Digital stethoscope technology to evaluate breath sounds in preterm neonates with respiratory distress syndrome

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List of Abbreviations

RDS	Respiratory distress syndrome
DST	Digital stethoscope technology
FRC	Functional residual capacity
SRT	Surfactant replacement therapy
CXR	Chest x-ray
EELV	End-expiratory lung volume
NICU	Neonatal intensive care unit
EIT	Electrical impedance tomography
CPAP	Continuous positive airway pressure
HFOV	High frequency oscillatory ventilation
RIP	Respiratory inductive plethysmography

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Declaration

I declare that the work presented here is my own and contains no material previously published or written by another person, except where due reference has been made in the text. I have written the literature review, with guidance and editing from my supervisors Dr Malhotra and Dr Marzbanrad. The concepts for the research project are a collaboration between the supervisors and myself.

Introduction

Preterm births make up 8.5% of all births within Australia (1). Babies born prematurely have a high prevalence of respiratory morbidity. A recent report from the Australian and New Zealand Neonatal Network demonstrated that 92.9% of babies born less than 32 weeks gestation required respiratory support (2). Within this group, respiratory distress syndrome (RDS) was the largest indication for respiratory support. RDS is a condition primarily affecting premature neonates that results from surfactant deficiency and dysfunction. Surfactant is a lipid rich substance that lines the distal airways and acts to reduce the alveolar surface tension. Surfactant deficiency results in alveoli collapse, which leads to decreased lung aeration within these babies. There are many clinical and experimental tools used to assess lung aeration in neonates with RDS. Digital stethoscope technology (DST) has yet to be trialled within this field.

This literature review explains lung aeration and the factors that influence it within the neonate. It describes techniques that can be used to assess lung aeration in the newborn with RDS and explains the benefits and limitations posed by each technique. DST and the current literature available on its use in neonates are then described.

1. Background

Lung aeration

Lung aeration, or functional residual capacity (FRC), is the volume of air within the lungs at the end of normal expiration (3). At the end of inspiration, the stretched lungs have an elastic drive to return to their original shape and the chest wall acts to resist this drive. The FRC of the lungs occurs at the point in which the two opposing forces reach an equilibrium. It is therefore dependent on factors that influence the elastic recoil of the lungs and the resistance of the chest wall. In the neonate, the chest wall is compliant and offers little resistance to the elastic recoil of the lungs (4). This elastic recoil of the lungs is largely determined by alveolar surface tension, which is a collapsing force that occurs as the result of the attraction between molecules at the air-liquid interface within the alveolus (5). Due to this strong relationship, the presence or absence of surfactant - a surface tension lowering substance - within the lungs has a large impact on lung aeration within the neonate.

Surfactant

The presence of surfactant within the lungs was first identified as early as 1929 by Kurt von Neergard (6). Von Neergard studied excised pig lungs and found that when the lungs were filled with liquid, the surface tension was reduced, allowing them to expand more easily. During the 1950s, Pattle studied the foam obtained from the trachea of rabbits with pulmonary oedema and discovered that the bubbles within the foam were more stable than bubbles obtained from other tissues (7). He concluded that the stability of the bubbles must be due to a surface tension lowering a substance found within the lining of the alveoli. Simultaneously, Clements was undertaking experiments on surface films extracted from the lungs of rats, cats and dogs. Clements also deduced the presence of a surfactant within the lungs and was able to demonstrate an inverse relationship between the surface area of the film and the measured surface tension (8).

The production, composition and role of surfactant is now considered well known. Surfactant undergoes a complex production and degradation cycle (5). It is produced by type 2 pneumocytes cells that line the alveoli and secreted into the alveolus. Surfactant undergoes degradation by type 2 pneumocytes and alveolar macrophages. The breakdown components are reused in the production of future surfactant. Surfactant is a substance primarily composed of phospholipids (9). Dipalmitoylphosphatidylcholine, a saturated phospholipid, is the major surface tension lowering component of surfactant. Dipalmitoylphosphatidylcholine forms a tightly packed lipid film over the lining of the alveoli that reduces the attractive forces of molecules at the air liquid interface. The surface tension lowering properties of dipalmitoylphosphatidylcholine are augmented by the other

components of surfactant. Proteins present in surfactant, particularly SP-B and SP-C, and other lipids work to promote the adsorption of the lipid film to the alveolar surface, thereby helping to stabilise the alveoli and the end of expiration (5).

Respiratory distress syndrome

RDS is a condition caused by surfactant deficiency and dysfunction that is a common cause of morbidity in preterm infants. In 1958 Avery and Mead studied the surface tension of lungs from neonates who had died from RDS and found that it was increased compared to the lungs of neonates who had died from other causes (10). They became the first to link the absence of surfactant to the pathophysiology of RDS. Surfactant deficiency and dysfunction occurs in neonates with RDS for several reasons. Type 2 pneumocyte cells begin differentiation in the fetal lung at around 24 weeks gestation (11). As a result there are decreased number of these cells within premature neonates. In premature neonates the surfactant metabolism pathway is slowed, leading to reduced surfactant pools (5). In addition, the composition of surfactant in preterm neonates is different to that of a term neonate (12). Surfactant from neonates born prematurely has a lower concentration of saturated phosphatidylcholine, such as dipalmitoylphosphatidylcholine, and therefore does not function as effectively at reducing surface tension as mature surfactant (12). Immature surfactant production leads to increased alveolar surface tension and alveolar collapse. This presents clinically in the neonate with the following non-specific signs of respiratory distress: tachypnoea, nasal flaring, grunting, tracheal tug and use of accessory muscles (13). Changes to the lung that occur as a result of surfactant deficiency are visible on pathological examination of the tissue; microscopically acini collapse is seen and macroscopically this consolidation is visualised as “hepatisation” of the tissue (13). The natural history of RDS has been substantially altered by advancements in the perinatal care of these neonates, including the introduction of antenatal glucocorticoids and surfactant replacement therapy (SRT) (14).

Surfactant replacement therapy

Goran Enhorning performed the first successful experiment involving SRT in 1972 (15). Enhorning administered surfactant obtained from adult rabbits into the trachea of premature rabbits and demonstrated that it had a positive effect on lung mechanics. In 1980 Fujiwara undertook a landmark study involving surfactant replacement in 10 neonates with RDS (16). After administering surfactant endotracheally to ventilated neonates, Fujiwara found that the neonates’ clinical status improved, oxygen requirements decreased and there was increased lung aeration on chest x-ray (CXR). SRT is now a well-established therapy in the neonatal intensive care unit (NICU). The 2016 European Consensus Guidelines on the Management of Respiratory Distress Syndrome (17) recommend early SRT for neonates with RDS with

concerning oxygen requirements. The clinical effects of SRT on neonates with RDS are well established within the literature. SRT in neonates with RDS has been shown in two separate Cochrane systematic reviews to reduce the complications associated with RDS and decrease neonatal mortality (18, 19).

