, with a further decrease in compliance, and thus setting up vicious circles. Access to airflow variables throughout the NICU stay of these infants would have been highly valuable.

In most studies of EP-born infants, low GA has been shown to be a risk factor for BPD, also within the already low GA interval of participants of the present study[173,174]. In this study, GA did not differ between the groups with and without BPD, and it is therefore unlikely that GA influenced the comparisons between these two groups. This can be considered a strength of the study, as differences in GA would have constituted a source of bias when it comes to assessing the ability of tidal breathing parameters to predict BPD.

Although, to our knowledge, no previous studies have reported on tidal flow volume loops obtained shortly after birth in EP-born neonates, several studies suggest that neonates who go on to develop different severity of BPD have innate lung differences. Those who develop the severe forms tend to need more prolonged mechanical ventilation[64,127], a larger proportion is perceived to need treatment with surfactant, they have different cytokine- and growth factor patterns[68,80], and a significant genetic predisposition has been proposed, although poorly understood[78,81].

To our knowledge, the present study is the first attempt to examine to what extent easily accessible flow data from a mechanical ventilator can be used to predict later development of BPD in EP-born neonates. A study by Bhutani et al. assessed the relationship between pulmonary compliance and resistance and subsequent BPD in low birth weight infants (≤ 1500 g) who required mechanical ventilation during their first week of life. They found that low dynamic pulmonary compliance and high total pulmonary resistance were related to BPD[175]. Of their various 'BPD prediction models' they concluded that a model including GA and dynamic pulmonary compliance had the best predictive accuracy[175]. A study by Kim et al. concluded that modified ventilator parameters, i.e. peak inspiratory pressure relative to birth weight (PIP/kg) and mean airway pressure relative to birth weight (MAP/kg) at 12 hours of

age were significant risk factors for the development of BPD[176]. Both the findings of Bhutani et al. and Kim et al. are very well consistent with our findings in study #4.

A general problem of various scoring systems that has been proposed for predicting BPD based on clinical variables and biomarkers, have been their relatively low specificity or low positive predictive values[177]. Furthermore, the need for prolonged observation and inclusion of complications or events that by nature simply cannot be predicted shortly after birth (such as later exposure to mechanical ventilation, sepsis and/or the persistence of a patent ductus arteriosus) obscure, unlike in our study, the significance of innate predispositions. To reveal innate predispositions are, in our opinion, fundamental when attempting to understand probable causes and thus ways of preventing a progression towards severe chronic lung disease in children born premature.

In conclusion, study #4 presents data that can be used to predict the clinical neonatal respiratory course reasonably accurately, based on information that is easily obtainable in all infants who require ventilator treatment shortly after birth. To what extent this information can be used to predict the clinical respiratory course later in life should be subject to investigation in carefully planned long-term follow-up studies. Specifically and importantly, flow variables obtained according to the same measurement principles can - and should - be followed longitudinally from birth through the NICU-stay and childhood, as was the original plan for the present study.

7. CONCLUSION

Interpretations of the main findings in relation to the hypotheses of this thesis:

1. The EIP method is well tolerated by term-born and preterm-born infants.
   Interpretation: The EIP method could successfully be applied in most instances, and did not disturb the infants in any noticeable way (study #1).
   Hypothesis sustained.

2. The repeatability of the EIP method is as good as those reported for traditional infant spirometry.
   Interpretation: the repeatability of the EIP method (FloRight) was acceptable and comparable to that reported for existing equipment (study #1).
   Hypothesis sustained.

3. The repeatability of the EIP method is as good for preterm as for term-born infants.
   Interpretation: The repeatability of the EIP method, as represented by FloRight, seemed to be better for term infants than for infants born premature. This probably reflects a more variable breathing pattern in neonates with lower GA rather than inaccuracies of the method itself, however this needs to be confirmed by further studies (study #1). Hypothesis rejected.

4. Reproducibility of the EIP method depends on the experience of the person who performs the measurement and on the temporal relationship to meals.
   Interpretation: The experienced nurse produced more repeatable data, and repeatability was better before than after meals (study #1).
   Hypotheses sustained.

5. The results of EIP measurements are *independent* on who is selecting the breath traces that are selected for the computerized analyses.
   Interpretation: Two independent persons who selected breath traces for analyses resulted in tidal breath variables that did not differ (study #1). Hypothesis sustained.

6. The differences between tidal flow values obtained with the EIP method (VoluSense Pediatrics) and traditional mask based spirometry utilizing an ultrasonic flowmeter (Exhalyzer D) are within 15%.
   Interpretation: The two methods produced data that were in the range of 15-20% of their mean values (study #2). Hypotheses partly sustained.

7. Application of a facemask will influence the measurement result when performing tidal breathing measurements in infants.
   Interpretation: When measured simultaneously (EIP and USFM) it became apparent that the mask did influence the tidal flow measurement (study #2). Hypotheses sustained.

8. The lung function at term-equivalent age of infants born EP, with or without BPD, will differ from the lung function of term-born infants.
   Interpretation: Most tidal breathing variables differed significantly between preterm and term-born infants, and the differences were more pronounced in preterm born infants with a neonatal history of BPD (study #3). Hypotheses sustained.

9. Tidal breathing parameters at term-equivalent age can predict respiratory morbidity during the first year of life of infants born EP.
   Interpretation: A combination of tidal breathing parameters and easily available characteristics did predict respiratory morbidity with a high degree of accuracy (study #3). Hypotheses partly sustained.

10. Early lung mechanics expressed by tidal breathing parameters obtained shortly after birth from ventilated EP-born neonates, will reflect susceptibility for later development of BPD.
    Interpretation: a combination of tidal breathing parameters obtained from ventilators and easily available characteristics did predict neonatal BPD with a high degree of accuracy (study #4). Hypotheses partly sustained.

8. FUTURE PERSPECTIVES

EIP emerges as a feasible method of infant lung function testing. Its strengths are the relative simplicity by which it can be put to use, and that it allows sampling of data for long periods of time without disturbing the baby. As it does not involve a facemask, it measures the undisturbed and natural way of breathing, and it can be applied in infants needing respiratory support such as CPAP and HFNC. Thus, EIP is likely to be well suited for NICUs and hospital wards treating neonates and infants with a wide spectrum of pulmonary illnesses. Potentially, it can be used in neonates with birthweight way below 1 kg, and thus facilitate large scale early tracking of lung function in preterm-born infants. If combined with tidal breathing variables obtained from ventilators, EIP provides the potential of creating longitudinal tidal lung function measurements from birth. Tidal lung function variables by nature bear interesting similarities with the variables obtained from standard spirometry used from mid-childhood until old age, opening for life-long tracking and potentially predicting COPD in these high-risk individuals. However, more extensive validation studies as well as procedural standardizations are clearly needed before the method can be applied clinically. We also need normative data for infants of all weight classes and postnatal ages, and by GA and weight at birth and for males and females.

This thesis has demonstrated that EIP can provide relatively easily accessible tidal breathing measurements that can aid the characterization of lung disease after preterm birth. The variables provide continuous numerical outcome measures that already shortly after birth may discriminate between neonates destined for a difficult or a less complicated clinical respiratory course during their NICU stay as well as during their first year of life. If subsequent evaluations were to confirm the findings of this thesis, EIP and tidal breathing parameters have the potential of early recognition of neonates at risk of severe forms of lung disease of prematurity and of later respiratory morbidity, thereby paving the way for lifelong targeted surveillance. Future clinical management and properly designed intervention studies - so urgently needed in these high-risk individuals - can be guided and assessed using properly scalable methods and not by vaguely defined grouping variables or categories, such as BPD.

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10. APPENDIX

APPENDIX 1

BabyPEP - SPØRRESKJEMA VED 3 MÅNEDER KORRIGERT ALDER

(sett kryss og kommentarer i aktuell rubrikk – ta med utfylt skjema til legen)

Navn:_____ F.dato:_____

Undersøkelsesdato:_____

Ernæring

- Kryss av de typer mat, vitaminer o.l barnet får <u>nå:</u>

☐ Morsmelk ☐ Grøt ☐ Vitaminer

☐ Morsmelkserstatning ☐ Grønnsaker/frukt ☐ Jern

Type: _____ ☐ Middager ☐ Annet: _____

Hvor mange måltider får barnet per døgn? _____	**JA**	**NEI**
• Ammes barnet ?	☐	☐
• Har det vært vedvarende ernæringsproblemer (stadig oppkast, diaré, spisevegring, mageknip o.l) Hvis ja; beskriv:_____ _____ _____	☐	☐

Lunger/luftveier	**JA**	**NEI**
• Har barnet <u>noen gang</u> etter utskrivelse fra sykehuset hatt pustevansker, tung pust eller piping/surkling/tetthet i brystet? Hvis ja; beskriv: _____ _____	☐	☐

- Hvor mange anfall av pustevansker, tung pust eller piping/surkling/tetthet i brystet har barnet hatt? _____
  _____

Søvn

- Hvordan er barnets døgnrytme? _____
  _____

	JA	NEI
• Våkner barnet om natten?	☐	☐
Hvis ja; hvor mange ganger: _____		
• Opplever du/dere at det er problemer relatert til barnets søvn? Hvis ja; beskriv:_____	☐	☐
_____		

Familiesituasjon

• Har det vært endringer i familieforhold siden siste kontroll (f.eks sykdom, flytting, skilsmisse, økonomiske problemer)?	☐	☐
Hvis ja; beskriv: _____		
_____		

Andre forhold

• Har barnet vært sykt siden forrige kontroll?	☐	☐
Hvis ja; beskriv: _____		
_____		
• Bruker barnet noen medikamenter?	☐	☐
Hvis ja; hvilke?_____		
_____		
_____		
• Har barnet vært vedvarende slapt eller stivt?	☐	☐
Hvis ja; beskriv: _____		

- Er det noe angående barnet som bekymrer deg? ☐ ☐

  Hvis ja; beskriv:_____
  _____
  _____
  _____
  _____
  _____
  _____
  _____

Dato: _____ Underskrift:_____

APPENDIX 2

BabyPEP - SPØRRESKJEMA VED 6 MÅNEDER KORRIGERT ALDER

(sett kryss og kommentarer i aktuell rubrikk – ta med utfylt skjema til legen)

Navn:_____ F.dato:_____

Undersøkelsesdato:_____

Ernæring

- Kryss av de typer mat, vitaminer o.l barnet får nå:

☐ Morsmelk ☐ Grøt ☐ Vitaminer

☐ Morsmelkserstatning ☐ Grønnsaker/frukt ☐ Jern

Type: _____ ☐ Middager ☐ Annet: _____

☐ Kumelk ☐ Brødmat _____

Hvor mange måltider får barnet per døgn? _____

	JA	NEI
• Ammes barnet ?	☐	☐
• Har det vært vedvarende ernæringsproblemer (stadig oppkast, diaré, spisevegring, mageknip o.l) Hvis ja; beskriv:_____ _____ _____	☐	☐

Lunger/luftveier	**JA**	**NEI**
• Har barnet noen gang etter utskrivelse fra sykehuset hatt pustevansker, tung pust eller piping/surkling/tetthet i brystet? Hvis ja; beskriv: _____ _____	☐	☐

- Hvor mange anfall av pustevansker, tung pust eller piping/surkling/tetthet i brystet har barnet hatt? _____
  _____

Søvn

- Hvordan er barnets døgnrytme? _____

  _____ **JA** **NEI**

- Våkner barnet om natten? ☐ ☐

  Hvis ja; hvor mange ganger: _____

- Opplever du/dere at det er problemer relatert til barnets søvn? Hvis ja; beskriv:_____ ☐ ☐

  _____

Familiesituasjon

- Har det vært endringer i familieforhold siden siste kontroll (f.eks sykdom, flytting, skilsmisse, økonomiske problemer)? ☐ ☐
  Hvis ja; beskriv: _____

  _____

Andre forhold

- Har barnet vært sykt siden forrige kontroll? ☐ ☐

  Hvis ja; beskriv: _____

  _____

- Bruker barnet noen medikamenter? ☐ ☐
  Hvis ja; hvilke?_____

  _____

  _____

- Har barnet vært vedvarende slapt eller stivt? ☐ ☐

  Hvis ja; beskriv: _____

- Er det noe angående barnet som bekymrer deg? ☐ ☐
  Hvis ja; beskriv:_____

  _____

  _____

  _____

_____
_____
_____
_____

Dato: _____ Underskrift_____-

APPENDIX 3

BabyPEP - SPØRRESKJEMA VED 12 MÅNEDERS KORRIGERT ALDER

(sett kryss og kommentarer i aktuell rubrikk – ta med utfylt skjema til legen)

Navn:_____ F.dato:_____

Undersøkelsesdato:_____

Ernæring

- Kryss av de typer mat, vitaminer o.l barnet får nå:
  ☐ Morsmelk ☐ Grøt ☐ Vitaminer

  ☐ Morsmelkserstatning ☐ Grønnsaker/frukt ☐ Jern

  Type: _____ ☐ Middager ☐ Annet: _____

  ☐ Kumelk ☐ Brødmat _____
- Hvor mange måltider får barnet per døgn? _____
- Ammes barnet ? ☐ ja ☐ nei
- Har det vært vedvarende ernæringsproblemer (stadig oppkast, diaré, spisevegring, mageknip o.l)? ☐ ja ☐ nei

  Hvis ja; beskriv:_____
  _____

Søvn

- Hvordan er barnets døgnrytme? _____
  _____
- Våkner barnet om natten? ☐ ja ☐ nei

  Hvis ja: Hvor mange ganger: _____
- Opplever du/dere at det er problemer relatert til barnets søvn? ☐ ja ☐ nei

  Hvis ja; beskriv:_____

Familiesituasjon

- Har det vært endringer i familieforhold siden siste kontroll (f.eks sykdom, flytting, skilsmisse, økonomiske problemer)? ☐ ja ☐ nei

  Hvis ja; beskriv: _____

  _____

Andre forhold

- Har barnet vært sykt siden forrige kontroll? ☐ ja ☐ nei

  Hvis ja; beskriv: _____

  _____

- Bruker barnet noen medikamenter? ☐ ja ☐ nei

  Hvis ja; hvilke:_____

  _____

- Har barnet vært vedvarende slapt eller stivt eller hatt rykninger? Hvis ja; beskriv: _____ ☐ ja ☐ nei

  _____

- Er barnet til tider urolig eller gråter mye? ☐ ja ☐ nei

  Hvis ja; beskriv: _____

  _____

- Er det noe ved barnet som bekymrer deg? ☐ ja ☐ nei

  Hvis ja; beskriv:_____

Spørsmål om lunge/luftveisproblemer

1.1 Har barnet <u>noen gang</u> hatt tung pust eller piping/surkling/tetthet i brystet ?

☐ ja ☐ nei

Hvis du har svart nei, gå til spørsmål 1.5

1.2 Hvor mange anfall av tung pust eller piping/surkling/tetthet i brystet har barnet hatt ? ☐ ingen ☐ 1 til 3 ☐ 4 til 12 ☐ >12

1.3 Hvor ofte har barnets søvn i gjennomsnitt blitt forstyrret på grunn av tung pust eller piping/surkling/tetthet i brystet?

☐ aldri våknet

☐ mindre enn 1 natt pr. uke

☐ 1 eller flere netter pr. uke

1.4 Har barnet hatt tung pust eller piping/surkling/tetthet i brystet under eller etter aktiv lek ? ☐ ja ☐ nei

1.5 Har barnet hatt tørr hoste om natten, utenom hoste i forbindelse med en forkjølelse eller andre luftveisinfeksjoner ?

