Hilo Trial: A Comparative Study of High versus Low Tidal Volume in Very Low Birth Babies with Respiratory Distress Syndrome

GUPTA, ANUPAM

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A thesis presented for the degree of

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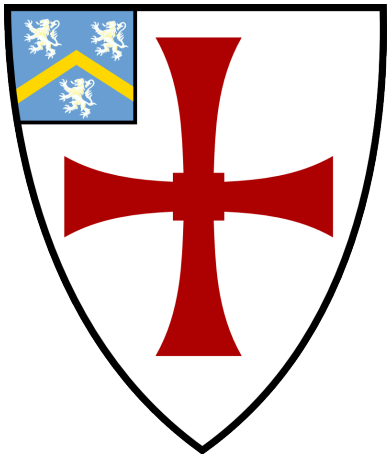

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Abstract

Background

Preterm infants often require mechanical ventilation. Volume targeted ventilation has been shown to reduce both complications and the duration of required mechanical ventilation. The recommended tidal volumes vary from 4-8 mL/kg, but the optimal tidal volume remains elusive.

Aims and objectives

To compare volume ventilation at a lower (4-5 mL/kg) with a higher (7-8 mL/kg) tidal volume during volume guarantee ventilation (VG) of respiratory distress syndrome (RDS) in very preterm infants.

Methodology

The randomised trial was conducted at North Tees Hospital in North East England from 2013 to 2016. Babies <32 weeks' gestation or <1500 grams birthweight requiring mechanical ventilation within 12 hours of life from RDS were included in the study. Babies were randomised to receive lower (4-5 mL/kg) or higher (7-8 mL/kg) tidal volume using Volume Guarantee (VG). The primary outcome was the time to achieve a 25% reduction from the initial peak inspiratory pressure (PIP). Secondary outcomes included the duration of mechanical ventilation, as well as respiratory and non-respiratory complications.

Results

Babies in both groups were similar at baseline with regard to maternal, demographic and clinical characteristics. There was no difference in the primary

outcome of time difference to reach a 25% reduction in baseline peak pressure between the two groups. There were no differences in short term secondary outcomes (air leak, pulmonary haemorrhage, sepsis, IVH, NEC, PDA and ROP) or medium term complications (Bronchopulmonary Dysplasia at 28 days' life and 36 weeks PMA, severity of Bronchopulmonary Dysplasia, amount of home oxygen, survival to discharge and survival without Bronchopulmonary Dysplasia at 36 weeks PMA). The minute volume, $paCO_2$ or FIO_2 requirements were not significantly different either.

Summary and conclusions

This trial did not find statistically significant differences between lower versus higher tidal volume delivery in a population of 70 infants with RDS. It is possible that both tidal volume ranges selected for study are at functional residual capacity and this might be one reason for negative results of the study.

Supervisors: Professor Samir Gupta and Professor A. P. S. Hungin

Dedicated

to

My Parents

Acknowledgements

I wish to thank my supervisors Professor Samir Gupta and Professor Pali Hungin. It would be an understatement to state that this would not have been completed without their guidance and support. Professor Samir Gupta's drive and clear thinking have contributed enormously to ensuring this project addresses the critical clinical questions. It has been a privilege to be supervised by Professor Hungin. He has been the driving force behind the thesis.

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Contents

Declaration

The work in this thesis is based on research carried out at the School of Medicine, Pharmacy, and Health, University of Durham, England.

No part of this thesis has been submitted elsewhere for any other degree or qualification, and it is the sole work of the author unless referenced to the contrary in the text.

The contribution of Dr Kasim Adetoyo, who helped to perform the statistical analyses, is formally acknowledged.

Ethical approval was granted by the NHS North east ethics committee for all work undertaken in this thesis. I confirm that no part of the material offered has previously been submitted by me for a degree in this or any other university. All material from the work of others has been referenced accordingly with no copyright infringements.