2. Tools to measure lung aeration in neonates with RDS

Systematic review

There are a number of tools that are used to measure lung aeration, with many of them applicable for use in neonates with RDS. A systematic review was performed to identify the recent literature available on such tools. The following databases were searched: OVID Medline, PubMed and Scopus. Where relevant articles were found, their reference lists were scoured for other relevant articles. The last search was performed on 15/04/2019.

Studies were included in the systematic review if the following criteria were met; participants were neonates with RDS at the time of investigation; the study measured lung aeration either visually or using FRC or end-expiratory lung volume (EELV); and the study was published in the last 15 years.

Studies were excluded from the systematic review if the participants weren't studied as neonates; the study population didn't have active RDS; the study did not measure a marker of lung aeration; the articles were review articles, systematic reviews or conference proceedings; and the study was not published within the last 15 years.

Search strategy

Search 1 - 'Functional Residual Capacity' was mapped to subject heading and included as a MeSH term. Keywords searched were 'functional residual capacity,' 'FRC,' 'end expiratory lung volume,' 'end expiratory thoracic volume,' and 'aeration.' These were all combined with the Boolean operator 'OR'.

Search 2 - 'Respiratory distress syndrome' was mapped to subject heading and the MeSH terms 'Respiratory distress syndrome, Newborn' and 'Hyaline Membrane Disease' were included. Keywords searched were 'respiratory distress syndrome,' 'RDS,' 'hyaline membrane disease,' 'HMD,' 'surfactant deficiency' and 'SDD.' These were all combined with the Boolean operator 'OR'.

Search 3 – 'Ultraso*' was mapped to subject heading and the MeSH term 'Ultrasonography' was included. The keyword 'Ultraso*' was combined with the MeSH term using the Boolean operator 'OR'

Search 4- ‘Electrical impedance tomography’ was mapped to subject heading and the MeSH term ‘Electrical Impedance’ was included. Keywords searched were ‘Electrical impedance tomography,’ ‘EIT,’ ‘applied potential tomography’ and ‘APT.’ These were all combined with the Boolean operator OR.

Search 5 – ‘Plethysmography’ was mapped to subject heading and the MeSH term ‘Plethysmography ’was included. Keywords searched were ‘plethysmography,’ ‘respiratory inductive plethysmography’ and ‘RIP.’ These were combined with the Boolean operator ‘OR’

Search 6 – ‘Multiple breath washout’ was mapped to subject headings and no relevant MeSH terms were found. Keywords searched were ‘Multiple breath washout,’ ‘multi breath washout’ and ‘MBW.’ These were combined with the Boolean operator ‘OR.’

Search 7 – ‘Nitrogen washout’ was mapped to subject heading and no relevant MeSH terms were found. Keywords searched were ‘Nitrogen washout,’ ‘fowler’s method’ and ‘single breath oxygen test.’ These were combined with the Boolean operator ‘OR.’

Search 7 – ‘Helium dilution’ mapped to subject heading and no relevant MeSH terms were found. Keywords searched were ‘Helium dilution’ and ‘gas dilution.’ These were combined with Boolean operator ‘OR.’

The following searches were combined with Boolean operator ‘AND’: Search 1 and 2, search 2 and 3, search 2 and 4, search 2 and 5, search 2 and 6 and search 2 and 7. Results were limited to newborn, humans, English language and last 15 years.

The PRISMA flow diagram for the systematic review is demonstrated in figure 1.

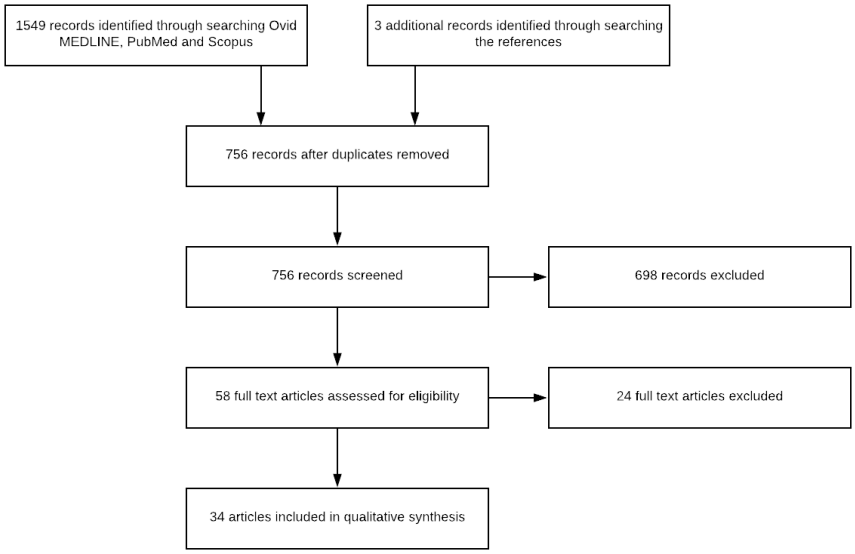


Figure 1 – PRISMA flow diagram for systematic review

A summary of the recent published literature on tools to measure lung aeration in neonates with RDS is depicted in table 1.

Table 1 – Literature on tools to assess lung aeration in neonates with RDS

Author (year)	**Principal aim**	**Pooled participants (n)**	**Main findings**
Ultrasound			
Soldati (2009) (20) Liu (2014) (21) Chen (2017) (22)	Characteristics of RDS on US	3701	Common findings are lung consolidation, pleural line abnormalities, absence of A-lines and white lung
Copetti (2008) (23) Liu (2014) (24) Abdelsadek (2015) (25) Sawires (2015) (26)	US features compared to CXR grade	325	No consensus on whether US findings correlate with CXR grade
Bober (2006) (27) Ahuja (2012) (28) Vergine (2013) (29) El Malah (2015) (30) Rachuri (2017) (31)	Diagnostic accuracy of US in identifying cases of RDS compared to CXR	472	US has high sensitivity and specificity for the diagnosis of RDS
Brat (2015) (32) Martino (2018) (33) Perri (2018) (34)	US score to predict surfactant need and oxygenation	319	US score is good at predicting surfactant need in premature neonates