☐ ja ☐ nei

Dato:_____Underskrift_____

11. Papers I, II, III and IV

I

ACTA PÆDIATRICA
NURTURING THE CHILD

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REGULAR ARTICLE

A new non-invasive method of infant spirometry demonstrates a level of repeatability that is comparable to traditional methods

Mariann H. L. Bentsen (Mariann.Haavik.Lysfjord.Bentsen@helse-bergen.no, Mariann.Bentsen@k2.uib.no)[1,2], Øystein A. Haaland[1,3], Lise B. Lønning[1], Hrefna K. Gudmundsdottir[1], Trond Markestad[1,2], Thomas Halvorsen[1,2]

1.Department of Pediatrics, Haukeland University Hospital, Bergen, Norway
2.Department of Clinical Science, Faculty of Medicine and Odontology, University of Bergen, Bergen, Norway
3.Department of Global Public Health and Primary Care, University of Bergen, Bergen, Norway

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Infant, Plethysmography, Premature, Respiratory function tests, Spirometry

Correspondence
M Bentsen, MD, Department of Pediatrics, Haukeland University Hospital, N-5021 Bergen, Norway.
Tel: +47 55970871 |
Fax: +47 55975147 |
Emails: Mariann.Haavik.Lysfjord.Bentsen@helse-bergen.no, Mariann.Bentsen@k2.uib.no

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ABSTRACT

Aim: The FloRight system provides novel non-invasive infant spirometry based on electromagnetic inductance plethysmography. We investigated the consistency of repeated measurements carried out in a Norwegian neonatal intensive care unit (NICU) using the system and how well these were tolerated.

Methods: Tidal flow-volume loops were obtained from 10 preterm infants at discharge, 10 stable growing preterm infants weighing about 1500 g and 10 term-born infants. A nurse experienced with the system measured all patients before and after meals, and these measurements were repeated by nurses new to the system.

Results: The measurements were well tolerated by the infants. The repeatability for the two parameters 'tidal volume' (Vt) and 'time to peak tidal expiratory flow to total expiratory time' (Tptef/Te) were relatively poor, similar to previous methods. However, the repeatability was good for the new 'flow-volume gravity mid-point' (FVg) parameter. Repeatability was better for term than preterm infants, when measurements were obtained by the experienced nurse and for measurements carried out before meals.

Conclusion: The FloRight system proved feasible in a NICU setting. The repeatability of the lung function measurements was similar to those reported for traditional infant spirometry. The nurse's experience and the relationship to meals appeared to be important.

INTRODUCTION

Lung diseases are common in neonates and early childhood. However, our understanding of the detailed lung mechanics in this age group is limited, during both health and disease, as methods for lung function measurements are complex and not readily available (1). This situation hampers our approach to medical care and also complicates lifelong tracking of lung function. Infant spirometry most commonly involves measuring airflow through a face mask, but other methods have also been used, such as whole-body plethysmography or respiratory inductance plethysmography (1–5). Most of these methods are time-consuming, require some form of sedation and disturb the infants, which means that they can only be used for brief periods and are not really suitable for clinical settings other than dedicated specialist laboratories. In addition, a mask may alter the pattern of breathing and the added dead space will influence the parameters measured (6–8).

The FloRight system (VoluSense, Bergen, Norway) is a novel method of infant spirometry, based on electromagnetic inductance plethysmography. The method is non-invasive, easy to calibrate, does not require sedation and is free of dead space artefacts.

Clinical validation studies comparing the FloRight system with ultrasonic flow meters (9,10) or pneumotachographs (11) have shown somewhat conflicting results for tidal volume measurements. Petrus et al. found that tidal volumes were, on average, 1.3 mL/kg lower with the FloRight system (10), while others found reasonable agreement between the FloRight system and mask-based methods (9,11). In all of these studies, the other tidal breathing parameters corresponded with each other and all the

Key notes

- The FloRight system is a novel non-invasive method for infant spirometry based on electromagnetic inductance plethysmography.
- We investigated the consistency of repeated measurements carried out on full and preterm infants by different nurses before and after meals and found that FloRight compared well with results from more intrusive methods.
- Repeatability was better for term infants, when measurements were obtained by a more experienced nurse and when carried out before meals.

systems were able to discriminate between healthy and diseased lungs.

The aim of this study was to assess the clinical applicability of the FloRight system, by examining the consistency of repeated measurements and how well the measurements were tolerated by infants.

PATIENTS AND METHODS

Subjects

We studied 30 infants in the neonatal intensive care unit (NICU) at Haukeland University Hospital, Bergen, Norway, who did not require assisted ventilation or oxygen supplementation at the time of study. These comprised 10 preterm infants examined at discharge and 10 stable growing preterm infants weighing approximately 1500 g, who were basically healthy, but still needed help to eat and regulate their temperature and were under surveillance for apnoeas. Six of the preterm infants examined at 1500 g and five of those examined at discharge had respiratory distress shortly after birth. The other ten were full-term infants who were in the NICU because of initial hypoglycaemia, anaemia, thrombocytopenia or suspected infection, but were diagnosed as healthy after short-term observation and, or, treatment. None of them had respiratory symptoms. The characteristics of the participants are shown in Table 1.

The study was approved by the Regional Committee on Medical Research Ethics of Western Norway and reported to *ClinicalTrials.com* (ID no. NCT01057472). Informed, written consent was obtained from the parents.

Equipment

The FloRight system consists of two thin metal wire coils that are sewn in a circular fashion into a thin, soft cotton vest, which is worn over the entire chest and abdomen. The upper coil covers the chest and the lower coil covers the abdomen, enabling separate analyses. A weak (100 mA) alternating electric current is passed through the coils, producing a magnetic field around the child's torso. The strength of this field is measured by a sensor positioned directly above the cot. The system is calibrated against a volume reference cylinder in the cot at the side of the infant, so that the system knows the exact volume contained in the vest. This volume expands and decreases in proportion to the infant's breathing movements. The volume signals are converted into flow signals by numerical differentiation, and flow-volume loops and tidal breathing parameters are calculated.

Table 1 Characteristics of the participants

	Term infants	Preterm infants at discharge	Preterm infants under NICU care
Number of infants	10	10	10
Gestational age at birth in weeks	39.0 (1.8)	32.3 (1.6)	31.0 (1.6)
Birthweight in grams	3598 (1096)	1833 (464)	1531 (159)
Age at test in days	7.9 (12.6)	27.6 (18.3)	4.4 (4.2)
Postmenstrual age at test in weeks	40.1 (2.0)	36.4 (1.6)	32.0 (1.9)
Weight at test in grams	3589 (1035)	2267 (341)	1479 (184)
Vt (mL/kg)	5.0 (1.8)	6.6 (2.1)	4.8 (1.4)
Tptef/Te	35.1 (9.6)	37.8 (13.9)	48.7 (13.0)
FVg	0.46 (0.03)	0.47 (0.04)	0.50 (0.03)

Vt, tidal volume; Tptef/Te, time to peak expiratory flow as a ratio of total expiratory time; Fvg, flow-volume gravity; NICU, neonatal intensive care unit. Values are group means and standard deviations (SD).

Measurements

We selected the correct-sized vest by measuring the infant's chest circumference at the level of the nipple. It was put over a thin layer of clothing and covered the infant from the armpits to the groin. The vest was tightened so that it provided an adequate fit but did not constrict the torso or inhibit the infant's breathing. All measurements were carried out at the cot with the infant in a supine position. The antenna was positioned straight above the bed at the height specified by the manufacturer, and before any measurements were carried out, the system was calibrated by placing a cylinder of known volume next to the child. Four measurement sessions, each lasting approximately five minutes, were carried out for each baby by two different nurses, Nurse A and Nurse B, in the 30 min before a planned meal in the following sequence: A1 before, B1 before, A2 before and B2 before. There was a five-minute interval between each of the four measurements. The measurements were then repeated twice by Nurse A approximately 10–15 min after a meal, and these were called measurements A3 after and A4 after. The vest was loosened and tightened between each measurement session, but not removed from the baby. The infants were relaxed, often sucking on a pacifier, and quietly awake or asleep during varying proportions of the measurement sessions. No sedation was used. The infant's oxygen saturation and heart rate were monitored by pulse oximetry and the respiratory rate by the FloRight system. The infant's behaviour, their facial expression and breathing patterns were subjectively observed and recorded by the examining nurse.

Seven nurses took part in the study, and they all received basic training in the equipment used in the study, including the manufacturer's operating instructions. Nurse A had independent experience in performing the test before the formal study was initiated and was the same for all the infants. Nurse B varied and was one of six different nurses. However, Nurse B was always the same for the same infant, meaning that each infant was measured by the same two nurses.

Data selection

The traces were visually inspected to select two or more segments that consisted of a minimum of 10 consecutive stable breaths to enable us to calculate the tidal breathing parameters. The selection criteria were no obvious artefacts, no sighs and no obvious changes in the depth of breathing

or baseline. Two raters, who were both physicians working in the NICU (HG and MB), independently selected breathing segments for computerised analysis using software provided by the manufacturer. The following parameters were recorded for each measurement session and averaged over the selected breathing segment: (i) tidal volume (Vt) in mL, (ii) time to peak tidal expiratory flow as a ratio of total expiratory time (Tptef/Te) and (iii) flow-volume gravity mid-point (FVg) (Fig. 1). FVg is the expiratory flow centroid, that is the dimensionless value given by the position of the centre of gravity of the expiratory flow-volume loop along the volume axis, where the start of expiration is defined as zero and the end as one. A symmetrical, normal curve will give an FVg of 0.5. Airway obstruction will prolong the end-expiratory phase and shift this point to the left and give values below 0.5, as shown in Figure 1. Calculation of the expiratory flow centroid has been found to provide a good description of the tidal expiratory waveform (12) and of changes in the tidal expiratory waveform seen in airflow obstruction (9,11,12).

Comparison of measurements

Repeatability, reproducibility and reliability are important properties when assessing how clinically useful a method is (13–16). *Repeatability* refers to the variations that occur when test and retest measurements are performed by the same person under identical conditions, and it provides an estimate of the minimum level of agreement between replicated measurements. *Reproducibility* is the degree of agreement between measurements performed by different examiners or under different conditions, in this case before and after a meal. *Reliability* is how much of the variations in the measurements are due to the sum of inborn within-subject variation and measurement errors as opposed to true differences in lung function mechanics. If reliability is high, the variability caused by measurement errors and inborn within-subject variations are low compared to the variations caused by subjects being different. Hence, subjects can be well distinguished from each other.

To estimate the repeatability of Nurse A and Nurse B, we compared the measurements of the respective nurses before the meal (A1 before versus A2 before and B1 before versus B2 before) and also after meal for Nurse A (A3 after versus A4 after). To determine the repeatability and reliability for the different categories of infants, comparisons were performed using the results from Nurse A, the most experienced nurse, before the meal. Interexaminer reproducibility was tested by comparing data collected by Nurse A and Nurse B before the meal using the measurements A1 before and A2 before versus B1 before and B2 before. Reproducibility before and after meal was analysed by comparing measurements A1 before and A2 before versus A3 after and A4 after. The results of the computerised analyses after the selection of the two raters were compared. As the breathing segments were obtained by different raters, but from the same measurement sessions, they can be referred to as both interrater repeatability and intrasession repeatability.

Statistical methods

To determine repeatability and reproducibility, we calculated the coefficient of repeatability (CR), which was defined as 1.96 standard deviations (SD) of the mean pairwise differences between measurements. We have reported these findings using CR%, which is CR as a percentage of the pairwise mean. The CR or CR% provides an estimate of the size of the test–retest

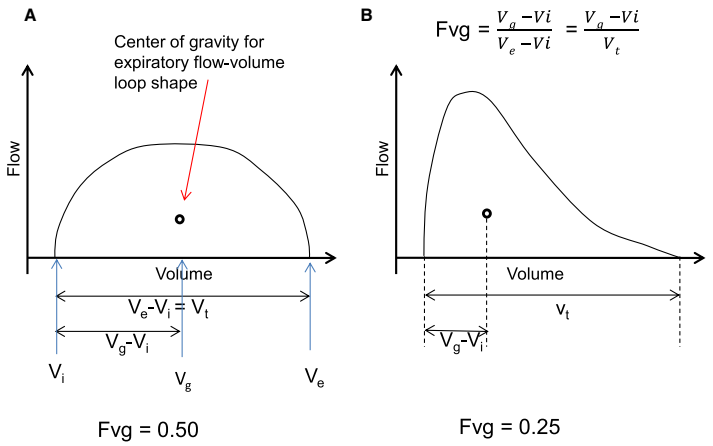

Figure 1 Flow-volume gravity mid-point (FVg). FVg is a dimensionless value given by the position of the centre of gravity of the expiratory flow-volume curve along the volume axis, using a linear scale where zero is the volume at start of expiration and one is the volume at end of expiration. FVg will therefore have a value between zero and one. (A) A symmetrical (normal) curve will give an FVg of 0.5. (B) Airway obstruction will shift the curve to the left and produce values for FVg below 0.5. Vg, volume of centre of gravity; Vi, inspiratory volume; Ve, expiratory volume; Vt, tidal volume.

measurement differences, for example a CR% of 10 means that 95% of all the test–retest differences were within ±10% of their pairwise mean. Paired-sample *t*-tests were applied to assess whether the mean pairwise differences of the various sessions differed significantly from zero. *F*-tests for the equality of variances were used to detect differences between the CRs. The coefficient of variation (CV) was also calculated for the pairwise measurements. The CV was calculated as the SD of all the measurements divided by their mean and is expressed as a percentage.

To examine reliability, the intraclass correlation coefficient (ICC) was calculated. The ICC is used to assess the consistency, or conformity, of measurements within a group, and it describes how strongly subjects in the same group resemble each other. It is defined as follows:

$$\mathrm{ICC} = \frac{(\text{SD between subjects})^2}{(\text{SD between subjects})^2 + (\text{SD within subjects})^2}$$

The ICC is expressed as a value between zero and one. Reliability was considered good if the ICC values were ≥0.75 and adequate or fair to good if $0.4 < \mathrm{ICC} < 0.75$ (17). One-way analysis of variance (ANOVA) was used to estimate between-subject and within-subject SDs. Data were analysed with MedCalc version 13.1 (MedCalc Software, Mariakerke, Belgium).

RESULTS

Feasibility and applicability

Having the vests fitted and wearing them did not cause any noticeable discomfort to the infants. There were no changes in respiratory rate, heart rate or oxygen saturation and no subjective changes in facial expressions, breathing patterns or other alterations in behaviour while wearing the vests (data not shown).

Of the 176 recordings, 126 (72%) provided adequate quality for the analyses. The reasons for irregular or variable tidal flow-volume (TFV) loops that could not be analysed were not always obvious, but the system was sensitive to movements from the child. It also reacted to interference from nearby electrical devices, such as spotlights, fluorescent lamps and battery chargers, but these problems were largely solved when we became aware of them. In two participants, measurements were only performed before the meal for practical reasons. A mean of 28 breaths (SD 9) was used to calculate mean values of the different tidal breathing parameters.

Intersession repeatability and reliability

Nurse A obtained successful paired measurements before the meal for 22 participants (A1 before versus A2 before) and Nurse B obtained them for 20 participants (B1 before versus B2 before). After the meal, Nurse A obtained 23 successful paired measurements (A3 after versus A4 after). There were no significant differences between the mean values of any paired measurement sessions for any of the breathing parameters (data not shown).

In terms of CR%, we found relatively large test–retest variability for Vt and Tptef/Te for nurses A and B, which indicates relatively poor repeatability (Table 2). FVg showed good repeatability. Nurse A achieved better repeatability (lower CR%) for all breathing parameters than Nurse B (Table 2), but the difference was only significant for Vt ($p < 0.05$, results not shown). After meals, the repeatability of Nurse A significantly decreased (CR% increased) for Vt ($p < 0.05$), but not for the other parameters (Table 2).

CR% tended to be better for the term-born than smaller premature infants, but the differences were not statistically significant (Table 3).

The reliability was good for Vt ($\mathrm{ICC} > 0.75$) for term infants and preterm infants under NICU care and adequate or fair to good ($0.4 < \mathrm{ICC} < 0.75$) for preterm infants at discharge. The opposite pattern was found for Tptef/Te and FVg, where the reliability was good for preterm infants at discharge and adequate or fair to good for term infants and preterm infants receiving NICU care (Table 3).