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Acronyms

A-aDO_2	Alveolar to arterial oxygen gradient
ALI	Acute Lung Injury
ARDS	Adult Respiratory Distress Syndrome
BAL	Broncho-alveolar Lavage
BD	Base Deficit
BE	Base Excess
BPD	Bronchopulmonary dysplasia
CI	Confidence Interval
CPAP	Continuous Positive Airway Pressure
DAMP	Damage Associated molecular Patterns
EPIPAGE	Epidemiological study on small gestational ages (French)
FIO_2	Fractional Inspired Oxygen
HFO	High Frequency Oscillation ventilation
HFV	High Frequency Ventilation
HHFNC	Humidified High Flow Nasal Cannula

HiP-LoV	High airway pressure with Low Volume
HiP-HiV	High airway pressure with high tidal Volume
IL-1	Interleukins 1
IL-6	Interleukins 6
IL-8	Interluekins 8
IMV	Intermittent Mandatory Ventilation
IQR	Inter Quartile Range
IVH	Intraventricular Haemorrhage
LFUPP	The Leiden Follow-Up Project on Prematurity, -1996/97
LISA	Less invasive surfactant administration
LoP-LoV	Low airway pressure with Low volume
MAP	Mean Airway Pressure
MAS	Meconium Aspiration Syndrome
nCPAP	nasal Continuous Positive Airway Pressure
NEC	Necrotising Enterocolitis
NICU	Neonatal Intensive Care Unit
NLR	Nodd like receptors
NNU	Neonatal Unit
OI	Oxygenation Index
OLV	One lung ventilation
PAMP	Pathogen associated molecular patterns
paO_2	Partial pressure of oxygen in arterial blood
$paCO_2$	Partial pressure of Carbon dioxide in arterial blood

PC-AC	Pressure control - Assist control
Pr AC	Pressure Assist control
PDA	Patent Ductus Arteriosus
PEEP	Peak End Expiratory Pressure
PIE	Pulmonary Interstitial Emphysema
PIP	Peak Inspiratory Pressure
pO_2	Partial Pressure of Oxygen in blood
pCO_2	Partial pressure of Carbon dioxide in blood
POPS	Project On Preterm and Small for Gestational Age Infants, -1983
PPV	Positive Pressure Ventilation
PRVC	Pressure Regulated Volume Control
PSV	Pressure support Ventilation
PVL	Periventricular Leukomalacia
RCT	Randomised Controlled Trial
RDS	Respiratory Distress Syndrome
SD	Standard Deviation
SIMV	Synchronised Intermittent mandatory Ventilation
SIPPV	Synchronised Intermittent Positive Pressure Ventilation
spt	Spontaneous
Te	Expiratory Time
Ti	Inspiratory Time
TLR	Toll like receptors
TNF-α	Tumour Necrosis Factor Alpha

VALI	Ventilator Associated Lung Injury
VAPS	Volume Assured Pressure Support
V_E	Minute Volume
VG	Volume Guarantee
VILI	Ventilator Induced Lung Injury
VLBW	Very Low Birth Weight
V_t	Tidal Volume
VTV	Volume Targeted Ventilation

Chapter 1

Introduction

The World Health Organisation (WHO) defines preterm birth as births before 37 completed weeks of gestation or fewer than 259 days from the first date of a woman's last menstrual period (LMP) (Who (1977)).

Blencowe et al. (Blencowe et al. (2012)) collated data from 99 countries and estimated the 2010 global prevalence of premature births at 11.1% (95% CI: 9.1%–13.4%). The authors also reported on the national variation on estimates of preterm birth rates, which ranged from approximately 5% in some European countries to 18% in some African countries. Premature births account for significant resource utilisation and bed occupancy. Though only 1% of all births are very low birth weight (less than 1500 grams) (Office of National Statistics (ONS), UK bulletin, 2017), this small group of infants are at particularly high risk of adverse outcomes; infant mortality rate at 10–15% and incidence of cerebral palsy at 5–10% (Larroque et al. (2008), Zeitlin et al. (2008)).

This is an area of modern medicine which has witnessed one of the most dramatic changes in practice and improvement in outcomes in the last century. It was not long ago, in 1960, when the perinatal mortality for very low birth weight babies was approximately 800 per 1,000 live births (Fryer and Ashford (1972)).