Raimondi (2018) [35]	US score to predict oxygenation	75	US scores are well correlated to oxygenation indices
Brusa (2015) [36]	Interobserver agreement	114	US has high interobserver agreement between interpreters with different levels of experience
Rodriguez-Fanjul (2016) [37]	US score to predict mechanical ventilation	105	US score is accurate at predicting mechanical ventilation
Electrical Impedance Tomography			
Bhatia (2017) [38]	Effect of recruitment procedure in neonates on CPAP	20	Lung hysteresis is present in majority of neonates with RDS
Miedema (2011) [39] Miedema (2012) [40]	Effect of recruitment procedure in neonates on HFOV	25	Lung hysteresis is present in neonates with RDS
Miedema (2011) [41] Chatziioannidis (2013) [42]	Effect of SRT in neonates on HFOV	32	SRT increases lung aeration
van der burg (2016) [43]	Effect of minimally invasive SRT in neonates on CPAP	16	Minimally invasive SRT increases lung aeration
van Veenendaal (2009) [44] Hough (2014) [45]	Effect of endotracheal suction in neonates on HFOV	31	Endotracheal suction results in an initial decrease in EELV. In the long term endotracheal suction results in an increase in EELV
Hough (2012) [46]	Effect of body position in neonates on CPAP	30	Position change did not affect lung aeration
van der Burg (2015) [47]	Effect of prone positioning after extubation	20	EELV increased with prone positioning after extubation
van der Burg (2014) [48]	Accuracy of EIT in measuring lung volume compared with RIP	23	There was correlation between EELV measured by EIT and RIP
Respiratory Inductive Plethysmography			
Tingay (2006) [49] Tana (2015) [50] Vento (2011) [51]	Effect of recruitment procedure in neonates on HFOV	28	Lung aeration initially increases with increasing continuous distending pressures but then decreases at high continuous distending pressures
Emeriaud (2010) [52]	Variability of EELV in neonates	18	There is high breath to breath variability in EELV in premature infants with and without RDS
Helium Gas Dilution			
Kumar (2005) [53]	Correlation between FRC measured by CXR and helium dilution	14	FRC measured by CXR is moderately correlated to FRC measured by helium dilution

Abbreviations: US – ultrasound, RDS – respiratory distress syndrome, CXR – chest X-ray, CPAP – continuous positive airway pressure, HFOV - high frequency oscillatory ventilation, SRT – surfactant

replacement therapy, EELV – end expiratory lung volume, EIT – electrical impedance tomography, RIP – respiratory inductive plethysmography, FRC – functional residual capacity

Chest X-ray

The principle of X-ray relies on the concept that different tissues within the body have variable densities. An X-ray beam is an electromagnetic wave that, when directed at a patient, is absorbed in different proportions depending on the tissue density. After passing through a patient, the resulting beams are absorbed by a photosensitive cassette, creating an image of the underlying body tissues.

Since its discovery in 1895 by Wilhelm Conrad Roentgen (54), X-ray has become a frequently used diagnostic tool, especially amongst neonates. X-rays are commonly used in the neonatal intensive care unit (NICU) in the initial assessment of respiratory distress. The number of X-rays received has been shown to be inversely correlated with gestation (55, 56). The most premature infants can be subjected to many X-rays within their first few weeks of life, with one study by Edison et al. (56) demonstrating that infants less than 750g received on average 30 X-rays over a six week period.

The typical chest X-ray (CXR) appearance of RDS demonstrates poorly inflated lungs with a reticulogranular or “ground glass pattern” that is consistent over the lung fields (57, 58). This pattern is thought to occur as the result of alveolar collapse and pulmonary oedema. Air bronchograms may be visualised on the radiograph (57). These are formed by the subsequent dilation of terminal bronchioles. The x-ray appearance of RDS can be graded one to four according to severity (59). The appearance of the different grades is as follows: grade one shows a fine reticulogranular pattern; grade two demonstrates air bronchograms; grade three illustrates consolidation of lung tissue; grade four demonstrates a white out appearance of the lung which impairs the ability to visualise the cardiac border (59). The CXR severity grade four is demonstrated in figure 2a. The correlation between CXR severity and clinical severity was evaluated in a study by Kero et al (60). The study found that the CXR severity matched clinical severity in 62% of cases. While the study mentions that the person assessing the CXR was blinded, it does not provide detailing on this blinding process to determine whether or not this was sufficient. SRT causes changes to the aeration of newborn lungs that are visible on CXR (58). Following SRT the lungs appear more radiolucent which correlates to an increase in lung aeration. The improvements are usually generalised but may be asymmetric due to the uneven distribution of surfactant. The appearance of the lungs after SRT is demonstrated in figure 2b.

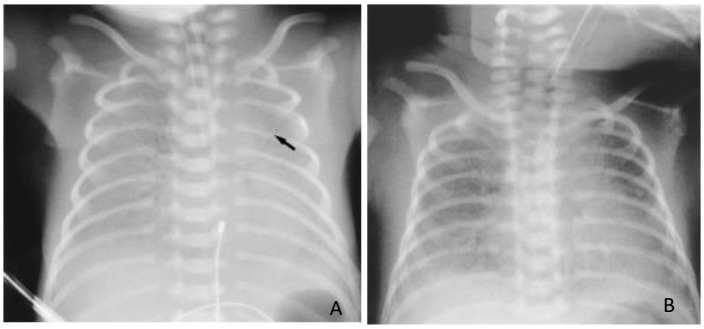


Figure 2 - Chest X-ray appearance of respiratory distress syndrome before and after surfactant replacement

(A) CXR of neonate showing grade 4 RDS. (B) CXR following surfactant therapy showing uneven improvements in white areas of the lung. Reproduced from Arthur R. Paediatr Respir Rev. 2001 (59)

CXR has many features that make it advantageous to use as a technique to measure lung aeration. It is a portable diagnostic tool that can be performed quickly and easily at the bedside. The images are automatic to allow for rapid diagnosis of disease. CXR also has a number of limitations. Despite the CXR features of RDS being well known, they can be hard differentiate from other causes of respiratory distress such as pneumonia (4). X-rays also pose a risk to health due to the effects of ionising radiation. Newborn infants are thought to be more sensitive to the harmful effects of ionising radiation compared with older children and adults (61). This is due to their small size (62), increased organ sensitivity (63) and longer lifespan for the negative health effects to manifest.

Lung ultrasound

Lung ultrasound is an imaging technique that uses the absorption and reflection of sound waves to provide information on the underlying tissue. Historically, lung ultrasound was not seen as a valued imaging technique as the normal aerated lung causes the ultrasound waves to be completely reflected, disabling the ability to visualise the lung parenchyma (64). In lung pathologies there are changes to the air-tissue interface which produce characteristic artefacts on lung ultrasound (65). Common artefacts and their appearance are illustrated in table 2. The ability to use these artefacts to differentiate causes of respiratory distress is a relatively new concept. As a result many recent studies have been undertaken on neonates to characterise the ultrasound findings in pathologies such as transient tachypnoea of newborn (66), pneumonia (67) and meconium aspiration syndrome (68).