Reproducibility for different examiners

There were no significant differences in mean values between the paired measurements performed by nurses A and B, which comprised 42 measurements of A1 before and A2 before versus B1 before and B2 before (Table 4). Interexaminer reproducibility, as expressed by the CR%, was similar to the repeatability of the experienced nurse, Nurse A, before meals ($p > 0.05$) (data not shown). Again, the CR% showed best reproducibility for FVg and larger variability for the parameters Vt and Tptef/Te.

Reproducibility before and after meal

There were no significant differences in the mean values obtained before and after meals, which comprised 44 measurements of A1 before and A2 before versus A3 after and A4 after (Table 4). However, the reproducibility was poor, especially for Vt and Tptef/Te, as demonstrated by CR % of 81.1 and 78.8, respectively (Table 4).

Interrater and intrasession repeatability

There were no significant differences between the results based on the blinded and independent selections by the two raters from all the 126 recordings (Table 2). The variability expressed by CR% was low for all parameters, suggesting that the raters had little influence on the results.

DISCUSSION

The FloRight system did not disturb the infants or interfere with their respiratory behaviour. The repeatability of the lung function measurements was similar to those reported for traditional infant spirometry using a face mask, which is much more intrusive (5,18–22). The repeatability of the FVg parameter was consistently good and FVg also showed good reliability for preterm infants at discharge. The more

Table 2 Inter- and intrasession repeatability

	Nurse A A1 versus A2[a]			Nurse B B1 versus B2[b]			After meal $A3_{after}$ versus $A4_{after}$[c]			Intrasession/interrater repeatability[d]		
	Difference (95% CI)	CR%	CV (%)	Difference (95% CI)	CR%	CV (%)	Difference (95% CI)	CR%	CV (%)	Difference (95% CI)	CR%	CV (%)
Vt (mL)	−0.92 (−2.1, 0.3)	37.5	14.1	−0.23 (−2.8, 2.3)	79.4*	28.0	0.36 (−1.4, 2.1)	57.2*	20.3	0.12 (−0.31, 0.07)	14.3	5.2
Tptef/Te	4.4 (−1.5, 10.4)	61.6	22.9	−0.33 (−7.5, 6.9)	76.0	26.7	−1.5 (−6.0, 3.1)	53.1	18.9	0.28 (−1.2, 0.7)	26.8	9.6
FVg	0.01 (−0.004, 0.03)	13.3	5.0	0.004 (−0.02, 0.02)	17.3	6.1	0.001 (−0.01, 0.01)	13.8	4.9	0.001 (−0.004, 0.001)	5.7	2.0

Nurse A was the same experienced nurse for all infants, Nurse B comprised several nurses, but the same nurse performed the two tests on the same infant.
Differences are group means with 95% CIs. CR%, coefficient of repeatability reported as a percentage of the pairwise means. CV, coefficient of variation, expressed as one standard deviation in percentage of the average value of the measurements. Vt, tidal volume; Tptef/Te, time to peak expiratory flow as a ratio of total expiratory time; Fvg, flow-volume gravity.
[a] n = 22.
[b] n = 20.
[c] n = 23.
[d] n = 126 (all recordings).
*Significantly higher than CR% for Nurse A before meal (p-value by *F*-test < 0.05).

experienced examiner, Nurse A, demonstrated improved repeatability, and the relation to when the infants received their meal appears to have been important.

It may seem confusing that the repeatability was limited despite similar mean values for the various group comparisons, but this apparent contradiction is thoroughly discussed in the statistical literature (13,15,16). Similar mean group values suggested that different examiners, the introduction of a meal or the somewhat extended time frames between measurements did not introduce systematic bias. Moreover, our mean values were basically what we expected when we compared them with those reported by others who used the FloRight system (9,23), except that the preterm-born group still receiving NICU care had smaller weight-corrected tidal volumes in our cohort (Table 1). There is a scarcity of tidal breathing data in infants under 36 weeks of postmenstrual age, partly due to the complexity of previous measurement methods. Therefore, the influence of factors such as gestational age, weight at birth and at the time of measurement, postnatal age, gender and neonatal lung disease is insufficiently understood and these issues certainly need to be explored further. Some of the inconsistencies regarding tidal volume measurements for electromagnetic inductance plethysmography are somewhat disturbing (9–11). However, even if the method underestimates true tidal volumes, the present data on repeatability and reliability are still helpful and indeed of practical importance.

Test and retest variability and intra-individual variations in lung function measures have commonly been described by the mean CV. In our view, this statistical measure is not a preferable way of expressing repeatability (14), but we used it to compare our results with other studies. The CVs in our study were comparable to those of Lødrup Carlsen et al. (19) and Fuchs et al. (24), which both used a face mask system when examining term-born infants. Olden et al. (9) used the FloRight system on term infants and reported similar repeatability for Vt and FVg, expressed as CR and CR%, as in the present study, but better repeatability for Tptef/Te (CR% 37% versus 51%). We may have improved the variability in Tptef/Te by calculating repeatability over a larger number of breaths (21), but few breaths cannot explain the difference between our results and those of Olden et al. (9), as their results were only based on 10 consecutive breaths.

As our repeatability figures are comparable to those reported for other methods, it is probable that the variability mainly reflects variable breathing patterns of the infants and not the method itself (5,18–22), although, for example, loosening and retightening the vest may have contributed to some of the variability. Newborn infants vary their breathing patterns over time, and this variation is influenced by factors such as gestational age and sleep stage (1). In our study, standardisation of sleep or awake states and shorter time intervals may have improved, but not eliminated, the effect of variations in breathing patterns. Large variations in breathing patterns in infants challenge all attempts to measure infant tidal breathing, and tidal breathing variables

Table 3 Repeatability and reliability of tidal breathing parameters in the different participant groups (repeated measurements by the same nurse: Nurse A1 versus A2)

	Term infants[a]				Preterm infants at discharge[b]				Preterm infants at under NICU care[c]			
	CR%	ICC	W-S SD/B-S SD	CV (%)	CR%	ICC	W-S SD/B-S SD	CV (%)	CR%	ICC	W-S SD/	CV (%)
Vt	22.2	0.98	1.4/9.7	7.4	48.7	0.62	2.5/3.2	17.7	47.3	0.81	1.33/2.71	18.7
Tptef/Te	50.9	0.64	7.7/10.2	22.1	61.8	0.80	8.6/17.3	20.8	66.0	0.59	11.7/14.1	4.9
FVg	9.2	0.65	0.020/0.027	4.4	14.5	0.83	0.023/0.051	24.6	14.9	0.55	0.027/0.030	5.4

CR%, coefficient of repeatability reported as a percentage of the pairwise means. ICC, the intraclass correlation coefficient. W-S SD, within-subject standard deviation; B-S SD, between-subject standard deviation; NICU, neonatal intensive care unit.
CV, coefficient of variation, expressed as one standard deviation in percentage of the average value of the measurements.
Vt, tidal volume; Tptef/Te, time to peak expiratory flow as a ratio of total expiratory time; Fvg, flow-volume gravity.
[a]n = 7.
[b]n = 8.
[c]n = 7.

Table 4 Mean values and reproducibility for different nurses (left) and before versus after meal (right)

	Nurse A before meal (n = 48)	Nurse B before meal (n = 45)	Difference Nurse A versus Nurse B (95% CI)	CR% reproducibility Nurse A versus Nurse B	Nurse A after meal (n = 50)	Difference before versus after meal (95% CI)	CR% reproducibility before versus after meal
Vt (mL)	13.2 (6.3)	13.3 (6.5)	−0.17 (−1.03, −0.78)	46.2	13.9 (6.3)	−0.38 (−2.2, 1.4)	81.1
Tptef/Te	40.9 (12.9)	40.2 (13.4)	0.58 (−3.0, 4.2)	56.8	39.8 (14.6)	−0.62 (−6.0, 2.8)	78.8
FVg	0.48 (0.03)	0.47 (0.04)	0.011 (−0.004, 0.025)	13.4	0.47 (0.04)	0.003 (−0.010, 0.015)	17.0

Figures are group means with standard deviations (SD) or 95% confidence intervals (95% CI). Nurse A before meal, Nurse B before meal and Nurse A after meal are mean values of two replicate measurements. Differences are measurements made by Nurse A minus Nurse B before meal (right table, n = 42) and Nurse A before meal minus Nurse A after meal (left table, n = 44). CR%, the coefficient of repeatability, reported as a percentage of the pairwise means. Vt, tidal volume; Tptef/Te, time to peak expiratory flow as a ratio of total expiratory time; Fvg, flow-volume gravity.

should, therefore, be cautiously interpreted irrespective of the measurement technique used. Nevertheless, methods with similar repeatability, such as plethysmographic measures of residual lung volume in children, have proved useful in assessing respiratory disorders (25,26).

We found that the interexaminer reproducibility did not differ from the repeatability of the experienced nurse. However, this does not mean that the experience of the examiner was unimportant. Repeatability was poorer for the six inexperienced nurses, and this showed that experience improved precision. The accuracy of the measurements will depend on the vest fitting properly and on the correct placement of the antenna and the calibration cylinder. Although all of the nurses felt that the system was easy to use, such details may improve with experience.

Our results suggest that the relationship to the meal was important when carrying out lung function testing in infants, as the repeatability after the meal was poorer. This was in contrast to authoritative advice, admittedly based on very limited experience (27). It may be that our finding was particularly relevant for the FloRight method due to abdominal distention and changes in torso topography after a meal. On the other hand, clinical experience suggests that infants breathe more irregularly shortly after meals and it is our opinion that poorer repeatability after a meal was mainly caused by changes in breathing patterns. The similar mean values obtained before and after meals certainly strengthen this assumption.

We found that term-born infants demonstrated a tendency towards better repeatability for all breathing parameters than preterm-born babies. This could be because there were more variable breathing patterns in the preterm-born infants in our cohort (28), but it is also conceivable that a smaller torso may contribute to relatively larger measurement errors. However, the study was vulnerable to type two statistical errors due to the small number of participants. On the other hand, the strengths of our study were the strictly defined patient groups and the meticulous adherence to the study protocol.

The FVg parameter should be investigated more thoroughly, as also pointed out by Olden et al. (9), as it had far better repeatability than Tptef/Te. FVg reflects the shape of the TFV loops, which has been shown to be unchanged during long-term measurements despite breath-to-breath variations (28). By definition, FVg must have a value between zero and one and, in most cases, it is between 0.3 and 0.55, which may limit its capacity to differentiate between health and disease. However, Olden et al. (9) studied prematurely born infants at discharge and showed that FVg differentiated infants with and without bronchopulmonary dysplasia. Their finding corresponds with our finding of better reliability, based on ICC, and larger

group heterogeneity for FVg, based on larger standard deviations between subjects, in our similar premature group at discharge. This group also had better reliability, based on ICC, for Tptef/Te than the other groups (Table 3). Although all three groups were considered healthy, the premature group at discharge probably had the largest variation in lung function, because of variations in early lung disease and abnormal lung maturation due to prematurity. The finding therefore suggests that the FloRight system can discriminate between patterns of lung function and be useful for exploring lung disease in infancy and early childhood.

The strengths of the FloRight system, as we see it, are its relative simplicity and the fact that it allows continuous and prolonged sampling of data without disturbing the baby and without using a face mask. The method is therefore suitable for lung function testing in large samples of infants (23) with a wide spectrum of respiratory diseases. It can be applied in situations where a face mask is not tolerated, for example in patients requiring continuous positive airway pressure (11,29) or high-flow nasal cannulas and if sedation may carry risks, such as in bronchiolitis. The method also appears to be well suited for monitoring the effects of clinical interventions. However, more studies are needed before the system is used widely, particularly in terms of validation against established methods, because there have been few previous validation studies, those that have been carried out have tended to be rather small and they have produced conflicting data.

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CONFLICTS OF INTEREST

The authors have no conflict of interests to declare.

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ORIGINAL ARTICLE
PAEDIATRIC PULMONOLOGY

Electromagnetic inductance plethysmography is well suited to measure tidal breathing in infants

Mariann H.L. Bentsen[1,2], Morten Eriksen[3], Merete S. Olsen[1], Trond Markestad[1,2] and Thomas Halvorsen[1,2]

Affiliations: [1]Dept of Pediatrics, Haukeland University Hospital, Bergen, Norway. [2]Dept of Clinical Science, Faculty of Medicine and Odontology, University of Bergen, Bergen, Norway. [3]VoluSense AS, Bergen, Norway.

Correspondence: Mariann H.L. Bentsen, Department of Pediatrics, Haukeland University Hospital, N-5021, Bergen, Norway. E-mail: bens@helse-bergen.no

ABSTRACT Reliable, accurate and noninvasive methods for measuring lung function in infants are desirable. Electromagnetic inductance plethysmography has been used to perform infant spirometry and VoluSense Pediatrics (VSP) (VoluSense, Bergen, Norway) represents an updated version of this technique. We aimed to examine its accuracy compared to a validated system measuring airflow *via* a facemask using an ultrasonic flowmeter.

We tested 30 infants with postmenstrual ages between 36 to 43 weeks and weights from 2.3 to 4.8 kg, applying both methods simultaneously and applying VSP alone. Agreement between the methods was calculated using Bland–Altman analyses and we also estimated the effect of applying the mask.

Mean differences for all breathing parameters were within ±5.5% and limits of agreement between the two methods were acceptable, except perhaps for peak tidal expiratory flow (PTEF). Application of the facemask significantly increased tidal volume, minute ventilation, PTEF, the ratio of inspiratory to expiratory time and the ratio of expiratory flow at 50% of expired volume to PTEF.

VSP accurately measured tidal breathing parameters and seems well suited for tidal breathing measurements in infants under treatment with equipment that precludes the use of a facemask.

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Accuracy of VoluSense Pediatrics
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Conflict of interest: Disclosures can be found alongside this article at openres.ersjournals.com

Introduction

Tidal breathing measurements and monitoring provide valuable information about lung function in infants, and can be used to evaluate lung development and management of respiratory diseases [1, 2]. However, these measurements are currently largely used as research tools because most techniques are complex and time-consuming, require sedation, or involve the application of a facemask that adds dead space and alters the breathing pattern of the infant [1, 3–6].

Electromagnetic inductance plethysmography (EIP) provides a relatively novel and noninvasive supplement to previous and more intrusive methods. The EIP system consists of an electromagnet and a patient vest encircling the torso in order to quantify chest and abdominal volume changes. It does not require sedation, and allows for continuous and prolonged recording of respiratory data without using a facemask. EIP can be seen as an extension of respiratory inductance plethysmography (RIP), a validated technique that has been available for decades [2, 7, 8]. The clinical use of RIP has been limited by complex calibration procedures, in contrast to EIP, which allows for simple and patient-independent calibration. FloRight (VoluSense, Bergen, Norway) was the first prototype to use the EIP principle, with several studies demonstrating its accuracy and usefulness [9–13], although Petrus *et al.* [14] reported relatively large tidal volume differences *versus* an ultrasonic flowmeter. VoluSense released an updated version of their method in 2015, called VoluSense Pediatrics (VSP), aiming to improve the accuracy of the volume measurements mainly by making changes to the patient vest and in the configuration of the magnetic field.

This study was performed in order to investigate the accuracy of tidal breathing parameters measured by VSP compared to a validated system measuring airflow *via* a facemask using an ultrasonic flowmeter, Exhalyzer D (Eco Medics, Duernten, Switzerland). We hypothesised that the differences between the two methods would be within 15% of each other, a range considered acceptable in a clinical setting. We also aimed to investigate how application of the facemask influenced the measurements.

Methods

Participants

Newborn infants without a need for assisted ventilation or oxygen supplementation and with a weight ⩾2.0 kg were eligible. The weight limit was set because Exhalyzer D is not approved for children <2 kg. Only healthy children were recruited, as simultaneous measurements with two methods were expected to be somewhat cumbersome and time consuming. The participants were mostly recruited from the maternity clinic at Haukeland University Hospital, Bergen, Norway, and studied either just before discharge or when returning to the outpatient clinic for routine screenings. Six participants were recruited from the hospital's neonatal intensive care unit where they initially had been admitted for either prematurity, hypoglycaemia or suspected infection, but they were ready for discharge when measured. Informed written consent was obtained from the parents. The study was approved by the Regional Committee on Medical Research Ethics of Western Norway.