According to the Office of National Statistics (ONS), UK bulletin 2015, very low birth weight babies, born of less than 32 weeks gestation, account for 15% of all

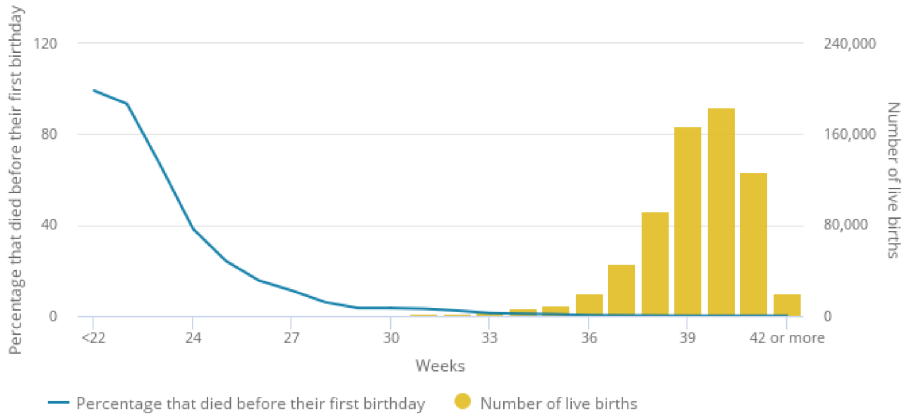


Figure 1.1: Percentage of infant deaths and number of live births by gestation week, babies born in 2014 (Source: Office of National Statistics, 2015)

preterm births; and approximately 50% of all neonatal deaths are due to prematurity related issues. The same bulletin also identified that 'of those live births that occurred at under 32 weeks' gestation, 14.9% resulted in an infant death' (Figure 1.1).

While mortality of preterm infants has come down in the last 50 years, there are concerns that this could be at the cost of increasing incidence of disabled children. This concern has refocussed our attention on reducing morbidity and long-term complications of prematurity.

Respiratory management of preterm infants is an integral part of neonatal intensive care. The most common immediate and long term complications of preterm infants include respiratory distress syndrome (surfactant deficient lung disease) and chronic lung disease respectively. Mechanical ventilation remains the standard management of respiratory distress syndrome although ventilation induced lung injury and the development of chronic lung disease are commonly associated with its use.

Historically, initial accounts of the use of intubation and ventilation date back to the 17th century (Stern et al. (1970)). Almost 200 years later, in the 19th century, a device dedicated to ventilation of newborn infants was used. In the 1940s, Dr

Julius Hess opened the first Premature Centre at the Michael Reese Hospital in Chicago. In 1953, Donald and Lord (Donald and Lord (1953)) reported their experience with a patient-cycled, servo-controlled respirator in the treatment of several newborn infants with respiratory distress. This heralded the era of modern ventilation in newborn infants. In 1963, a premature son of President Kennedy, born at 34 weeks weighing 2.1kg, died at 39 hours of age. The infant had received hyperbaric oxygen to deal with lung disease. This unfortunate event brought into focus the needs of premature infants' ventilation and this decade is regarded as that of the birth of neonatology.

The success in dealing with respiratory distress syndrome has led to improved survival of premature babies with the antecedent complication identified as bronchopulmonary dysplasia (BPD) (Northway et al. (1967)). The association of oxygen therapy and retinopathy of prematurity was already reported, and this highlighted the complications secondary to intensive care interventions to deal with respiratory distress syndrome.

Subsequently, development of continuous distending pressure (Gregory et al. (1971)), the concept of longer inspiration (Reynolds and Taghizadeh (1974)) and the use of continuous gas flow (Kirby et al. (1972)) led to the design of a ventilation technique combining mechanical and spontaneous breaths called the "Intermittent Mandatory Ventilation" (IMV).

In the 1990s, further development in technology led to the ability to use synchronisation, a patient-triggered mode of ventilation and pressure support ventilation in newborn infants. This helped to reduce the sedation requirement and bedside adjustment of ventilation. The next generation of ventilators used electronic controls and microprocessors to enable delivery of very small inspiratory time while facilitating the monitoring of ventilatory parameter measurements (inspiratory: expiratory ratio, mean airway pressure). This triggered the debate for the best modality to provide ventilation.