Table 2 - The appearance of major artefacts on lung ultrasound

Artefact	Appearance

Pleural line	Smooth white line < 0.5 cm thick. Moves horizontally with respiration.
A - lines	Many horizontal white lines, situated below the pleural line and running equal distance apart.
B- lines (lung comets)	Vertically orientated white lines beginning at the pleural line and extending out
Alveolar interstitial syndrome	Three or more B-lines
Lung consolidation	Lung takes on the appearance of the liver on ultrasound.
Air bronchograms	Visible distal airways
White lung	Many B-lines situated close together causing a white appearance on ultrasound

Adapted from Liu J et al. Iran J Pediatr. 2014 (24) and Liu J at al. Chest 2016 (66)

The appearance of RDS on lung ultrasound has been characterised by several studies (20-22). A large study was performed in 2015 including 657 cases of RDS and 747 controls (22). This study found that lung consolidation, thickened irregular pleura and absent A-lines occurred in 100% of cases of RDS and no controls. Pleural effusion, interstitial syndrome, pulmonary oedema and lung pulse were seen in 31.7%, 70.3%, 85.2% and 22.2% of cases respectively. While the clinician performing and interpreting the ultrasound was said to be blinded to patient's clinical information, as ultrasound was performed at the bedside, complete blinding to patient's clinical scenario is not possible. Despite these limitations, similar findings have been repeated in other smaller studies (20, 21). The appearance of RDS on lung ultrasound is demonstrated in figure 3.

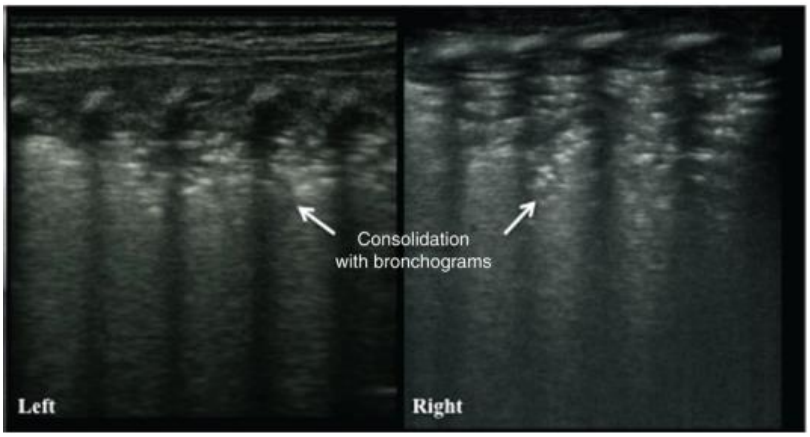


Figure 3 - Ultrasound appearance of RDS

The ultrasound appearance of RDS showing consolidation and air bronchograms. Reproduced from Liu J et al. Medicine 2014 (21)

The correlation between lung ultrasound findings and CXR grade of severity has been evaluated by several studies. An early study in 2008 found no correlation between the ultrasound appearance of RDS and radiological grade of severity (23). This study was limited by the fact that the sonographers performing and interpreting the ultrasound were not blinded to the clinical and radiological status of the patient. It is also unclear as to when the CXR was taken in relation to SRT that may influence results. A later study found that the extent of lung consolidation and visibility of air bronchograms increased with increasing x-ray severity (24). In this study the sonographer was blinded to the participants' clinical information and the CXR was performed immediately following ultrasound and interpreted by a radiologist blinded to ultrasound result. Another study found that the occurrence of lung consolidation and white lung increased with increasing x-ray severity (26). While sonographers in this trial were blinded to the radiological findings, it is unclear as to whether they were blinded to the clinical scenario.

Several studies have been performed to determine whether lung ultrasound patterns correlate to oxygenation indices and can be used to predict the need for SRT in neonates with RDS. Raimondi et al. (35) found that lung ultrasound scores were correlated to oxygenation indices when images were interpreted by two neonatologists both blinded to the patients' clinical scenario. Two other studies have had similar findings (32, 33). In addition to looking at oxygenation indices, Brat et al. (32) found that lung ultrasound score was significantly better at predicting SRT in neonates less than 34 weeks gestation compared to those more than 34 weeks gestation. Within this study, the lung ultrasound score was given by the physician performing the scan, and therefore complete blinding of clinical status was not possible. To make up for this limitation, the study was able to demonstrate high interobserver agreement between these scores and additional scores that were made using recorded images by blinded investigators away from the bedside. A study with a similar methodology was also able to identify that lung ultrasound scores were highly accurate at predicting surfactant treatment in neonates less than 30 weeks gestation (33). Additionally, this study found that the lung ultrasound scores were accurate at predicting surfactant retreatment, but that this accuracy was lower compared to initial treatment. Perri et al. (34) compared the accuracy of lung ultrasound scores to chest x-ray scores in the prediction of surfactant administration in preterm neonates. They were able to demonstrate that lung ultrasound scores had a greater sensitivity, specificity and predictive values when compared to chest x-ray.

On a similar note, the ability for lung ultrasound patterns to predict the need for invasive ventilation has also been assessed. A study by Rodriguez-Fanjul et al. (37) found that high risk ultrasound patterns, including the pattern seen in RDS, had a sensitivity and specificity of 95 and 82.5% respectively for the prediction of mechanical ventilation. The pattern was determined by an investigator who did not perform the ultrasound and was blinded to clinical scenario and CXR findings. This study found that sensitivity and specificity for lung ultrasound in predicting mechanical ventilation was greater than that found for chest radiography, but the difference between the two was not found to be statistically significant.

The accuracy of lung ultrasound in diagnosing RDS has been evaluated by several studies (27-31). A 2014 study by Vergine et al. (29) found the sensitivity and specificity of lung ultrasound in diagnosing RDS to be 95.6% and 94.4% respectively when compared to clinical and radiological diagnosis. In this study, the ultrasound images were sent to an external referee to make the final diagnosis and therefore this referee was able to be completely blinded to clinical scenario. Similar results were found by other studies (27, 30, 31). Ahuja et al. (28) reported the lowest sensitivity and specificity for lung ultrasound in the diagnosis of RDS. This may be due to the fact that this study used the transabdominal ultrasound approach, unlike the majority of the other studies, which used the transthoracic approach or a combination of both. A systematic review combined the results of six studies and found the sensitivity and specificity of lung ultrasound in diagnosing RDS to be 97% and 92% respectively when compared to chest x-ray (69). It should be noted, however, that there was significant heterogeneity between the articles used within the systematic review.

Ultrasound has many features that make it advantageous for use within the NICU. It is a low-cost, radiation-free imaging technique. Ultrasound is considered a safe diagnostic technique for neonates (70). It can be performed quickly and easily by the bedside and gives automatic results which can be interpreted at the time of examination. Lung ultrasound has been shown to have high interobserver agreement for the diagnosis of RDS (36). A retrospective study found that there were high levels of agreement between a primary interpreter and three different interpreters, regardless of the level of experience. Ultrasound is limited by the fact that physicians require formal training to interpret and perform the scans. The ultrasound probe has the potential to introduce bacterial pathogens into the cot if not disinfected prior to and following use. Additionally, the propagation of ultrasound waves can be limited by dressings on the chest.