Equipment and modifications compared to the old EIP model

VSP consists of a patient vest shaped as a rectangle and made from very elastic fabric with two thin conductive elements attached to the outside in a wide zig-zag pattern (figure 1). The upper zig-zag wire covers the chest and the lower the abdomen, enabling separate analyses and detection of thoracoabdominal asynchrony. The vest is wrapped around the torso of the baby and fastened with Velcro. An electromagnet positioned above the cot at a precise height and parallel to the baby's spine generates a weak magnetic field that alternates with a frequency of ∼100 kHz. The magnetic field induces a voltage in the metal wires of the vest that is proportional to the instantaneous average cross-sectional area of the torso covered by the wires, and increases and decreases proportionally to the infant's breathing movements. The system is initially calibrated against a calibration coil with a known area (A_C) positioned at the side of the infant in the cot to establish the relationship between the induced voltage (U_C) and this area. During measurements, the thoracic or abdominal volumes are calculated as

$$V = \frac{U_M A_C W_Z}{U_C}$$

where U_M is the voltage that is measured and W_Z is the width of the zig-zag pattern of the conducting loop in the vest. According to Faraday's law of induction, the induced voltage of a conductor loop will be proportional to the area regardless of its shape, given a homogeneous magnetic field. The volume signal is converted by a computer into flow by numerical differentiation, and flow–volume loops and tidal breathing parameters are calculated [15]. All calculations and storage of raw data are performed at a rate of 100 Hz. The volume variations of the torso are assumed to be caused by variations in the volume of air in the lungs.

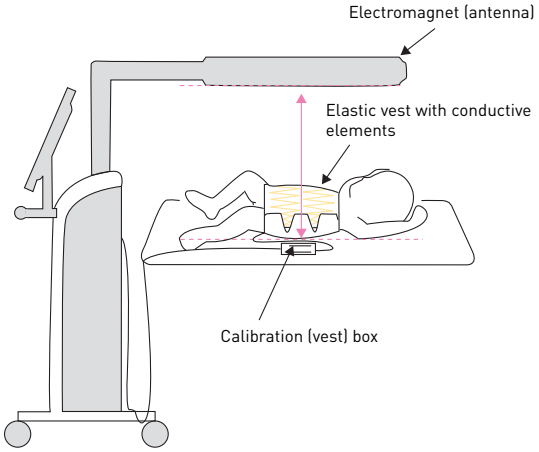

FIGURE 1 Schematic drawing of the VoluSense Pediatrics (VoluSense, Bergen, Norway) measurement system. The supine infant is wearing an elastic vest with conductive elements. An electromagnet positioned above the cot at a precise height generates a weak magnetic field that induces a voltage in the metal wires of the vest. This voltage is proportional to the cross-sectional area of the torso, and will increase and decrease proportionally to the breathing movements of the infant. The system is initially calibrated against an area reference coil positioned at the side of the infant. A computer controls the system during collection and logging of data.

Compared to FloRight, which was described in detail previously [13], VSP is different mainly in the design of the patient vest and in the configuration of the magnetic field, but these changes have also led to upgrades of hardware and software. For a detailed description of the differences between FloRight and VSP, please see the online supplementary material. An ultrasonic flowmeter (Exhalyzer D) with a flow head suitable for infants >2 kg body weight and a flow-range of ±0.5 $L \cdot s^{-1}$ was used as a reference method. The system was calibrated according to the instructions from the manufacturer with a 10-mL volumetric syringe. A neonatal facemask with soft edges allowing a good seal was applied. The total dead space of the system (including the mask) was 9 mL. Measurements were performed according to the latest European Respiratory Society/American Thoracic Society standards of infant lung function testing [16].

Protocol/study design

Validation of VSP was based on simultaneous measurements with VSP and Exhalyzer D, as illustrated in figure 2. The effect of the facemask was assessed by comparing the VSP measurements obtained before to those obtained after the facemask was put on. The infants were dressed in the appropriate-sized vest selected according to the length from the armpit to the hip. Care was taken to ensure that the vest fitted snugly around the torso, and that the width of the copper wire zig-zag covered the entire thorax and abdomen including the pubic region. The vest was applied directly to the skin; neither a body nor a nappy was worn underneath, but a nappy and a warm blanket were put on outside the vest. The infants were fed before being placed supine in a cot and encouraged to sleep. No sedation was used. Once the infant fell asleep, data were collected with VSP alone for 2–3 min. The VSP measurement was continued while the Exhalyzer D neonatal facemask was placed gently, but firmly, over the infant's mouth and nose, ensuring a good seal. Once the facemask was in place, a marker was inserted in the VSP recording. The Exhalyzer D measurement was started ~30 s after the facemask was put on, to allow adaptation to the facemask. Simultaneous recording using both devices was then performed for ~1 min before the mask was removed. New markers were inserted in the VSP recording as the Exhalyzer D measurement was started and stopped.

Data analysis

The markers in the VSP recording (figure 2) enabled visual identification of the corresponding respiratory cycles from the VSP and the Exhalyzer D recording. This allowed for comparison of the same flow, volume and timing parameters from each device. Segments with movement artefacts, apnoeas or sighs were removed from both recordings before the breathing segments were analysed using the software provided by the respective manufacturers.

Effect of the facemask was assessed by comparing the breathing parameters obtained from the VSP before *versus* after putting on the Exhalyzer D mask.

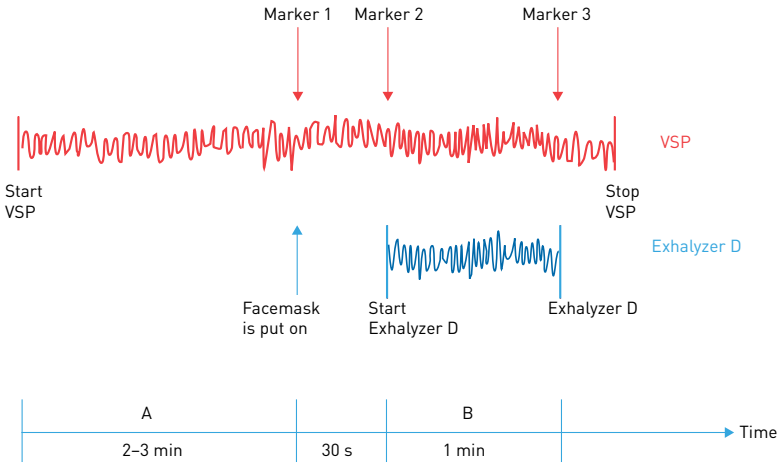

FIGURE 2 Schematic illustration of the study design. When the infant fell asleep, data were first collected with VoluSense Pediatrics (VSP) (VoluSense, Bergen, Norway) alone for 2–3 min (A) before the Exhalyzer D (Eco Medics, Duernten, Switzerland) neonatal facemask was placed over the infant's mouth and nose. Once the facemask was in place, a marker was inserted in the VSP recording. To allow adaptation to the facemask, the Exhalyzer D measurement was started ~30 s after the facemask was put on. Simultaneous recording using both devices was done for ~1 min (B) before the mask was removed. New markers were inserted in the VSP recording as the Exhalyzer D measurement was started and stopped.

Statistical methods

Data were analysed and graphs were created with SPSS version 22 (IBM SPSS Statistics, Armonk, NY, USA) and MedCalc version 13.1 (MedCalc Software, Mariakerke, Belgium).

We constructed Bland–Altman plots to visualise agreement between the two measurement techniques [17, 18]. After the inclusion of 18 cases, the Bootstrap resampling method [17] was used to estimate the number of participants needed in the study. We concluded that the reliability of the 95% limits of agreement would not improve by including >30 participants in the study. One-sample t-tests were used to assess if the mean differences between data obtained with the two methods differed significantly from zero, indicating the presence of a consistent bias. To evaluate the existence of proportional bias, *i.e.* that the methods did not agree equally through the range of measurements, the difference between the methods was regressed on the average of the two methods. To evaluate the effect of the facemask, we compared the means of the different breathing parameters from the VSP recording with and without facemask using paired sample t-tests.

Results

Participants

30 infants with postmenstrual ages ranging between 36 and 43 weeks and a weight range of 2.3–4.8 kg were studied. Their characteristics are shown in table 1. Successful simultaneous measurements were obtained for all participants. In 14 cases, the simultaneous measurement was successful at the first attempt; in 13 cases, at the second attempt; and in three cases, at the third attempt. The most common cause of unsuccessful measurements was babies waking up, disapproving of application of the facemask. Other causes were movement artefacts in the VSP recordings or obvious mask leakage in the Exhalyzer D recording.

TABLE 1 Characteristics of the participants (n=30)

Males	18 (60%)
Gestational age at birth weeks	38.0 (36.5–39.5)
Postnatal age at study days	13.1 (3.4–22.8)
Postmenstrual age at study weeks	39.9 (39.2–40.6)
Birth weight kg	3.2 (2.9–3.6)
Weight at study kg	3.4 (3.2–3.7)
Length at study cm	49.5 (48.7–50.4)

Data are presented as mean (95% CI) unless otherwise stated.

Comparison of the two methods

Agreements for all tidal breathing parameters are presented in figure 3.

Tidal volumes (per kg) showed a nonconsistent and nonsignificant mean difference (VSP minus Exhalyzer D) of −0.15 mL·kg^{-1}, with limits of agreement within ±0.93 mL·kg^{-1}. This corresponds to a difference of −3.0% and limits of agreement within ±16.0% (figure 3a and b).

Mean minute ventilation was 3.8% lower for VSP and the difference was consistent based on a one-sample t-test (figure 3c). Measured respiratory rates differed by <1% (figure 3d). PTEF was higher when measured by VSP than by Exhalyzer D; on average, the difference was +5.0%. The difference was consistent but with a relatively large standard error and wide limits of agreements (figure 3e).

Time to peak tidal expiratory flow (*t*PTEF)/total expiratory time (*t*E) ratio differed, on average, by −5.4% (VSP minus Exhalyzer D), with limits of agreement of ±18.0% (figure 3f). Regression analysis revealed a small proportional bias of the mean difference (p=0.0008, regression slope 0.51 and r^2=0.33). The inspiratory time (*t*I)/*t*E ratio and the tidal expiratory flow at 50% of expired volume (TEF50)/PTEF ratio both corresponded well, with small mean differences (−3.1% and −3.0%, respectively; VSP minus Exhalyzer D) and relatively narrow limits of agreement (figure 3g and h). A small proportional bias was found for both parameters (p=0.005, regression slope 0.23 and r^2=0.25; and p=0.034, regression slope 0.38 and r^2 =0.15, respectively).

Influence of the facemask

Application of the facemask significantly increased the tidal volume and the minute ventilation. *t*PTEF, *t*I/*t*E and TEF50/PTEF were also significantly increased. No difference was found for respiratory rate or *t*PTEF/*t*E (table 2).

Discussion

The principle of EIP, as utilised by VSP, accurately measured tidal breathing parameters in infants when compared to data obtained from Exhalyzer D, which is based on measuring airflow through a facemask. Mean differences for all tidal breathing parameters were within ±5.5%. The limits of agreement between the two methods were within ranges that, in our opinion, were acceptable given a clinical context, with the possible exception of PTEF. Application of the facemask significantly increased tidal volume, minute ventilation, *t*PTEF, *t*I/*t*E and TEF50/PTEF.

Comparison of the two methods and possible influences

This is the first published study using VSP, and thus, comparison with previous validation data is impossible. However, compared to results from previous validation studies of FloRight, the results generated by VSP are just as accurate. Petrus *et al.* [14] included 37 infants in their study and found that tidal volumes, on average, were 1.3 mL·kg^{-1} lower using FloRight compared to an ultrasonic flowmeter and a facemask. This contrasts with the results of Olden *et al.* [9], who compared FloRight to an ultrasonic flowmeter, and the results of Williams *et al.* [11], who compared FloRight to a pneumotachograph. Both these studies found close agreement between the methods. It also contrasts with the difference of 0.15 mL·kg^{-1} that was found in the present study testing VSP. However, substantiated by these findings, and considering the fact that it is difficult to completely avoid a mask leak, which will lead to the opposite volume bias, both FloRight and VSP probably measure volumes that might be somewhat lower than the actual volumes. The most plausible reason for this is the inability of the vest to capture breathing movements at the top of the thorax, above the armpit level, as well as at the back towards the underneath surface where the magnetic field fails to create voltage changes. The accuracy of Exhalyzer D is, according to the manufacturer, better than 2% for flow- and volume-derived parameters, which is not expected to contribute substantially to the calculated mean differences between the two methods or to their limits of agreement.

For all parameters except *t*PTEF, *t*I/*t*E and TEF50/PTEF, we found a consistent bias, which means that the difference between the methods can be adjusted for by subtracting the mean difference from the VSP method. The proportional biases found for *t*PTEF/*t*E, *t*I/*t*E and TEF50/PTEF, which indicate that the limits of agreement will be wider as the numerical level of the pairwise mean increases, were small and probably of limited clinical relevance when testing infants.

Compared to the results of Petrus *et al.* [14], we found smaller mean differences but somewhat wider limits of agreement for all breathing parameters. In addition, Olden *et al.* [9] found limits of agreement for tidal volume and *t*PTEF/*t*E that were narrower than in our study. In the validation study by Williams *et al.* [11], however, where FloRight was compared to a pneumotachograph, the limits of agreement were wider than in our study. In other words, studies comparing FloRight to either an ultrasonic flowmeter or a pneumotachograph show divergent results regarding the variability between paired measurements. This is as expected, as the studies have all used different "gold-standard methods". It should be emphasised that no