At this time, animal studies by Dreyfuss and Hernandez et al. (Dreyfuss et al. (1988), Hernandez et al. (1989), Dreyfuss and Saumon (1998)) addressed the question of which of the two, volutrauma or barotrauma, contributed more towards ventilator-induced lung injury. This prompted a number of clinical studies, leading to evidence of the superiority of volume targeting in newborn infants (Wheeler et al. (2011)). This marked the shift in the technique of ventilation in the last decade to volume targeting and this is now widely used as the default mode for newborn respiratory management.

With continuing development in ventilation technology, it is now feasible to combine different modes of ventilation and deliver respiratory support using "hybrid modes" of ventilation, e.g., Volume Guarantee (VG), Pressure Regulated Volume Control (PRVC) and Volume Assured Pressure Support (VAPS). With the development of these modes, the target tidal volume can be set as per the baby's needs and advanced microprocessor technology delivers the set parameters. This brought us to a new question now being asked on a regular basis – what is the appropriate tidal volume for respiratory support using mechanical ventilation? Tidal volume is the volume of medical gas delivered by the ventilator per breath. Its importance lies in the fact that it is crucial to deliver the tidal volume within a narrow range and avoid fluctuations. The new generation of ventilators have the capability to target the set tidal volume. However, high tidal volume can lead to volutrauma and hypocarbia, while low tidal volume can cause atelectotrauma and hypercarbia.

In adults and older children, this dilemma has been extensively studied. However, there is a paucity of studies in neonatal population which can be explained by the lack of availability of technology until recently, to deliver such small tidal volumes.

Williams et al. (Williams et al. (2011)) studied V_t of 43 non-ventilated (CPAP or spontaneous breathing) preterm newborns and noted that the range of tidal volume, measured with two different methods, was between 4–11 ml/kg.

In a recent international cross-sectional study by van Kaam et al. (van Kaam et al.

(2010)) of the local ventilation practices in Neonatal Intensive Care Units authors reported that although the majority of infants were ventilated with tidal volumes between 4 and 7 ml/kg, 18% were ventilated with tidal volumes >7 ml/kg.

Experts have suggested that the tidal volume range should be 4-8 ml/kg, but there is limited evidence to guide clinicians as to whether low or high normal tidal volumes should be targeted (van Kaam (2011)).

The lower end of tidal volume has been extrapolated from studies in adults, but this strategy of employing low tidal volume is not without its shortfalls. A study by Chowdhury et al. (Chowdhury et al. (2012)) evaluated work of breathing in preterm babies using tidal volumes of 4-6 ml/kg. It reported that a V_t of 4 ml/kg led to increased work of breathing and therefore recommended an increased targeted tidal volume of 6 ml/kg. Hence, while the lower tidal volumes could limit volutrauma, this study suggests that such low total volumes in turn could lead to increased work of breathing.

In summary, while volume targeting appears to be superior to pressure targeting, it is not clear what the optimal tidal volume set is on the hybrid modes widely used across the world. This remains a vital question that needs investigation.

After this literature search, we found that it was an appropriate question to address in a randomised control setting. We decided to use the volume guarantee modality, the commonest and most widely used hybrid mode, to address this question. The volume guarantee applies principles of pressure and volume ventilations using a software algorithm which helps to maintain expiratory tidal volume delivered to the baby with the aid of a feedback loop. Arguably, volume guarantee is the most commonly used and researched of the hybrid modes. Therefore, its use makes the results of the study more widely applicable than it could have been if any other mode were to have been used. Such a study has not been done so far, and with volume guarantee being a relatively new mode, we decided to go for a pilot project which would help us address the feasibility of performing this research on a much

larger multicentre basis.

This prospective randomised controlled trial allowed collection of important data on various respiratory and non-respiratory parameters. We could also compare the outcome with the babies who could not be enrolled for any reason. The study was carried out in a clinical setting hence replicating the common issues which could arise during clinical management of preterm infants and aimed to provide practical solutions. This made the study highly relevant to clinical practice. The study also used a standardised protocol which can be used in managing infants with volume-targeted ventilation.