Electrical impedance tomography

Electrical impedance tomography (EIT) measures regional lung aeration by assessing the electrical resistance through a cross section of the lung (71). To measure electrical

resistance, multiple electrodes are positioned across the neonate's chest, as seen in figure 4. Paired electrodes deliver small currents to the body and the resulting electrical potentials between other pairs of electrodes are measured. The ratio of the applied current to the electrical potential forms the electrical impedance. Changes in lung aeration results in changes in the electrical resistance of the lung tissue (72). These changes will therefore be picked up as changes in the electrical impedance. Barber and Brown are credited with inventing the first EIT device during the 1980s (73). For many years, EIT devices were used solely as a research tool (74). There are now different models of EIT devices commercially available and being used in adult intensive care units (75), however they are not yet routinely used within the NICU. In neonates, EIT is mainly used experimentally in the evaluation of different interventions and their effects on lung aeration.

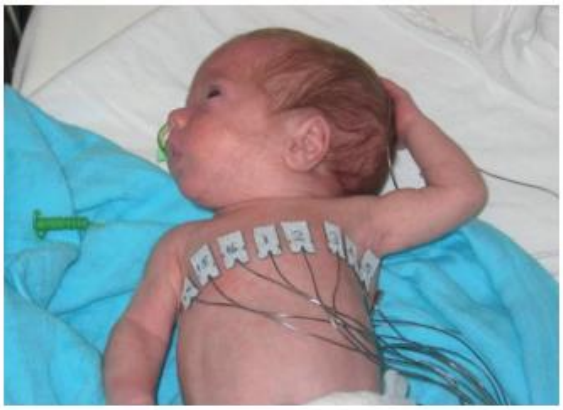


Figure 4 – Electrode placement on an infant in electrical impedance tomography

Reproduced from Wilmott R et al. Kendig & Chernick's Disorders of the Respiratory Tract in Children 2012. (76)

EIT has been used to identify regional differences in lung aeration in neonates with RDS. In an Australian study, Hough et al (46) found that there was increased ventilation in the posterior and right lung regions in preterm neonates on continuous positive airway pressure (CPAP). The study also found that preterm neonates on CPAP had greater variation in ventilation across the lung field than spontaneously breathing preterm neonates. These findings appear to be different in neonates on high frequency oscillatory ventilation (HFOV). Two studies involving neonates on HFOV found that lung aeration appeared to be greater in the anterior and right lung regions (41, 42). The effect of body position on lung aeration has also been studied. One study found that body position had no effect on regional lung aeration in neonates on CPAP (46). In contrast, another study found that there was an increase in EELV following prone positioning of infants who were extubated to CPAP (47).

The increase in EELV occurred in both anterior and posterior lung regions but was greatest in the posterior region. This study was limited by a lack of control and as a result it is hard to differentiate the prone positioning as the cause for increased EELV.

Miedema et al used EIT to map out the pressure volume curve of neonates on HFOV during a recruitment procedure (39). A recruitment procedure aims to open and stabilise alveoli at the lowest possible distending pressure. The study found that lung hysteresis was present in all of the participants; the recruitment procedure allowed ventilation to be moved onto the deflation limb of the pressure volume curve, where a greater lung volume was able to be maintained at a lower distending pressure. This finding was confirmed by a later study with similar methodology (40). A 2017 Australian study examined the effect of a recruitment procedure in neonates on CPAP (38). The study was able to demonstrate lung hysteresis in 13 out of 20 neonates. The study found no difference in the baseline characteristics of the infants who demonstrated hysteresis versus those who did not. The study also measured oesophageal pressure as a proxy for intrapleural pressure. Additionally there was no difference in the intrapleural pressures measured between the groups that could account for the absent of hysteresis.

The effect of SRT has on lung aeration has also been studied using EIT (41-43). One study found that SRT increased lung volume by a mean of 61% (41). The plateau in lung volume was reached within 5 minutes of SRT. Lung volume increase was greatest in the posterior region of the lung, thought to be due to the effects gravity had on the displacement of surfactant. Another study demonstrated similar findings (42). As well as showing an increase in lung volume most pronounced in the posterior lung area, they were also to demonstrate greater volume change in the left lung region compared to right. Van der Burg et al (43) assessed the effects of minimally invasive SRT on lung aeration in neonates on CPAP. Following minimally invasive SRT there was an initial increase in the volume of the posterior lung over the anterior regions, but at 60 minutes this difference was not statistically significant. They did not find any difference in lung volume changes between the left and right lung with SRT. All studies were limited by a lack of control, which made it difficult to isolate whether surfactant was the cause of effect.

The effect of endotracheal suction on lung aeration has also been explored using EIT. One study found that endotracheal suction caused a decrease in electrical impedance, correlating to a decrease in lung aeration, of which majority was regained after cessation of the suctioning (44). The study was limited by its small sample size of only 11 neonates. Another study found similar short term effects of endotracheal suctioning (45). This latter study also

found that following the cessation of endotracheal suctioning, EELV was increased from baseline, and this increase was maintained for the 2 hours following.

EIT has many properties, which make it an attractive tool for use in the NICU. It is a radiation free technique that can be performed at the bedside. EIT takes frames rapidly and can assess regional changes in ventilation, making it a suitable tool for monitor the effects different interventions have on lung aeration (71). The long term use of EIT in a clinical setting has been evaluated in a by a recent study run across multiple countries and centres (77). The study demonstrated stability in contact impedance measurements over time. Other than a mild skin rash near the placement of the electrode belt in six out of 30 neonates, no other adverse events were seen to occur. EIT has several limitations to its use. The positioning of the electrodes can be technically difficult in the neonate, especially if the electrodes are not in a belt form. In addition, the image resolution of EIT is decreased when compared to other techniques (78).

EIT measures the change in resistance and conductivity over a cross section of the chest only, which creates concerns about whether these results are able to be extrapolated to provide information on lung aeration globally. A study by found that cross sectional lung volume measured by EIT correlated to whole lung volume measured by respiratory inductive plethysmography (RIP) in neonates with RDS on HFOV (48). RDS is thought to be a homogenous disease. Therefore, while EIT measurements are accurate for the whole lung in this group, more research needs to be undertaken to see if this correlation is true for other neonatal pathologies, which are not homogenous in nature.

Respiratory inductive plethysmography

Respiratory inductance plethysmography (RIP) can be used to measure FRC by assessing changes in chest and abdominal circumference (79). Two belts containing transducers are worn; one at the level of nipple and one at the level of umbilicus as shown in figure 5. The changes in the resistance of flow of the transducers during respiration can be used to determine changes in the volume of the chest and abdomen. Cohn is credited with introducing the first RIP device in 1977 as a tool to monitor respiration (80). RIP devices are still mainly used as an experimental tool in neonates.