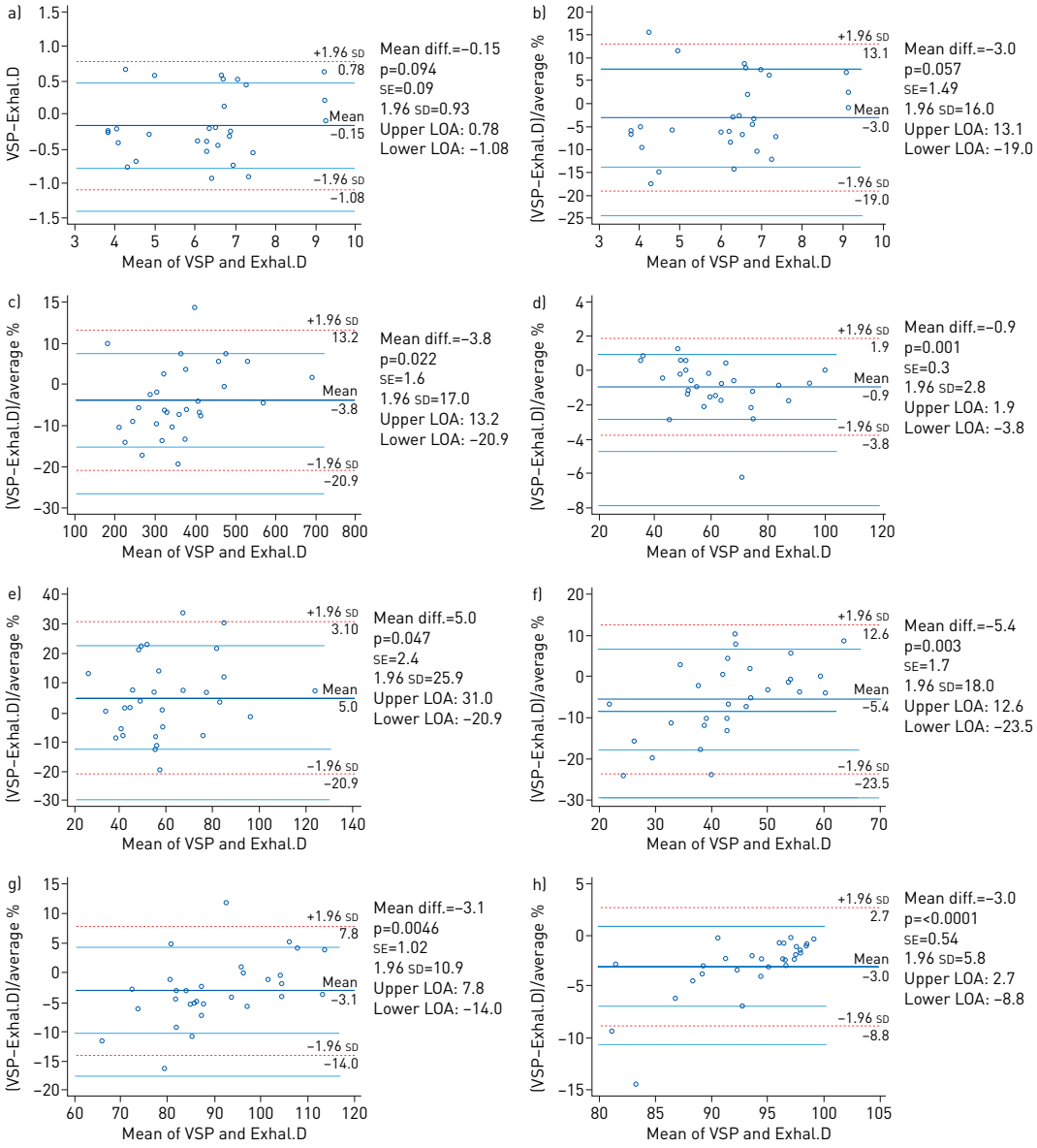

FIGURE 3 Agreement regarding tidal breathing parameters between VoluSense Pediatrics (VSP) (VoluSense, Bergen, Norway) and Exhalyzer D (Exhal.D) (Eco Medics, Duernten, Switzerland) expressed as Bland–Altman plots. a, b) Tidal volume per kg body weight; c) minute ventilation per kg body weight; d) respiratory rate (per min); e) peak tidal expiratory flow (PTEF) ($mL \cdot s^{-1}$); f) time to PTEF/expiratory time (t_E) ratio (%); g) inspiratory time/t_E (%); h) tidal expiratory flow at 50% of expiratory volume/PTEF ratio (%). Horizontal axes represent the mean of the two measurement values ((VSP+Exhal.D)/2). Vertical axes: a) difference in tidal volume between the two methods as raw data (VSP minus Exhal.D); b–h) differences as percentages of their mean value, *i.e.* the relative difference between the methods. p-values are from one-sample t-tests, testing the mean difference between the two methods against zero. Mean diff.: mean difference between the two methods; LOA: limits of agreement.

gold-standard method can be expected to produce a "true value" for infant airflow variables. Thus, the limits of agreement reflect how much the two methods differ from each other for most individuals, not how much VSP differs from the true value.

TABLE 2 Tidal breathing parameters in 30 infants at term age measured with Volusense Pediatrics (VSP) (VoluSense, Bergen, Norway) without and with simultaneous application of a facemask

	VSP only	VSP+mask simultaneously	Mean difference (95% CI)	Per cent difference (95% CI)	p-value
Number of breathing cycles analysed	44.1±14.1	60.2±26.3			
Tidal breathing parameters					
V_T mL	15.0±3.1	20.1±4.3	5.0 (4.2–5.9)	34.3 (29.2–39.5)	0.0001
V_T mL·kg^{-1}	4.5±1.2	6.1±1.7	1.5 (1.3–1.8)	34.3 (29.2–39.5)	0.0001
V'_E·mL·min^{-1}·kg^{-1}	275.7±91.8	358.7±113.8	83.0 (57.0–109.0)	33.8 (24.2–43.4)	0.0001
Respiratory rate per min	63.0±19.7	61.4±16.0	−1.6 (−4.8–1.6)	−0.2 (−5.4–4.9)	0.31
PTEF mL·s^{-1}	46.6±16.9	62.1±22.7	15.5 (9.9–21.1)	37.5 (24.9–50.2)	0.0001
t_{PTEF}/t_E %	41.5±10.8	42.2±11.4	0.7 (−2.8–4.2)	3.7 (−5.5–13.0)	0.69
t_I/t_E %	78.9±9.7	89.4±13.4	10.4 (6.4–14.5)	13.7 (8.5–18.9)	0.0001
TEF_{50}/PTEF %	90.3±5.7	92.2±6.0	2.0 (0.5–3.4)	2.2 (0.6–3.9)	0.009

Data are presented as mean±SD unless otherwise stated. V_T: tidal volume; V'_E: minute ventilation. PTEF: peak tidal expiratory flow; t_{PTEF}: time to peak tidal expiratory flow; t_E: total expiratory time; t_I: inspiratory time; TEF_{50}: tidal expiratory flow at 50% of expiratory volume. p-values are from paired-sample t-tests.

For PTEF, the limits of agreement were relatively wide in the present study, indicating that the differences between the two methods varied relatively much among the participants. One possible explanation may have been variable degrees of air leakage through the Exhalyzer D facemask, which is most likely to occur during peak expiration and therefore particularly likely to influence PTEF. The VSP recordings may have been influenced by unnoticed and variable use of accessory respiratory muscles being incorrectly recognised as thoracic volume changes, although most such artefacts are easily recognised. Moreover, the VSP measurements could have been influenced by variable displacement of venous truncal blood volume into the head and limbs during respiration. VSP calculates flow from volume changes based on the assumption that all internal volume variation of the torso is caused by airflow within the respiratory system, and that all such airflow induces corresponding external volume variations of the torso. These are small but not insignificant simplifications that can explain some of the discrepancies we found for most of the tidal breathing parameters. Precise control over these issues requires whole-body plethysmography.

Software differences are also a potential source of bias that may influence the agreement between the two measurement methods. Determining the beginning and end of inspiration and expiration is a well-known problem when it comes to computer-based analysis of tidal breathing [18]. We believe that Exhalyzer D interprets the start of expiration slightly later than VSP. Such phase shifts can explain some of the minor differences found for t_{PTEF}/t_E, t_I/t_E and TEF_{50}/PTEF.

Strengths and limitations of VSP

The major weakness of the EIP method is the high sensitivity to a poor fit of the vest. It must be applied directly to the skin, as the fabric has to enclose the skin tightly with no wrinkles. Any body surface moving with respiration that is not sufficiently covered by the vest will lead to an underestimation of measured volumes. As a consequence, the infants must have their hips extended during the measurements, because flexion of the hips will cause folding of the fabric. The experience of the personnel performing the measurements will therefore have an impact on the accuracy of the results [13]. Skin lesions like surgical wounds, gastroschisis or stomas and drainage tubes will limit its clinical application.

The major advantage of the method is the avoidance of a facemask. This allows extended recordings in babies who are respiratory unstable or use equipment that may preclude the use of a facemask, primarily continuous positive airway pressure (CPAP) or high-flow nasal cannulas (HFNCs). In accordance with previous studies, we found that applying a facemask significantly increased minute ventilation [2, 4, 7]. This finding is physiologically plausible and most likely explained by increased dead space ventilation induced by the facemask or by tactile stimulation of the facial skin. We found that parameters reflecting airway obstruction, *i.e.* t_I/t_E and TEF_{50}/PTEF, were also significantly increased by the facemask. We believe that this apparent "anti-obstructive effect" was caused by a slight positive expiratory counter pressure generated mainly by the resistance of the flowmeter. This might increase the average diameter of small and medium-sized airways, and thereby cause t_E to decrease and TEF_{50} to increase, which is in accordance with our findings.

Conclusions

VSP accurately measures tidal breathing parameters if used correctly by experienced personnel. As inductive plethysmography does not involve a facemask, it can be applied in infants needing respiratory support such as CPAP and HFNCs. Thus, VSP is likely to be well suited for neonatal intensive care units and hospital wards treating children with pulmonary illnesses such as bronchiolitis. However, validation studies and normative data from children of all weight classes, and by gestational age, weight and sex will be needed. The validity of the relatively few existing sets of reference equations for infant spirometry parameters is probably limited to the methods that were used to obtain them [19–21].

Acknowledgements

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Online supplement

The following changes have been made from the FloRight ™ to the Volusense Pediatrics (VSP) ™ system.

- The VSP vests are made from a thinner and more flexible fabric than the FloRight vests, making it easier to enclose the skin tightly without creating wrinkles. This is of practical importance, as folds or wrinkles of the fabric that lead to insufficiently covered torso surfaces would lead to underestimation of the volume measurements.
- The VSP vests are no longer circular, making them easier to dress as they do not require being pulled over the head (as for FloRight). Dressing and/or undressing VSP vests will thus not come into conflict with other equipment, e.g. CPAP or feeding tubes.
- When no longer circular, the wires of the VSP vests had to be attached in a zig-zag pattern instead of a multi-turn helix (as in FloRight) in order to produce a voltage proportional to the instantaneous average cross-sectional area of the torso covered by the conductive elements (according to Faradays law of induction).
- A major difference is that the VSP vest is no longer energized to produce the magnetic field, but is instead used for recording an induced voltage originating from the field produced by the electromagnet antenna above the bed. This can mathematically be shown to give the same overall performance as in FloRight, with the added advantage of exposing the patient to far lower magnetic field strengths.
- The apparatus has overall been made smaller in size and is now easier to maneuver, making measurements more feasible in a NICU setting.

- The computational methods are unchanged, but the development tools used have been updated to harmonize with current advances in computer technology. The hardware has been re-designed to adapt to the change in configuration of the magnetic field.

III

BMJ Open

Lung function at term in extremely preterm-born infants: a regional prospective cohort study

Mariann Haavik Bentsen,[1,2] Trond Markestad,[1,2] Knut Øymar,[2,3] Thomas Halvorsen[1,2]

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[1]Department of Pediatrics, Haukeland University Hospital, Bergen, Norway
[2]Department of Clinical Science, Faculty of Medicine, University of Bergen, Bergen, Norway
[3]Department of Pediatrics, Stavanger University Hospital, Stavanger, Norway

Correspondence to
Dr Mariann Haavik Bentsen; bens@helse-bergen.no

ABSTRACT

Objectives To compare lung function of extremely preterm (EP)-born infants with and without bronchopulmonary dysplasia (BPD) with that of healthy term-born infants, and to determine which perinatal characteristics were associated with lung function at term and how predictive these measurements were for later respiratory health in EP-born infants.

Methods Perinatal variables were recorded prospectively, and tidal breathing parameters were measured at term-equivalent age using electromagnetic inductance plethysmography. Respiratory morbidity was defined by hospital readmissions and/or treatment with asthma medications during the first year of life.

Results Fifty-two EP-born infants (mean gestational age 26^{1}, range 22^{6}–27^{6} weeks) and 45 term-born infants were included. There was evidence of significant airway obstruction, higher tidal volumes and increased minute ventilation in the EP-born infants with and without BPD, although generally more pronounced for those with BPD. Male gender, antenatal steroids and number of days on continuous positive airway pressure were associated with lung function outcomes at term. A prediction model incorporating two unrelated tidal breathing parameters, BPD, birth weight z-score and gender, predicted respiratory morbidity in the first year of life with good accuracy (area under the curve 0.818, sensitivity and specificity 81.8% and 75.0%, respectively).

Conclusion Lung function measured at term-equivalent age was strikingly abnormal in EP-born infants, irrespective of BPD. Tidal breathing parameters may be of value in predicting future pulmonary health in infants born premature.

Trial registration number NCT01150396; Results.

Strengths and limitations of this study

- This is one of very few studies from this decade comparing lung function in infants born extremely preterm and healthy term-born infants using tidal breathing parameters.
- The data reflect infants' natural breathing pattern, with no disturbances from sedation or a face mask, which has been applied in most previous studies.
- The applied measuring device is relatively new and the number of participants is relatively low, and the results should therefore be tested in larger studies.

INTRODUCTION

Survival after extremely preterm (EP) birth, that is, before 28 weeks' gestational age (GA), has steadily increased since the 1970s, and most infants born after 24–25 weeks' GA are now discharged alive from well-equipped neonatal intensive care units (NICUs). When born EP, the normal in utero pulmonary development is disrupted in a vulnerable phase,[1–4] and gas exchange must take place in developmentally fetal lungs. Combined effects from pulmonary immaturity and the trauma inflicted by life-saving neonatal interventions lead to inflammatory responses that disturb lung growth and development. The result is altered lung structure with alveolar hypoplasia, disrupted pulmonary vasculature and interstitial cell proliferation.[5]

Respiratory outcome in EP-born neonates is heterogeneous and insufficiently understood, and predictors for later respiratory health are poorly defined. Bronchopulmonary dysplasia (BPD) is defined by the use of oxygen supplementation at specific time points, and has long been used as a predictor of subsequent increased risk of respiratory disease.[6] However, it is now increasingly recognised that BPD is a relatively poor measure of neonatal lung injury.[7–11] Thus, new and feasible diagnostic methods are needed to improve our understanding of lung injury in these vulnerable infants and to enhance our ability to predict their later respiratory health. Such knowledge is also needed to objectively assess interventions aimed at early prevention and treatment of lung disease.

In this study we compared lung function at term-equivalent age in EP-born and healthy term-born infants using tidal breathing parameters obtained with electromagnetic inductance plethysmography (EIP). The

aim was to investigate if, in what way, and to what extent, survivors of EP birth exhibit lung function abnormalities, and if infants with and without BPD differ in that respect. Additionally, we explored if lung function at term was associated with perinatal clinical characteristics or with respiratory morbidity during the first year of life.

METHODS

Subjects and study design

This study was part of a larger, prospective, population-based cohort study (project extreme prematurity; BabyPEP) that has been ongoing since 2011 in two tertiary referral hospitals in Western Norway (Haukeland University Hospital and Stavanger University Hospital). Women with threatening preterm delivery before 28 weeks' gestation are invited, and the infants are included if they are born before 28 weeks' GA. Detailed prenatal, perinatal and neonatal history, examinations and treatments are consecutively recorded in a database during the hospital stay. At discharge or term age, whatever comes first, the lung function is assessed by EIP. Follow-up at 3, 6 and 12 months' corrected age, that is, age calculated from expected term birth, includes clinical examinations and parental questionnaires addressing various problems, including respiratory symptoms and treatments.

Additionally, 45 healthy infants born at term (37–41 weeks' GA) at Haukeland University Hospital in the period of November 2015–March 2016 were recruited as controls. Infants returning to the maternity outpatient clinic for routine metabolic screening or weight control on days when our study nurse was available were asked to participate, provided they appeared healthy and breathed without efforts. Exclusion criteria were significant perinatal disease, malformations or syndromes.

Definitions

BPD was defined as being dependent on oxygen supplementation at 36 weeks' GA.[6] Oxygen supplementation was provided according to similar algorithms in the two participating NICUs, and usually by low-flow nasal cannulas guided by pulse oximetry targeting 90%–95% saturation at 36 weeks' GA.

Small for gestational age (SGA) was defined as birth weight (BW) <10th percentile for GA according to Norwegian growth curves.[12] z-Scores for BW were calculated with reference to the 2013 Fenton growth charts.[13]

Respiratory morbidity during the first year of life was defined by need for hospital readmission because of respiratory symptoms and/or parental report of treatment with inhaled asthma medications.

Lung function measurements

Lung function was measured using EIP, made commercially available by VoluSense (Bergen, Norway). The EIP system basically consists of an electromagnet and a patient vest encircling the torso in order to quantify chest and abdominal volume changes. EIP does not require sedation and allows continuous and prolonged recording of respiratory data without using a face mask. Data were obtained as described previously,[14] partly (in the period from 2011 to 2013) by using the first released version from VoluSense (FloRight),[15–20] and partly by using the upgraded version released in 2015 (VoluSense Pediatrics, VSP).