This thesis is divided into different chapters describing a critical review of published literature, methods including inclusion and exclusion criteria, results, discussion, summary and conclusion. The illustrative tables and flow diagrams have been used to present the data of the findings and the appendices give the details of other materials used in the study.

Chapter 2

Resume of Literature

2.1 Premature babies and respiratory distress syndrome

Preterm birth is defined by the World Health Organisation as the delivery of an infant before 37 completed weeks (259 days) of gestational duration. Its incidence has been steadily rising before plateauing in 2008 when it occurred in 12.3% of births in the United States (Martin J.A. (2010)). It is also considered to be the most common cause of perinatal (McCormick (1985)) and infant (Callaghan et al. (2006)) mortality in the United States. It is associated with about one third of all infant deaths in the United States and accounts for approximately 45% of children with cerebral palsy, 35% of children with vision impairment and 25% of children with cognitive or hearing impairment.

2.1.1 Very low birth weight infants

Very low birth weight infants are defined as newborn infants with birth weight less than 1,500 grams. These infants are known to have higher morbidity and mortality due to significant immaturity and circumstances surrounding birth. While it was widely known that these infants have a high risk of morbidity and mortality, Stewart et al. (Stewart et al. (1981)) compiled the data from 22 reports from developed

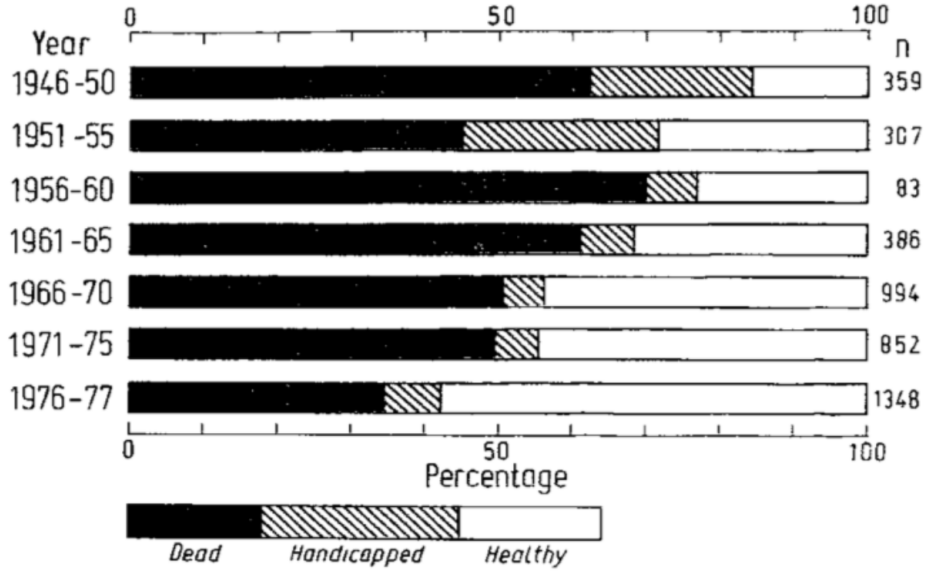


Figure 2.1: Historical data about secular trends of morbidity and mortality in VLBW infants (≤1500 grams) - adapted from Stewart et al. (Stewart et al. (1981))

world describing the outcome for infants of very low birth weight born since 1946. They pooled the data according to the quinquennium of birth and subjected it to statistical tests and analysis. The authors then divided the historical outcome into three phases (Figure 2.1):

Phase 1 - 1940s and 1950s, very few VLBW infants survived because of inadequate treatment and knowledge of neonatal diseases.

Phase 2 - In the late 50s and 60s, knowledge concerning the normal physiology of VLBW infants and derangement which causes death and damage in newborns increased. Treatments were developed and the mortality rate started declining but sometimes at the expense of increased prevalence of handicap amongst the survivors.

Phase 3 - From the early 1960s onward, chances of healthy survival of VLBW infants increased and prevalence of handicap remained stable and relatively low, 6 to 8% of total VLBW births.