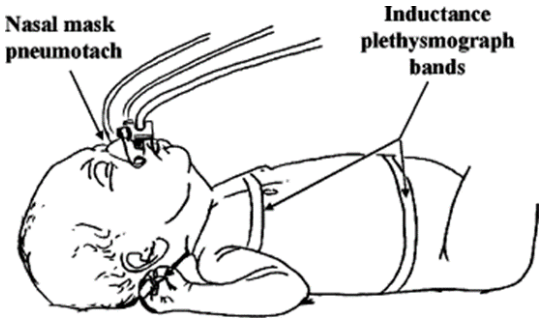


Figure 5 – Respiratory inductive plethysmography bands

Reproduced from Di Fiore J.M. Semin Neonatol 2004 (81)

An early Australian study mapped out the pressure volume curves of neonates undergoing a recruitment procedure using RIP (49). The study was able to demonstrate the presence of lung hysteresis in 11 out of the 12 neonates studied. The only neonate in whom lung hysteresis could not be demonstrated was the only neonate within the study who had RDS. The study was limited by its small sample size and also by the fact that neonates were given muscle relaxants, which may have affected results. A later study involving neonates with RDS found that FRC initially increased with increasing distending pressure, but then was seen to decrease as higher distending pressures were applied (51). RIP can't differentiate between changes in blood flow and changes in aeration. As the oxygen requirements were seen to decrease with increasing distending pressure, the study hypothesised that the decrease in FRC was actually due to a reduction in pulmonary blood flow. They were not able to prove this theory within the constraints of their study design. As well as using RIP to measure EELV, another study assessed pulsatile index, oxygen saturation and cerebral and renal tissue oxygenation during a recruitment procedure (50). The study also found that EELV decreased at high distending pressures, and that this was accompanied by decrease in pulsatile index, post ductal saturation and renal tissue oxygenation. While the latter findings may suggest a decrease in left ventricular output as a result of increased pulmonary vasculature resistance, a direct measure of haemodynamic changes such as a Doppler echocardiogram is needed to confirm the pulmonary vascular changes with high continuous distending pressures.

RIP was used to determine the difference in the breath to breath variability of EELV amongst different groups of premature neonates (52). The study found that there was large variations in breath to breath EELV in self-ventilating premature neonates with and without RDS. While not statistically significant, the breath to breath variability of EELV in neonates with RDS on respiratory support appeared to be lower. There appeared to be different

patterns of variability amongst the groups; the EELV in neonates with RDS would vary from the baseline for longer periods when compared with neonates without RDS, which demonstrated less respiratory control.

RIP has similar qualities to EIT that make it desirable for use in the neonate; it is a non-invasive bedside test that can monitor dynamic changes in lung aeration. Additionally, like EIT, RIP has many limitations, which have resulted in it being a largely experimental tool. It can be difficult to maintain the position of the bands and the recordings are affected by heat and movement (49). Additionally measurements are affected by changes in both lung aeration and pulmonary blood flow, which creates an added complexity in interpreting the results (51).

Helium dilution

The helium dilution technique measures lung aeration through utilising the concept of diffusion (82). The neonate breathes into a circuit with a known concentration helium. As the neonatal lungs do not usually contain helium, the helium gas diffuses into the lungs down the concentration gradient until the concentration of helium in the circuit and the lungs reach an equilibrium. The initial and final helium concentrations can be used to calculate the FRC of the lungs.

A single study using helium dilution in neonates with RDS was identified in the systematic review. This study compared FRC measured by helium dilution to lung area measured on CXR and identified a moderate correlation between the two in both the supine and prone positions (53). Within the study, the lung area was interpreted by two different investigators, with only one of the investigators blinded to the FRC values. As the lung areas were averaged, this has the ability to affect the results.

Helium dilution technique has been used in the past to assess lung aeration in neonates with RDS (83, 84). The technique is supported in neonates as it is non-invasive and can be performed in the unsedated and sick neonate (82). The lack of use of the helium gas dilution in recent times may be due to the limitations the technique poses; measurements by helium gas dilution may be affected by gas trapping and mask leaks.

Other tests

There are a number of other tests which are available to measure lung aeration in neonates, but no recent literature on their use in neonates with RDS was identified. Available techniques to measure lung aeration, their advantages and limitations are listed below in table 3.

Table 3 - Tools available to measure lung aeration

	Available tools						
	X-ray	Ultrasound	Electrical impedance tomography	Respiratory inductive plethysmography	Gas techniques	Whole body plethysmography	Computed tomography
Advantages	Cheap Bedside Instant results	Cheap Bedside Instant results Radiation free	Bedside Radiation free Dynamic monitoring Assess regional changes	Bedside Radiation free Dynamic monitoring	No sedation	Measures trapped gas	High quality image resolution
Disadvantages	Ionising radiation	Training Infection	Appliance of electrodes Technical analysis Cross sectional only	Technical analysis Sensitive to heat and movement Influenced by blood flow	Doesn't measure trapped gas Affected by mask leaks Alter breathing patterns Costly	May require sedation Bulky machine Unsuitable for unstable or small neonates	Ionising radiation Transfer to machine Unsuitable for unstable neonates Costly Time consuming

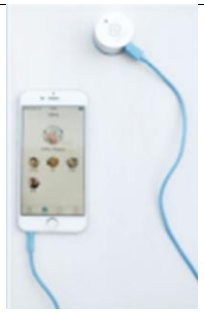
3. Digital stethoscope technology

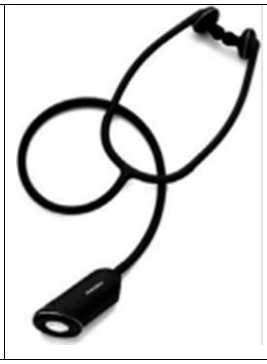
The humble stethoscope, first invented by Rene Laënnec over 200 hundred years, ago has undergone modern advancements to keep up with today's technological world. Digital stethoscopes technology (DST) is now available as clinical tool to monitor the sounds of the heart, lungs and gastrointestinal system. Like their acoustic counterparts, digital stethoscopes have a diaphragm which functions to capture sounds (85). Instead of the sound being transmitted via acoustic vibrations, these sounds are converted to electrical signals, which can then be amplified and processed to optimise the information they contain. These electrical signals can be converted back to sound waves for listening. They can also be transferred to an electronic device where they can be stored, transferred to health professionals or be presented visually as phonocardiogram or spectrogram. There are currently many different types of digital stethoscopes available commercially (86). The stethoscopes previously used in the paediatric population are demonstrated in table 4.

Commenté [FM1]: Can technically be also recorded and shared with other clinicians' review and even enables crowd-sourcing diagnosis.