The infants were dressed in the appropriate-sized vest selected according to their armpit–hip length. All measurements were performed with the baby quietly awake or asleep and in supine position in a cot. No sedation was used, but some of the infants were given oral sucrose for relaxation. Tidal breathing was recorded for 5–10 min.

Analysis of flow volume loops

The traces were inspected visually to select a minimum of 50 stable breaths based on the following criteria: (1) no obvious artefacts, (2) no sighs and (3) no obvious changes in the depth of breathing or baseline. The following tidal breathing parameters were calculated and averaged by the computer software: tidal volume (Vt), minute ventilation (V'E), respiratory rate (RR), peak tidal expiratory flow (PTEF), time to peak tidal expiratory flow as a ratio of total expiratory time (Tptef/Te), ratio of tidal expiratory flow at 50% of expired volume to peak tidal expiratory flow (TEF_{50}/PTEF), ratio of tidal expiratory flow at 75% of expired volume to peak tidal expiratory flow (TEF_{75}/PTEF) and flow volume gravity (FVg), described in details previously.[20] Weight at measurement differed between groups, and hence data for volume and flow parameters were related to body weight.

Statistical methods

Data were analysed and graphs created with SPSS V.22 and MedCalc V.13.1 (MedCalc Software, Mariakerke, Belgium). Power calculations were based on the tidal breathing parameter Vt/kg, with data on distribution obtained from a previous study.[20] It was estimated that 44 infants were needed in each group to provide a power of 80% to show a 20% difference (0.6 SD) between the EP and term-born groups. After testing for normal distribution, groups were compared by independent samples t-tests or Mann-Whitney U tests, as appropriate. Categorical data were analysed with the X^2 test. Differences between the EP-born and term-born control group in terms of gender, GA at the time of measurement as well as body weight at the time of measurement were adjusted for in multiple regression analyses. We used linear regression analyses to assess if perinatal variables were associated with lung function at term. The following tidal breathing parameters were tested as outcomes: Vt/kg, RR, V'E/kg, PTEF/kg, TEF_{50}/PTEF and Tptef/Te. Potential explanatory variables were first entered into univariate models, and those associated with outcome with $p<0.10$ were entered into multivariate backward stepwise linear regression analyses. Receiver-operator characteristic (ROC) analyses were used to assess the ability of tidal

breathing parameters and selected clinical variables to discriminate between groups, and to predict respiratory morbidity during the first year of life. To create the best possible prediction model of later respiratory morbidity, we estimated a prognostic score (predicted probabilities) using multiple logistic regression incorporating two unrelated tidal breathing parameters (TEF_{50}/PTEF and Vt/kg), BPD, BW z-score and gender. This prognostic score was subsequently used as the independent variable in an ROC analysis, while respiratory morbidity in the first year of life was used as the dependent variable.

Approvals

The study was approved by the Regional Committee on Medical Research Ethics of Western Norway (REC West) and reported to the ClinicalTrials.com (ID NCT01150396).

RESULTS

Subjects

All EP-born infants born during the time periods we had the EIP method available (FloRight or VSP) and survived to be discharged were included: 41 at Haukeland University Hospital and 11 at Stavanger University Hospital. Clinical characteristics and perinatal data are provided in table 1. FloRight was used in 33 (63%) and VSP in the remaining 19 EP-born infants and all the 45 term-born controls. There were no differences between test results obtained using FloRight and VSP in comparable preterm-born groups (see online supplementary table S1).

Comparisons of tidal breathing parameters and discrimination between groups

All EP-born versus term-born control infants

Mean GA and body weight at the time of lung function assessment and the proportion of male were lower in the EP-born than the term-born group, and thus adjusted for in the regression analyses (table 2). Vt/kg, RR, V'E/kg and PTEF/kg were higher while the variables indicating airway obstruction (TEF_{50}/PTEF, TEF_{75}/PTEF, Tptef/Te and FVg) were all lower in the EP-born compared with the term-born control group (table 2). ROC analyses

Table 1 Perinatal data* of the infants studied (n=52)

	All preterm-born infants	Preterm-born infants without BPD	Preterm-born infants with BPD	p Value†
Number of subjects	52	20 (38%)	32 (62%)	
Male gender	18 (35%)	8 (40%)	10 (31%)	p=0.73
Gestational age at birth, weeks	26* (22–27)	26† (24‡–27)	25 (22–27§)	p=0.33
Birth weight, g	788 (370–12 809)	909 (650–1280)	713 (370–1045)	p<0.0005
Birth weight z-score	−0.17 (−2.58 to 1.88)	0.40 (−0.53 to 1.80)	−0.53 (−2.58 to 1.88)	p=0.004
Small for gestational age§	12 (23%)	0	12 (38%)	p=0.005
Antenatal steroids	48 (92%)	18 (90%)	30 (94%)	p=0.99
Chorioamnionitis	10/18 (56%)	12/30 (40%)	22/48 (45.8%)	p=0.79
Latency, days§	3.4 (0–55)	5.1 (0–55)	2.3 (0–21)	p=0.28
Caesarean section	27 (52%)	8 (40%)	19 (59%)	p=0.29
Apgar score 1 min	4.4 (1–9)	4.5 (1–9)	4.4 (1–9)	p=0.87
Apgar score 5 min	6.0 (1–9)	6.0 (2–9)	6.0 (1–9)	p=0.97
Surfactant, treated	48 (92%)	16 (80%)	32 (100%)	p=0.036
Patent ductus arteriosus, treated	17 (33%)	8 (40%)	9 (28%)	p=0.55
Septicaemia, verified or suspected	22 (42%)	5 (25%)	17 (53%)	p=0.089
Mechanical ventilation, days	6.6 (0–33)	2.7 (0–11)	9.1 (0.5–33)	p=0.004
CPAP or HFNC, days	48.7 (13–75)	38 (13–62)	55.4 (31–75)	p<0.0005
Supplementary oxygen, days	75.6 (0–127)	53.5 (0–79)	89.4 (61–127)	p<0.0005
Postnatal steroids	14 (26.9%)	0	14 (44%)	p=0.0016
Pathological chest X-ray at PMA 36 weeks	21/42 (50%)	7/18 (39%)	14/24 (58%)	p=0.36

*Means with ranges, or numbers (%).
†Independent samples t-tests, Mann-Whitney U test or X^2 test.
‡Days from rupture of membranes to delivery.
§Birth weight <10th percentile for gestational age.
BPD, bronchopulmonary dysplasia; CPAP, continuous positive airway pressure; HFNC, high-flow nasal cannulae; PMA, postmenstrual age.

Table 2 Lung function at term-equivalent age in all infants

	Healthy term-born controls (n=45)	All extremely preterm-born infants* (n=52)	Preterm-born infants without BPD* (n=20)	Preterm-born infants with BPD† (n=32)
Male gender	24 (53.3%)	18 (34.6%) p=0.035	8 (40%) p=0.28	10 (31.3%) p=0.73
Gestational age at time of measurement, weeks (ranges)	40.6 (37.3–43.1)	38.8 (36.0–42.9) p<0.001	38.7 (36.1–41.3) p<0.0001	38.9 (36.0–42.9) p=0.50
Postnatal age in days (ranges)	6.5 (3–21)	88.9 (60–115) p<0.001	86.9 (62–103) p<0.0001	90.2 (60–115) p=0.42
Body weight at time of measurement, g (ranges)	3494 (2465–4700)	2768 (1900–3800) p<0.001	2862 (2177–3800) p<0.0001	2709 (1900–3500) p=0.22
Vt (mL)/kg	4.6 (4.3 to 4.9)	6.0 (5.5 to 6.5) p=0.019	5.8 (5.0 to 6.6) p=0.026	6.1 (5.3 to 6.8) p=0.63
RR (/min)	58.2 (54.6, 61.9)	67.8 (63.4, 72.2) p=0.012	71.1 (62.8, 79.4) p=0.002	65.7 (60.7, 70.8) p=0.23
V'E (mL)/kg	258.5 (236.4 to 280.6)	394.0 (358.1 to 429.8) p<0.0001	405.0 (336.7 to 473.3) p<0.0001	387.1 (344.3 to 429.9) p=0.63
PTEF (mL/s)/kg	14.2 (12.5 to 15.9)	27.5 (24.6 to 30.5) p<0.0001	25.6 (21.3 to 30.0) p<0.0001	28.7 (24.6 to 32.8) p=0.31
TEF_{50}/PTEF (%)	84.1 (82.2 to 86.0)	78.2 (75.9 to 80.4) p=0.008	81.6 (78.7 to 84.4) p=0.51	76.1 (73.0 to 79.1) p=0.015
TEF_{75}/PTEF (%)	67.6 (64.5 to 70.7)	53.1 (48.7 to 57.5) p<0.0001	59.9 (53.8 to 66.1) p=0.026	48.8 (43.1 to 54.5) p=0.012
Tptef/Te (%)	40.9 (37.6 to 44.3)	30.1 (26.4 to 33.8) p<0.0001	35.1 (29.2 to 41.1) p=0.056	26.9 (22.2 to 31.6) p=0.029
FVg	0.48 (0.47 to 0.49)	0.44 (0.43 to 0.45) p<0.0001	0.46 (0.44 to 0.48) p=0.019	0.43 (0.42 to 0.45) p=0.013

Data are presented as means with 95% CIs, unless stated otherwise.
*Significance tested versus the term-born control group and adjusted for differences in terms of GA and body weight at the time of measurement.
†Significance tested versus the EP-born group without BPD.
BPD, bronchopulmonary dysplasia; EP, extremely preterm; FVg, expiratory flow volume loop centre of gravity (dimensionless); GA, gestational age; PTEF, peak tidal expiratory flow; RR, respiratory rate; TEF_{50}/PTEF, flow at 50% expired volume as a per cent of PTEF; TEF_{75}/PTEF, flow at 75% expired volume as a per cent of PTEF; Tptef/Te, time to peak tidal expiratory flow as a ratio of total expiratory time; V'E, minute ventilation; Vt/kg, tidal volume per kilogram body weight.

showed that most tidal breathing parameters discriminated between the EP-born and term-born control groups (figure 1).

Non-BPD versus term-born control infants

As for the complete EP-born group, Vt/kg, RR, V'E/kg and PTEF/kg were significantly higher and TEF_{75}/PTEF and FVg were significantly lower in the non-BPD group when compared with the term-born control group; however, they were numerically less pronounced (table 2). TEF_{50}/PTEF and Tptef/Te did not differ significantly.

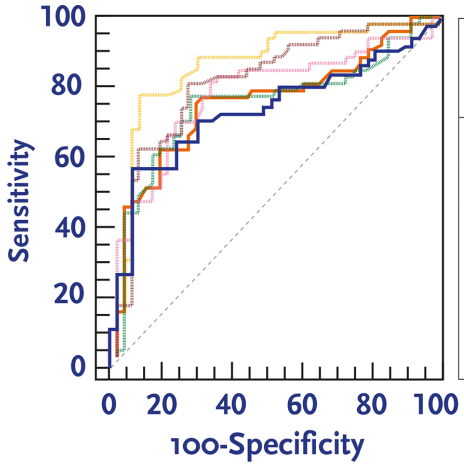
BPD versus non-BPD infants

Thirty-two (62%) of the 52 EP-born infants had BPD (table 1). The BPD infants had lower mean BW, and higher proportions were SGA and treated with surfactant and postnatal corticosteroids compared with infants without BPD. The BPD infants also required more days on mechanical ventilation, continuous positive airway pressure (CPAP) or high-flow nasal cannulas (HFNC), and supplementary oxygen.

TEF_{50}/PTEF, TEF_{75}/PTEF, Tptef/Te and FVg were all lower in the infants with BPD (table 2), but on ROC analyses none of these tidal breathing parameters could distinguish better between infants with and without BPD than the clinical variables 'days on mechanical ventilation', 'days on CPAP/HFNC' and 'BW z-scores' (figure 2).

Perinatal clinical determinants of tidal breathing parameters in EP-born infants

Explanatory variables associated with one or more of the tidal breathing outcome parameters in univariate linear regression analyses are provided in the online supplementary table S2. In the multivariate regression model, male gender was related to lower (more obstructive)



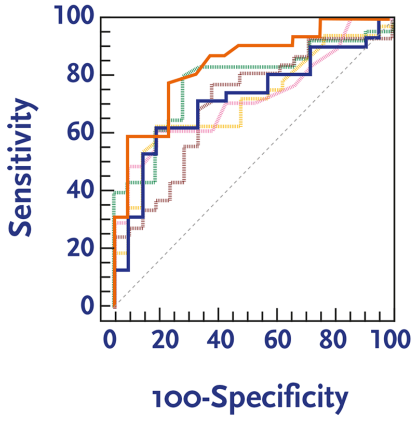
	Tidal breathing parameter	AUC with 95% CI	Optimal cut-off point	Sensitivity	Specificity
	Vt/kg	0.742 (0.643, 0.825)	5.64	57.7	93.3
	V'E/kg	0.829 (0. 739, 0.898)	282.7	80.8	77.8
	PTEF/kg	0.881 (0.800, 0.938)	19.98	76.9	93.3
	Tptef/Te	0.760 (0.663, 0.841)	34.4	76.9	77.8
	TEF_{75}/PTEF	0. 790 (0.696, 0.866)	64.7	80.8	71.1
	FVg	0.767 (0.671, 0.847)	0.463	75.0	75.6

Figure 1 Receiver-operator characteristic (ROC) curves comparing the ability of different tidal breathing parameters to discriminate between extremely preterm-born infants (n=52) and healthy term-born controls (n=45). If the 95% CI of the area under the ROC curve (AUC) includes 0.5 (no discrimination), the tested variable does not distinguish between the two groups. The optimal cut-off point is the point for which sensitivity+specificity is maximal. For abbreviations of lung function variables, please see list in table 2.

TEF_{50}/PTEF and Tptef/Te, as well as lower RR, whereas number of days on CPAP/HFNC was related to lower RR and lower TEF_{50}/PTEF. Antenatal treatment with steroids was related to lower (more normal) Vt/kg. The results from the multivariate regression model are shown in table 3. Neither SGA nor BW z-score influenced the lung function measures (see online supplementary table S2 and table S3).

Prediction of later respiratory morbidity

At the time of data analysis, 35 of the 52 EP-born children had reached a corrected age of 1 year, and 11 (31%) of them had either been hospitalised and/or treated with asthma medication. Mean TEF_{50}/PTEF was significantly lower and TEF_{75}/PTEF, Tptef/Te and FVg tended to be lower in the group with respiratory morbidity (table 4). The ROC analyses showed that the tidal breathing variable TEF_{50}/PTEF, but not BPD, predicted respiratory morbidity during the first year of life (figure 3). The prediction model that incorporated TEF_{50}/PTEF, Vt/kg, BPD, BW z-score and gender gave an area under the curve (AUC) of 0.818 (95% CI 0.651 to 0.928), predicting respiratory morbidity in the first year of life with a sensitivity and specificity of 81.8% and 75.0%, respectively.



		AUCwith 95% CI	Optimal cut-off point	Sensitivity	Specificity
	Tptef/Te	0. 727 (0.585, 0.841)	25.7	62.5	85.0
	TEF_{50}/PTEF	0. 715 (0.573, 0.831)	82.0	78.1	65.0
	FVg	0. 710 (0.568, 0.828)	0.426	59.4	85.0
	BWz-score	0. 793 (0.658, 0.893)	0.04	81.2	75.0
	Ventilation days	0. 736 (0.595, 0.848)	4.0	62.5	85.0
	CPAP/HFNC days	0.840 (0. 712, 0.927)	44	78.1	80.0

Figure 2 Receiver-operator characteristic (ROC) curves comparing the ability of tidal breathing parameters and clinical variables to discriminate between extremely preterm-born infants with and without bronchopulmonary dysplasia. If the 95% CI of the area under the ROC curve (AUC) includes 0.5 (no discrimination), the tested variable does not distinguish between the two groups. The optimal cut-off point is the point for which sensitivity+specificity is maximal. For abbreviations of lung function variables, please see list in table 2.

Table 3 Final multivariate regression model* testing relations between perinatal variables and lung function parameters at term in extremely preterm-born infants (n=52)

	Multivariable model			
	Coefficient	95% CI	p Value	
Vt/kg (mL)				
Antenatal steroids	−2.4	−4.3 to 0.5	0.014	Adjusted R^2=10%
RR (/min)				
Male gender	−7.6	−15.9 to 0.65	0.070	Adjusted R^2=19%
Days of CPAP/HFNC	−0.45	−0.72 to 0.19	0.001	
Tptef/Te (%)				
Male gender	−7.1	−14.7 to 0.5	0.07	Adjusted R^2=5%
TEF_{50}/PTEF (%)				
Male gender	−5.8	−9.9 to 1.6	0.007	Adjusted R^2=23%
Days of CPAP/HFNC	−0.22	−0.35 to 0.09	0.002	

*Explanatory variables that were associated with a p<0.10 in univariable analyses were included in the final multivariate regression model (ie, male gender, birth weight z-score, antenatal steroids, days of CPAP/HFNC and days of oxygen). Only variables associated with one or more of the tidal breathing outcome parameters in the final model (p<0.10) are presented in the table.
CPAP, continuous positive airway pressure; HFNC, high-flow nasal cannulas; PTEF, peak tidal expiratory flow; RR, respiratory rate; TEF_{50}/PTEF, flow at 50% expired volume as a per cent of PTEF; Tptef/Te, time to peak tidal expiratory flow as a ratio of total expiratory time; Vt/kg, tidal volume per kilogram body weight.

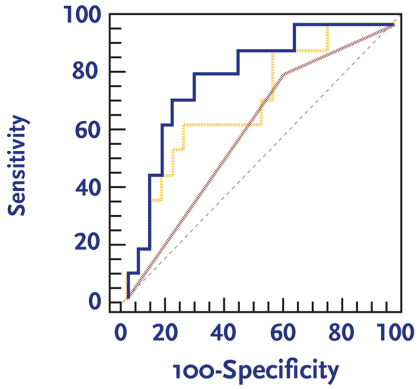
DISCUSSION

The EP-born infants had primarily obstructive pulmonary abnormalities at term-equivalent age when compared with healthy term-born controls. Lung function abnormalities were present irrespective of BPD, although more pronounced within the BPD group. Male gender and days on CPAP/HFNC were associated with airway obstruction at term, while treatment with antenatal steroids was associated with lower Vt, that is, more similar to the control group. The parameter TEF_{50}/PTEF, which reflects airway obstruction, showed a promising ability to predict respiratory morbidity during the first year of life in infants born EP. A compound prediction model, incorporating TEF_{50}/PTEF, Vt/kg, BPD, BW z-score and gender, showed the best accuracy to predict respiratory morbidity in the first year of life (AUC=0.818, 95% CI 0.651 to 0.928).

Table 4 Presence or absence of respiratory morbidity during the first year of life* dependent on early clinical characteristics and lung function data obtained at near-term gestational age after extremely preterm birth

	Respiratory morbidity (n=11)	No respiratory morbidity (n=24)	p Value†
Gestational age at birth	25.6 (24.5 to 26.7)	25.9 (25.4 to 26.4)	0.53
Birth weight z-score	−0.21 (−1.11 to 0.70)	−0.29 (−0.60 to 0.02)	0.81
Male gender	4 (36.4%)	8 (33.3%)	0.84
Pathological chest X-ray at term	3/8 (37.5%)	12/19 (63.2%)	0.42
Diagnosis of BPD	8/11 (72.7%)	15/24 (62.5%)	0.84
Vt/kg (mL/kg)	5.5 (4.4 to 6.6)	5.9 (4.9 to 6.8)	0.63
RR (/min)	67.7 (58.7 to 76.8)	66.2 (59.2 to 73.2)	0.79
V'E/kg (mL/kg/min)	366.2 (286.3 to 446.0)	378.6 (318.0 to 439.2)	0.80
PTEF/kg (mL/s/kg)	26.8 (19.0 to 34.6)	27.2 (22.3 to 32.1)	0.93
Tptef/Te (%)	25.8 (19.2 to 32.5)	34.2 (27.2 to 41.2)	0.13
TEF_{50}/PTEF (%)	73.5 (68.5 to 78.6)	79.9 (76.6 to 83.1)	0.03
TEF_{75}/PTEF (%)	47.9 (40.5 to 55.2)	56.5 (48.5 to 64.5)	0.17
FVg	0.43 (0.41 to 0.46)	0.45 (0.43 to 0.47)	0.21

*Defined by the need for hospital readmission because of respiratory symptoms and/or a parental report of treatment with inhaled asthma medications. Data are given as means with 95% CIs or numbers (% in brackets).
†Independent sample t-tests or the X^2 test.
BPD, bronchopulmonary dysplasia; RR, respiratory rate; V'E, minute ventilation; Vt/kg, tidal volume per kilogram body weight.



		AUC with 95% CI
	Compound model	0.818 (0.651, 0.928)
	BPD	0.617 (0.438, 0.776)
	TEF_{50}/PTEF	0. 723 (0.547, 0.861)

Figure 3 Receiver-operator characteristic (ROC) curves comparing the ability of TEF_{50}/PTEF and BPD, and additionally a compound model incorporating TEF_{50}/PTEF, Vt/kg, BPD, birth weight z-score and gender, used to predict development of respiratory distress requiring readmission or treatment with asthma medication during the first year of life of extremely preterm-born individuals (n=35). The compound model achieved the best sensitivity and specificity, that is, respectively, 81.8% and 75.0% at a cut-off value of 0.34. If the 95% CI of the AUC includes 0.5 (no discrimination), the parameter does not predict later respiratory distress. The optimal cut-off point is where the sensitivity and specificity are maximal. This value corresponds with the point on the ROC curve farthest from the diagonal line. AUC values for other breathing parameters and clinical variables are given in (online supplementary table S4). AUC, area under the curve; BPD, bronchopulmonary dysplasia; TEF_{50}/PTEF, ratio of tidal expiratory flow at 50% of expired volume to peak tidal expiratory flow; Vt/kg, tidal volume per kilogram body weight.

Strengths and limitations

This is one of few studies comparing lung function in EP and healthy term-born infants using tidal breathing parameters obtained with a non-invasive method that does not involve sedation and/or the application of a face mask. In our opinion, the use of this method is an important strength of this study, since a face mask inevitably adds dead space and alters the breathing pattern of the baby, possibly in different ways in health and disease.[21–24] None of the participants in this study were sedated, and the data reflect the infant's natural breathing pattern. As few studies have applied comparable methods, our data cannot easily be compared with those of others. The mean GA and body weight at the time of the measurements and the gender ratio differed slightly between the EP and term-born groups, characteristics that may have influenced the findings, although adjustments did not influence statistical conclusions. Unfortunately, the manufacturer upgraded the equipment during the study and we were forced to comply. However, we found no systematic differences between the two models for any of the tidal breathing parameters, suggesting that bias was not introduced. A relatively low number of participants made the study vulnerable to type II statistical errors, possibly explaining some of the non-significant differences, for example, no difference in respiratory morbidity in the first year of life between infants with and without a neonatal diagnosis of BPD (table 4 and figure 3). Finally, differences regarding birth year between the EP and term-born infants (2011–2013 and 2015–2016 vs 2015–2016, respectively) and the use of a convenience sample as control group were weaknesses that possibly may have influenced outcome.

Comparisons of tidal breathing parameters and discrimination between groups

Vt and V'E were higher in the EP than in the term-born group. This corresponds with the findings of Olden *et al*,[15] who also used EIP and found the mean Vt/kg was 5.4 mL in healthy term-born infants and 7.0 mL in prematurely born infants with BPD. Increased Vt is also consistent with results from studies using the multiple-breath washout method.[10 25] However, it contrasts the findings of studies where tidal breathing parameters were assessed with mask-based methods, such as the studies by Schmalisch *et al*[26] and Hjalmarson and Sandberg.[27] They found that preterm-born infants with chronic lung disease had *lower* Vt compared with term-born controls, and that a higher V'E was instead related to a substantially higher RR. Higher V'E in EP-born infants is compatible with the histological picture of BPD with few but large alveoli and a cell-rich interstitial space,[5] and thus presumably a greater physiological dead space ventilation that necessitates larger exchanges of volumes to maintain adequate gas exchange. To conclude on this issue, our data suggest that a *combination* of increased Vt and RR is the most likely adapted breathing pattern in EP-born infants. Increased Vt may not be captured by mask-based measurements as the face mask alters the breathing pattern.

Evidence of small airway obstruction has been found in most studies comparing EP-born infants at term-equivalent age with term-born controls, especially for infants with BPD. This applies to studies using EIP as well as mask-based methods.[15 26 28] This is consistent with our findings, and also with long-term follow-up studies that show persistent airway obstruction in older EP-born children.[9]

Clinical determinants of lung function at term in EP-born infants

Our findings suggest that antenatal steroids have a positive effect on Vt measured at term-equivalent age. Thus, antenatal steroids might possibly improve later respiratory mechanics. Male gender and prolonged respiratory support were negatively associated with the tidal breathing parameters reflecting airway obstruction. This is plausible considering what is known from studies on causes and prevention of BPD.[1 29] Contrasting some recent studies,[9 25 28] GA at birth, SGA status and BW z-score were unrelated to the assessed tidal breathing parameters, findings for which we have no good explanation.

Prediction of later respiratory morbidity

The proportion of EP-born infants readmitted to hospital or treated for asthma-like symptoms during their first year of life corresponds with data from other studies.[11 30 31] Infants with tidal breathing parameters compatible with airway obstruction were more likely to develop respiratory symptoms perceived to require treatment in their first year of life, significantly so for the parameter TEF_{50}/PTEF. This is in agreement with Proietti *et al*, [30] who found that reduced Tptef/Te in preterm infants assessed near term was associated with wheezing during the first year of life. It is also consistent with Drysdale *et al*, [32] who used the single-breath occlusion technique and found higher airway resistance (Rrs) at 36 weeks' GA in preterm-born infants who were later hospitalised because of viral lower respiratory tract infections. A compound prediction model consisting of easily accessible variables including two tidal breathing parameters could predict respiratory morbidity in the first year of life with high accuracy.

Data from several studies have suggested a lifelong tracking of lung function, also in people born EP.[33–36] We suggest that non-invasive and clinically acceptable methods of assessing lung function in infants will facilitate large-scale early assessment of lung function after preterm birth. Hopefully, such measurements may prove to be so closely related to future lung function that they can be used as a proxy of long-term pulmonary outcome in intervention studies aiming at prevention or treatment of neonatal lung disease.

CONCLUSIONS

EP-born infants exposed to contemporary advanced NICU care had strikingly abnormal lung function at term age. Lung disease of prematurity appears to represent a continuum, and classifying lung morbidity simply by BPD or not seems too imprecise when predicting future pulmonary health. Tidal breathing measurements at term-equivalent age using the EIP method gave important additional information about later respiratory morbidity in EP-born infants. A prediction model combining tidal breathing parameters, a diagnosis of BPD and readily available clinical parameters could predict later respiratory morbidity with good accuracy. EIP emerges as a feasible method to obtain lung function data of value for targeted follow-up and also for objective assessment of effects of interventions aimed to prevent or treat lung disease associated with prematurity.

Contributors All authors contributed to the conceptualisation and design of the study. MHLB contributed to the data collection, carried out the analyses and is the responsible author of the manuscript. TM, KO and TH participated in the interpretation of the results, and critically reviewed and revised the manuscript. All authors approved the final manuscript as submitted.

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Competing interests None declared.

Ethics approval The Regional Committee on Medical Research Ethics of Western Norway (REC West).

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IV

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ORIGINAL ARTICLE
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Ventilator flow data predict bronchopulmonary dysplasia in extremely premature neonates

Mariann H. Bentsen[1,2], Trond Markestad[1,2] and Thomas Halvorsen[1,2]

Affiliations: [1]Dept of Pediatrics, Haukeland University Hospital, Bergen, Norway. [2]Dept of Clinical Science, Faculty of Medicine and Odontology, University of Bergen, Bergen, Norway.

Correspondence: Mariann Bentsen, Dept of Pediatrics, Haukeland University Hospital, N-5021, Bergen, Norway. E-mail: bens@helse-bergen.no

ABSTRACT Early prediction of bronchopulmonary dysplasia (BPD) may facilitate tailored management for neonates at risk. We investigated whether easily accessible flow data from a mechanical ventilator can predict BPD in neonates born extremely premature (EP).

In a prospective population-based study of EP-born neonates, flow data were obtained from the ventilator during the first 48 h of life. Data were logged for >10 min and then converted to flow–volume loops using custom-made software. Tidal breathing parameters were calculated and averaged from ⩾200 breath cycles, and data were compared between those who later developed moderate/severe and no/mild BPD.

Of 33 neonates, 18 developed moderate/severe and 15 no/mild BPD. The groups did not differ in gestational age, surfactant treatment or ventilator settings. The infants who developed moderate/severe BPD had evidence of less airflow obstruction, significantly so for tidal expiratory flow at 50% of tidal expiratory volume (TEF_{50}) expressed as a ratio of peak tidal expiratory flow (PTEF) (p=0.007). A compound model estimated by multiple logistic regression incorporating TEF_{50}/PTEF, birthweight z-score and sex predicted moderate/severe BPD with good accuracy (area under the curve 0.893, 95% CI 0.735–0.973).

This study suggests that flow data obtained from ventilators during the first hours of life may predict later BPD in premature neonates. Future and larger studies are needed to validate these findings and to determine their clinical usefulness.

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Introduction

Bronchopulmonary dysplasia (BPD) is the most common severe complication of premature birth and affects two out of three infants born extremely premature (EP), *i.e.* born before 28 weeks' gestational age [1]. It is associated with lifelong health consequences, including poor neurodevelopmental outcome and chronic respiratory conditions such as asthma, pulmonary hypertension and, possibly chronic obstructive pulmonary disease [2–4]. BPD is defined by need of oxygen supplementation for ⩾28 days and is graded as mild, moderate or severe depending on the requirement for oxygen supplementation or ventilatory support at 36 weeks' gestational age [5]. BPD is characterised by arrested lung growth with alveolar hypoplasia, abnormal pulmonary vasculature and interstitial cell proliferation [6] due to disruption of the normal *in utero* pulmonary development [2, 7, 8]. The disease process is multifactorial, but inflammation caused by various neonatal conditions, traumas and treatments is an essential driving force of the pathogenesis [9].

The risk of BPD is statistically linked to low gestational age, low birthweight and male sex [2, 4, 10], but EP-born neonates are probably born with variable susceptibilities or predispositions [4, 11]. We lack reliable clinical methods that might reflect such risk factors during the very early postnatal period; such methods are needed in order to improve our understanding of the disease process and thus provide a basis for prevention and targeted early management in neonates at particular risk. Our hypothesis was that early lung mechanics may reflect a susceptibility for later development of BPD, and our aim was to investigate whether flow data that are easily obtained from a ventilator during the first few hours of life can predict development of the severe forms of BPD in EP-born neonates in need of mechanical respiratory support.

Methods

Subjects and study design

This study was part of a larger prospective population-based cohort study (Project Extreme Prematurity; BabyPEP) that has been ongoing at Haukeland University Hospital (Bergen, Norway) since 2011. Pregnant females threatening preterm delivery before 28 weeks' gestation are invited and their children included if born <28 weeks gestational age. Since 2014, flow data have been logged from the ventilator for the neonates who received conventional ventilator support during their first 48 h of life.