Table 4 - Digital stethoscope brands used in paediatric population

Stethoscope name	Image

Littman Electronic Stethoscope Model 3200/400	
Thinklabs One Digital Stethoscope	
Clinicloud Digital Stethoscope	
ViScope Visual stethoscope	
EkoCore and EkoDuo Digital Stethoscopes	

Welch Allyn Meditron/Meditron M30 Stethoscope	

Adapted from Ramanathan et al Acta Paediatrica 2018 (86)

The current literature involving the use of DST in the paediatric population has been reviewed recently by Ramanathan et al. (86). Digital stethoscopes have been used previously in the paediatric population to evaluate the breath sounds in both healthy and diseased lung states but none of these studies have included neonates in their sample group. One previous study has looked at the spectral and temporal characteristics of breath sounds in children (87). This study involved 186 children aged 2 months to 5 years with no respiratory symptoms and a normal respiratory exam. The study found a linear relationship between the children's age, height and weight and the spectral and temporal characteristics of the breath sounds. In addition, this study was able to validate the use of DST as a clinical tool in a busy hospital environment.

In terms of pathological breath sounds, DST has been shown in several studies to have moderate interobserver agreement for the interpretation of wheeze and crackles (88-90). An Australian study found that DST was more accurate than standard auscultation at detecting paediatric wheezing (91). It is unclear as to whether the clinician interpreting the breath sound recordings within the study was blinded to the patients' clinical situation. This study is also limited by its small sample size. Another study performed across multiple centres in Nepal found DST to have a sensitivity and specificity of 56% and 73% respectively for the diagnosis of pneumonia when compared to radiography (90). These low values may be attributed to the recruitment method. The recruitment occurred in an outpatient clinic where patients were presenting with less severe pneumonia and many of which had already received antibiotic treatment, and as a result may have had cases of pneumonia that were not picked up radiographically. When comparing DST to clinical diagnosis of pneumonia the sensitivity remained similar but the specificity increased to 85%.

The available literature on the use of DST in neonates is lacking (86). Several studies have been performed using DST technology to assess the heart rate of neonates. Two studies

have found that the heart rate recorded by DST was well correlated to heart rate recorded by ECG (92, 93). Another study found that heart rate recorded by DST was slower than the heart rate recorded by standard auscultation but this difference was not found to be statistically significant (94). All studies demonstrated some limitations associated with DST; crying, loss of contact with chest and cord wirings were seen to affect recordings within these studies (92-94). A study assessing the use of DST in the diagnosis of pathological heart murmurs included neonates in its sample group (95). The study used a computer algorithm to analyse the phonocardiograms of 106 children and found and found that it had a sensitivity and specificity of 87% and 100% respectively when compared with echocardiograms. DST has also been studied in the evaluation of neonatal bowel sounds to monitor peristalsis, with studies demonstrating audible changes in bowel sounds during feeding (96, 97).

Monash Children's Hospital has several studies in manuscript stages involving the use of DST in the evaluation of neonatal breath sounds. These include the "CLEAR" study, which evaluates the difference in the breath sounds of neonates born by vaginal delivery versus caesarean section and the "Neo-Auscultate" study that characterises the differences in the breath sounds of term and preterm neonates over time.

4. Summary and rationale of the research

Lung aeration in the neonate is greatly determined by the presence or absence of surfactant within the lung. Surfactant is a lipid rich substance that lines the alveoli and acts to reduce alveoli surface tension. RDS is a condition that occurs as a result of immature surfactant production systems. Neonates with RDS have increased alveolar surface tension resulting in alveoli collapse and decreased lung aeration. SRT has been shown in these groups to improve lung aeration and clinical outcomes.

Many tools have been used previously to assess lung aeration in neonates with RDS. X-ray is currently the most commonly used clinical tool in this group but its repeated use is largely limited by the effects of ionising radiation. Lung ultrasound is taking off as a diagnostic tool for many causes of respiratory distress in the neonate, including RDS. It is a radiation free technique that can be performed at the bedside and has been shown to have high accuracy in diagnosing RDS and predicting SRT. EIT and RIP are tools that have been used to monitor the effect different interventions have on lung aeration in neonates with RDS. Currently their use is limited to experimental tools due to the difficulty in performing and interpreting these tests.

In today's age of technology, digital stethoscopes have evolved from their humble acoustic counterpart and offer the advantages of sound amplification, filtering of artefacts, ~~and~~

spectrographic/phonocardiographic representation of the sound, and artificial intelligence for clinical decision support. While there is increasing literature on the use of digital stethoscopes to evaluate pathological and non-pathological breath sounds in paediatrics, evidence on the use of DST in neonates is lacking. Currently no published studies exist involving the use of DST in the evaluation of breath sounds in neonates. DST has many qualities that make it desirable for use as a tool to monitor lung aeration in neonates with RDS. It is a simple, fast, non-invasive bedside technique. Additionally its use in evaluating breath and heart sounds in busy clinical environments has be validated by several studies (87, 92, 94).

While no available literature exists involving the use of breath sounds in the evaluation of lung aeration, there is evidence within the literature to suggest that changes in lung aeration may alter the acoustics of sound. Several studies on animal lungs in vitro have demonstrated an increase in the attenuation of sound and decrease in velocity of sound with increased inflation of the lung (98, 99). We wish to evaluate whether changes in lung aeration will result in acoustic differences in the breath sounds recorded using DST. A similar concept has been studied once before previously. A small study analysed the acoustic reflection of sound before and after SRT in neonates with RDS (100). The study was able to demonstrate an increase in the reflection of sound from the lung tissue following SRT, as shown in figure 6. This study was limited by its small sample size and only two neonates were analysed before and after SRT. Additionally, the study did not measure physiological breath sounds; it used a transducer and receiver to deliver and record sounds. In this study we aim to use a novel clinical tool, the digital stethoscope, to evaluate the breath sounds of neonates with RDS. We aim to identify differences in the acoustic characteristics of the sound with changes in lung aeration.

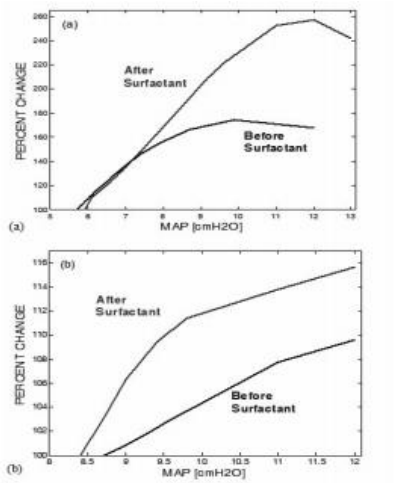


Figure 6 - Reflection of sound before and after surfactant replacement therapy

Change in the acoustic reflection of the sound in two neonates (a and b) before and after surfactant replacement therapy. Following surfactant replacement therapy there is evidence of increase in the acoustic reflection. Reproduced from Luria et al. Proceedings of the 29th Annual International Conference of the IEEE EMBS Cité Internationale, 2007 (100).

5. Project outline

Aims

To identify acoustic differences in the breath sounds of preterm neonates with RDS before and after SRT.

To identify differences (if any) in the acoustic characteristics of breath sounds recorded at 24 – 48 hours of life between preterm neonates who received SRT and those who did not receive SRT.

Hypothesis

The acoustic characteristics of breath sounds of neonates recorded using DST may be different before and after SRT.

The acoustic characteristics of breath sounds of neonates recorded using DST at 24-48 hours of life may be different in neonates who received SRT compared to those who did not receive SRT.

Study design

This is a prospective-observational study. Neonates involved in this study will have no changes made to their usual care.

Setting - The study will be conducted at Monash Medical Centre and Monash Children's Hospital

Study population – The population being studied are preterm neonates less than 32 weeks gestation. We aim to study two groups within this population; neonates that require SRT and those that do not require SRT.

Recruitment – Parents of neonates that fulfil the inclusion criteria will be invited to participate in the study during the antenatal or early postnatal period. Parents will be recruited on the wards and written, informed consent will be obtained from either parent of the child. We will recruit a minimum of 20 participants within each group. The recruitment period will run from March 2019 until August 2019.

Inclusion criteria

- All neonates delivered at Monash Medical Centre during the recruitment period born at
- Gestational age between 24 and 32 weeks inclusive whose
- Parents have given informed consent to involvement in the study

Exclusion criteria

- Neonates born at or above 32 weeks gestation
- Known fetal lung pathology
- Known major fetal anomaly or genetic diagnosis
- Parents unable to give informed consent

Data collection will be ceased at any time if it is found to be obstructing any necessary treatment of the neonate or parents have withdrawn their consent for involvement within the study.

Methodology

This trial has ethics approval from both Monash Health and Monash University Human Research Ethics Committees.

Procedure:

Neonates who require SRT will have breath sound recordings made at three distinct times; within one hour prior to SRT, within an hour post SRT and between 24 – 48 hours of life. Neonates who do not require SRT will have breath sound recordings made between 24 – 48 hours of life. A schematic of this is procedure is illustrated in figure 7. Some of the data involving neonates who do not require SRT will be sourced from the "Neo-Auscultate" study

that is currently in its final stages at Monash Medical Centre which characterises the difference in the breath sounds of preterm and term neonates over time.

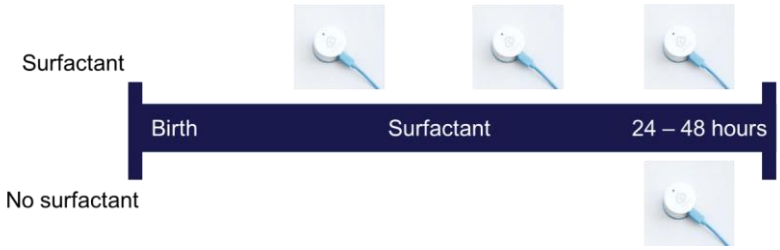


Figure 7 - Recording procedure schematic

Breath sound recordings will be performed by placing a digital stethoscope on the right anterior or posterior chest. If feasible, an additional recording will also be made from the left anterior or posterior chest. Each recording will be one minute in duration. Additional recordings may be performed in the same position if breath sounds are not audible on the initial recording. Basic demographic data as well as the neonates length, weight and head circumference, vital signs (heart rate, respiratory rate, temperature and oxygen saturation), mode of respiratory support and fraction of inspired oxygen will be recorded at the time of breath sound recording using observational charts and patient notes.

Monash University Department of Computer Systems and Electrical Engineering will be assisting in the spectral analysis of the raw audio recordings. The analysis team will be blinded to patient clinical data, surfactant administration and the timing of the recordings. This will be achieved through randomly numbering the recordings.

The analysis of the raw recordings will involve three stages. First segments of crying will be detected and removed. Crying detection will be achieved using an automated computer algorithm. Once detected, these segments will be extracted to allow for analysis of the segments where the newborn is breathing normally. Secondly, other non-crying artefacts will be removed by applying a Butterworth band pass filter at ~~0.5 – 350 Hz~~100-1000Hz across the sound recordings. Finally, the acoustic features of the sound will be extracted for comparison. The features extracted for each recording are listed in Table 4.

Commenté [FM2]: I don't think these babies cry much, as Lindsay mentioned. You can remove this part.

Table 5 - Acoustic characteristics identified in analysis of breath sounds

Feature	**Explanation**

MeanPSD	The average frequency calculated as the sum of product of the power spectrum and the frequency divided by the total sum of the power spectrum
stdPSD	The deviation of the spectrum frequencies from the mean frequency
MedPSD	The frequency which separates higher half of the spectral power from the lower half
bw	The difference between the upper frequency where the power is 3 dB lower maximum and the lower frequency where response is 3 dB lower
p25	The frequency below which a quarter of the spectral power lies
p75	The frequency below which three quarter of the spectral power lies
IQR	Interquartile range, i.e. ~~T~~the frequency range between p25 and p75
TP	Total power in the 100-1000 Hz range
p100-200	Power in the 100-200 Hz range divided by TP
p200-400	Power in the 200-400 Hz range divided by TP
p400-800	Power in the 400-800 Hz range divided by TP
spectrum-slope~~2~~	The rate at which the sound spectrum power tails off or decreases from mean frequency to higher frequencies. The value represents the gradient of the linear regression line fitted to the power in logarithmic octave scale
r-square2	Statistical measure of how close the data is to the fitted regression line

IPM SPSS Statistics 25 (SPSS, Inc, Chicago, USA) will be used to perform statistical analysis. Independent sample t-tests will be used to compare the means between neonates

who received SRT and those who did not. Paired sample t-tests will be used to compare the means in neonates before and after SRT. Subgroup analysis may be performed if there is found to be differences in the baseline characteristics of the two groups.

Measurement tools:

Breath sound recordings will be collected using a Clinicloud (Pty Ltd, Melbourne, Australia) digital stethoscope. Data will be collected in a de-identified manner onto a smart phone device using the app Voice Recorder & Audio Editor (TapMedia Ltd, London, England). It will then be transferred to a Monash Health sever, where it will be stored under password protection. Additional information on pregnancy, birth and complications of prematurity will be collected using maternal and newborn medical charts and hospital records. This includes but is not limited to maternal diabetes, maternal GBS status, antenatal corticosteroids, mode of delivery, meconium stained liquor, Apgar scores, neonatal resuscuitations, patent ductus arteriosus, late onset sepsis and bronchopulmonary dysplasia.

Commenté [FM3]: Data processing and spectral analysis will be performed using Matlab R2019 (The MathWorks, Inc.)

Safety considerations:

The digital stethoscope has the potential to introduce pathogens into the neonate's environment. To lessen this risk, all hospital hand hygiene precautions will be adhered to. Hands will disinfected with 70% alcohol or 2% chlorhexidine solution prior to and following breath sound recordings. Additionally, the Clinicloud digital stethoscope and cord will be cleaned using a 70% isopropyl alcohol wipe prior to and following any breath sound recordings.

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