Ventilator flow data

All neonates were ventilated using BabyLog VN500 (Dräger, Lübeck, Germany). Raw bidirectional flow data with a frequency of 50 Hz were logged for >10 min during the neonates' first 48 h of life using custom-made software (MedLink 4.4; Nortis Ingenieurbüro, Nürnberg, Germany) provided by the Dräger company. The software was installed on a computer connected to the ventilator by a Medibus cable. Flow–volume curves were constructed and breathing parameters analysed using another custom-made software package (VoluSense, Bergen, Norway). The mode of mechanical ventilation was "assist control with volume guarantee" during all data acquisitions. All neonates had uncuffed endotracheal tubes of sizes 2.5 mm (n=31) or 3.0 mm (n=2). The individual tube size was decided at the discretion of the attending neonatologist. All neonates were sedated and given morphine pain relief at doses 10–15 $\mu g \cdot kg^{-1} \cdot h^{-1}$; they were all clinically stable at the time of data acquisition, and laryngeal air leaks calculated by the ventilator were <15%.

Analysis of flow–volume loops

The analyses of the flow–volume loops and the selection of the traces upon which the analyses were based took place consecutively as the infants were included and before the patients were old enough to qualify for a BPD diagnosis, and thus were blinded to outcome. The traces were inspected visually to select ⩾200 stable breath cycles based on the following criteria: 1) no obvious artefacts; 2) no sighs; and 3) no obvious changes in the depth of breathing or baseline (figure 1a). The following breathing parameters were calculated and averaged by the computer software: tidal volume (V_T), minute ventilation (V'_E), respiratory rate, peak tidal expiratory flow (PTEF), time to PTEF as a ratio of total expiratory time (t_{PTEF}/t_E), ratio of tidal expiratory flow at 50% of expired volume (TEF_{50}) to PTEF (figure 1b), ratio of tidal expiratory flow at 75% of expired volume (TEF_{75}) to PTEF and flow volume gravity, which is a parameter described in detail previously [12]. We excluded V_T, respiratory rate and V'_E from comparisons/statistical analyses, as these parameters primarily reflect support from the ventilator.

Definitions

BPD was defined according to the National Institute of Child Health and Human Development and National Heart, Lung, and Blood Institute workshop criteria from 2000, and the infants were retrospectively divided into two groups: no/mild BPD and moderate/severe BPD [5].

z-scores for birthweights were calculated with reference to the 2013 Fenton growth charts [13].

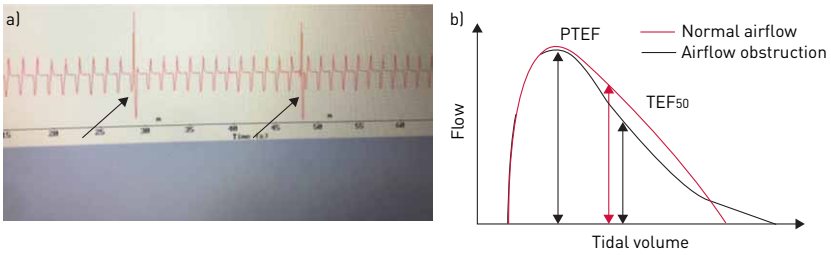


FIGURE 1 Flow–volume loop analysis. a) Example of flow traces selected for computerised analysis. The arrows indicate breaths that were excluded from analysis (sighs). b) Airflow obstruction gives a concave flow–volume loop and lower tidal expiratory flow at 50% of expired volume (TEF_{50})/peak tidal expiratory flow (PTEF) ratio.

Statistical methods

Data were analysed and graphs created using SPSS (version 22; IBM SPSS Statistics, Armonk, NY, USA) and MedCalc (version 13.1; MedCalc Software, Mariakerke, Belgium).

The data were tested for normal distribution, and means with standard deviations or 95% confidence intervals were calculated for the various patient variables and breathing parameters. Group comparisons were made with independent sample t-tests or the Chi-squared test, as appropriate. Receiver operator characteristic (ROC) analyses were used to assess the ability of the different expiratory breathing parameters, as well as a compound model consisting of TEF_{50}/PTEF, birthweight z-score and sex, to predict BPD severity. As regards the compound model, a prognostic score (predicted probabilities) was estimated by multiple logistic regression, and this score was subsequently used as the test variable in the ROC analysis. Since gestational age and birthweight are highly correlated confounders, we chose to include birthweight z-score in this model.

Approvals

The study was approved by the regional committee on medical research ethics of Western Norway (REC West) and registered at www.ClinicalTrials.gov (identifier number NCT01150396).

Results

Subjects and perinatal data

72 EP-born neonates were included in the BabyPEP study during the inclusion period (July 2014 to November 2016). 40 neonates received conventional ventilator support during their first 48 h of life, and 37 of them were included in this study. Flow data from three patients could not be recorded due to practical reasons. Data from four patients were excluded from the analyses due to death before 36 weeks gestational age preventing grading of BPD. Clinical characteristics and perinatal data for the remaining 33 patients are provided in table 1. 18 neonates developed moderate/severe BPD and 15 no/mild BPD. There were more males, and also a tendency for lower gestational age, birthweight and birthweight z-score in the preterm-born neonates who developed moderate/severe BPD; however, differences were not statistically significant. Ventilatory support in terms of pressures and fractions of oxygen supplementation did not differ between the two BPD subgroups at the time of data acquisition.

Comparisons between BPD subgroups and prediction of later BPD

The moderate/severe BPD group had higher numerical values for all the expiratory breathing parameters than the no/mild BPD group, *i.e.* suggesting less obstruction, but the difference was only significant for TEF_{50}/PTEF (table 2). This finding remained highly significant after performing a Bonferroni adjustment (adjusted p-value of 0.01).

ROC analyses showed that TEF_{50}/PTEF predicted later development of moderate/severe BPD with an area under the curve (AUC) of 0.774 (95% CI 0.596–0.901), but that gestational age (0.606, 0.421–0.771), birthweight (0.672, 0.487–0.825), birthweight z-score or sex did not (figure 2). The AUC (95% CI) of a compound model incorporating TEF_{50}/PTEF, birthweight z-score and sex was 0.893 (0.735–0.973). The optimal cut-off point for the combined model was 0.785, meaning that a prognostic score >0.785 predicted BPD with sensitivity and specificity of 66.7% and 100%, respectively, corresponding to positive and negative predictive values of 100% and 71.4% in this study.

TABLE 1 Perinatal characteristics of the extremely premature infants studied

	Preterm-born neonates who later developed no/mild BPD	Preterm-born neonates who later developed moderate/severe BPD	p-value#
Subjects n	15	18	
Male	7 (47)	11 (61)	0.65
Gestational age at birth weeks^{days}	$26^{2}\pm1^{1}$	$25^{5}\pm1^{3}$	0.24
Birthweight g	864±164	754±154	0.06
Birthweight z-scores	0.11±0.54	-0.29±0.67	0.08
Antenatal steroids	13 (87)	17 (94)	0.93
Surfactant treatment	15 (100)	18 (100)	1.00
Age at data acquisition h	17.8±13.3	17.1±12.4	0.87
PIP¶ cmH_2O	16.1±2.3	16.8±4.2	0.55
PEEP¶ cmH_2O	4.9±0.48	4.8±0.62	0.74
Set tidal volume per kg¶ $mL\cdot kg^{-1}$	5.4±0.78	5.2±0.66	0.34
Fraction of inspired oxygen¶ %	32±15	28±10	0.40

Data are presented as n (%) or mean±SD, unless otherwise stated. BPD: bronchopulmonary dysplasia; PIP: ventilator peak inspiratory pressure; PEEP: ventilator positive end-expiratory pressure. #: independent samples t-test or Chi-squared test; ¶: during data acquisition.

Discussion

The expiratory flow ratio TEF_{50}/PTEF obtained during the first 48 h of life was significantly higher, *i.e.* suggesting a less obstructive airflow pattern, for the EP-born neonates who later went on to develop moderate/severe BPD than for those who developed no or mild BPD. In addition, the TEF_{50}/PTEF ratio significantly predicted moderate/severe BPD, in contrast to other neonatal variables such as gestational age, birthweight and birthweight z-score. A compound model estimated by multiple logistic regression incorporating TEF_{50}/PTEF ratio, birthweight z-score and sex predicted moderate/severe BPD with good accuracy (AUC 0.893).

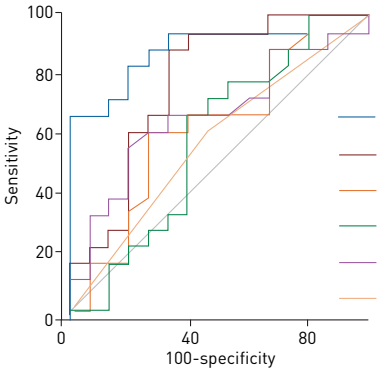
Strengths and limitations

Like any single-centre study with relatively few participants, the results of this study must be interpreted cautiously, as features particular to the participants or institutional practices may have introduced bias difficult to control for. The likelihood of selection bias was reduced to the extent possible by the inclusion procedure, *i.e.* convenience based and consecutive recruitment with few dropouts from a population-based sample of EP-born neonates. We only studied EP-born neonates who required early ventilatory support, and neonates with relatively little lung disease shortly after birth may still develop BPD [14]. Obviously, other prognostic methods are needed for this group, and electromagnetic inductance plethysmography is emerging as a promising method in this respect, since it can be applied in patients needing continuous positive airway pressure or high-flow nasal cannulas [15–17].

TABLE 2 Expiratory breathing characteristics calculated from flow data from a mechanical ventilator during the first 48 h of life in 33 extremely premature infants with or without later development of moderate/severe bronchopulmonary dysplasia (BPD)

	Preterm-born neonates later developing no/mild BPD	Preterm-born neonates later developing moderate/severe BPD	p-value#
Subjects n	15	18	
PTEF per kg $mL\cdot s^{-1}$	27.6 (22.1–33.2)	30.2 (25.7–34.7)	0.45
t_{PTEF}/t_E	21.1 (15.7–26.5)	23.4 (19.8–26.9)	0.44
TEF_{50}/PTEF %	71.8 (62.9–80.6)	85.1 (81.8–88.4)	0.007
TEF_{75}/PTEF %	44.1 (33.7–54.5)	51.3 (44.2–58.5)	0.22
FV_g	0.42 (0.39–0.44)	0.44 (0.42–0.45)	0.12

Data are presented as mean (95% CI), unless otherwise stated. t_{PTEF}: time to peak tidal expiratory flow; t_E: expiratory time; TEF_n: tidal expiratory flow at n% of expired volume; PTEF: peak tidal expiratory flow; FV_g: flow volume gravity. #: independent samples t-test.



	AUC (95% CI)	Optimal cut-off	Sensitivity	Specificity
Compound model	0.893 (0.735–0.973)	0.785	66.7	100
TEF_{50}/PTEF	0.774 (0.596–0.901)	77.5	88.9	66.7
FV_g	0.626 (0.441–0.787)			
t_{PTEF}/t_E	0.576 (0.392–0.745)			
Birthweight z-score	0.667 (0.482–0.820)			
Sex	0.572 (0.389–0.742)			

FIGURE 2 Receiver operator characteristic (ROC) curves comparing the ability of different expiratory breathing parameters and perinatal variables (birthweight z-score and sex) to predict development of bronchopulmonary dysplasia (BPD) (n=33). The combination model incorporates tidal expiratory flow at 50% of expired volume (TEF_{50}) to peak tidal expiratory flow (PTEF) ratio, birthweight z-score and sex. If the 95% confidence interval of the area under the ROC curve (AUC) includes 0.5 (no discrimination) the parameter does not predict BPD. The optimal cut-off point is where the sensitivity and specificity are maximal. FV_g: flow volume gravity; t_{PTEF}: time to peak tidal expiratory flow; t_E: expiratory time.

We cannot exclude that our findings reflect flow conditions or secretions in the endotracheal tubes. However, there were no clinical signs of tube obstruction in any of the participants during the time of data acquisition, and all neonates had an appropriately sized tube with minimal laryngeal air leaks. Furthermore, the obtained expiratory flow patterns do not agree with the pattern expected with tube obstructions or leaks. Expiration is primarily a passive process, and expiratory breathing parameters were thus considered to reflect natural lung mechanics. The positive end-expiratory pressure provided by the ventilator could theoretically bias the data, but it was almost identical in the two BPD subgroups.

Comparisons between the two subgroups of BPD

The neonates who went on to develop moderate/severe BPD had the least obstructive airflow pattern in the first few hours of life. Only the variable describing mid-expiratory flow (TEF_{50}/PTEF) differed significantly between the two groups of BPD, but the overall pattern was strengthened by the nonsignificant but coherent group differences also for the other expiratory flow parameters. In contrast, BPD in preterm-born infants is usually associated with increased expiratory airflow obstruction at term-equivalent age as well as throughout childhood, adolescence and early adulthood [18–20]. We do not have data to explain this unexpected finding. However, it is not unreasonable to assume that those neonates who later went on to develop the more severe forms of BPD were those who were born with the most immature lungs, characterised by low compliance due to low surfactant production and less developed and stiffer small and medium sized airways and distal respiratory airway structures [21, 22]. This is in accordance with major studies of EP-born neonates which show that low gestational age (*i.e.* increasing degree of prematurity) is a risk factor for severe BPD development within this already low gestational age group [23, 24]. It may further be speculated that this scenario could lead to more intense early interstitial and peribronchial inflammatory responses to positive pressure ventilation with a further decrease in lung and airway compliance. Additionally, the chest wall has a major influence on the underlying lungs, as its outward recoil is generally extremely low in preterm-born infants because of the soft rib cage and scarce intercostal muscles [22]. The overall effect for the most severely ill neonates could be an initial phase characterised primarily by high pulmonary elastic recoil pressures and reduced compliance, creating tidal airflow patterns that are less obstructive. This line of thinking is compatible with the clinically well recognised concept of "stiff lungs"; a term often used to describe the breathing pattern observed in the most severely ill preterm-born neonates with the more severe forms of neonatal respiratory distress syndrome.

Several studies suggest that neonates who go on to develop different severity of BPD have innate lung differences. Those who develop the severe forms tend to need more prolonged mechanical ventilation [20, 25], a larger proportion are treated with surfactant, they have different cytokine and growth factor patterns [26, 27] and a significant genetic predisposition has been proposed, although it is poorly understood [4, 11].

Prediction of later BPD from early airflow parameters

To our knowledge, the present study is the first attempt to examine to what extent easily accessible flow data from a mechanical ventilator can be used to predict later development of BPD in EP-born neonates.

Bhutani *et al.* [28] studied the relationship between pulmonary compliance and resistance and subsequent BPD in low birthweight infants (⩽1500 g) who required mechanical ventilation during their first week of life, and found that low dynamic pulmonary compliance (C_L) and high total pulmonary resistance were related to BPD. In addition, they created different BPD prediction models and found that a model dependent on gestational age and C_L had the highest positive predictive accuracy. Kim *et al.* [29] found that modified respiratory parameters, *i.e.* peak inspiratory pressure relative to birthweight and mean airway pressure relative to birthweight at 12 h of age were significant risk factors for the development of BPD.

Various scoring systems for predicting later BPD based on clinical variables such as gestational age, birthweight, sex, fraction of inspired oxygen, exposure to mechanical ventilation, sepsis and presence of patent ductus arteriosus have been proposed, but generally they have not been adopted in clinical practice. A general problem of these scoring systems has been their relatively low specificity or low positive predictive values [30]. Furthermore, the need for prolonged observation and inclusion of unpredictable complications obscure the significance of innate predispositions which, in our opinion, is fundamental in an attempt to understand probable causes and thus ways of preventing a progression towards severe chronic lung disease in children born prematurely.

Conclusions

Flow data easily obtained from a ventilator could be used to compute breathing parameters that discriminated between neonates who went on to develop the severe forms of BPD and those who did not. The parameter TEF_{50}/PTEF showed promising predictive abilities in this respect, and a model combining this parameter with birthweight z-score and sex could predict later respiratory morbidity with good accuracy. Future and larger studies are needed to validate these findings and to determine their clinical usefulness. If subsequent evaluations were to confirm the findings, this would open for targeted management and institution of customised preventive and early therapeutic measures in neonates at particular risk of developing severe BPD.

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