Stoelhorst et al. (Stoelhorst et al. (2005)) also studied the secular trends (data over

long term) in the mortality pattern of preterm infants of less than 32 weeks (The Leiden Follow-up Project on Prematurity, LFUPP-1996/97). The authors found that whilst comparing infants born in 1996-97 (The Leiden Follow-Up Project on Prematurity, LFUPP-1996/97) with ones born in 1983, (Project On Preterm and Small for Gestational Age Infants, POPS-1983), the absolute number of preterm births in the study region increased by 30%. 73% of the LFUPP-1996/97 infants were treated antenatally with glucocorticosteroids compared with 6% of the POPS-1983 infants. 42% of the LFUPP-1996/97 infants received surfactant. In-hospital mortality decreased from 30% in the 1980s to 11% in the 1990s. Mortality of the extremely preterm infants (<27 weeks) fell from 76% to 33%. The incidence of respiratory distress syndrome remained the same; approximately 60% in both groups. Mortality from respiratory distress syndrome, however, decreased from 29% to 8%. The incidence of bronchopulmonary dysplasia increased from 6% to 19%. For the surviving infants, the average length of stay in the hospital and the mean number of NICU days stayed approximately the same (approximately 67 days total admission time and 44 NICU days in both groups). Including the infants who died, the mean NICU admission time increased from 27 days in the 1980s to 41 days in the 1990s. There was no change in adverse outcome (dead or an abnormal general condition) at the time of discharge from hospital (+/-40% in both groups). The authors concluded that the secular trends demonstrated an increase in the absolute number of very preterm births in this study region, leading to a more significant burden on the regional NICUs. They also speculated as to whether improvements in perinatal and neonatal care had led to increased survival of especially extreme preterm infants; which in turn had resulted in more morbidity, mainly bronchopulmonary dysplasia, at the moment of discharge from the hospital.

In a multi-centre prospective study of preterm infants born in France “EPIPAGE (epidemiological study on small gestational ages)” (Pierrat et al. (2017)), the authors reported that survival at two years corrected age was 51.7% at 22-26 weeks’ gestation and 93.1% at 27-31 weeks’ gestation. Survival without severe or moder-

ate neuro-motor or sensory disabilities among live births increased between 1997 and 2011, from 45.5% to 62.3% at 25-26 weeks' gestation. At 32-34 weeks' gestation, there was a non-statistically significant increase in survival without severe or moderate neuro-motor or sensory disabilities (p=0.61), but the proportion of survivors with cerebral palsy declined (p=0.01). In <32 weeks group, an incidence of severe neuro-motor or sensory disability was 1.7% and incidence of survival without moderate to severe disability was 96.4%. Survival at two years corrected age was 84.3%. When comparing the same group of babies to 14 years earlier (2011 versus 1997) (Larroque et al. (2001)), the authors noted that there was an increase in two year survival from 79.4% to 84.1% (p=0.001). Both adjusted as well as unadjusted mean differences were highly significant. Similarly, the proportion of babies surviving without major disability increased from 74.5% to 80.5%. This was also statistically significant.

During the past decade, survival has improved, particularly in infants with extremely low birth weight (Lemons et al. (2001), Fanaroff et al. (2007)). Whilst there have been improvements in survival, the incidence of most short term major medical complications associated with prematurity has remained relatively stable.

2.1.2 Respiratory distress syndrome

Respiratory distress syndrome (RDS), formerly known as hyaline membrane disease, is a common problem in preterm infants. This disorder is caused primarily by a deficiency of pulmonary surfactant in an immature lung. RDS is a major cause of morbidity and mortality in preterm infants.

The incidence of RDS increases with decreasing gestational age. The risk is highest in extremely preterm infants as illustrated by a study from the National Institute of Child Health and Human Development Neonatal Research Network. This study found a 93% incidence of RDS in a cohort of 9,575 extremely preterm infants (gestational age 28 weeks or below) born between 2003 and 2007 (Stoll et al. (2010)).

The European agency for neonatal data in 2010 reported respiratory distress syndrome incidence of 92% at 24–25 weeks' gestation, 88% at 26–27 weeks, 76% at 28–29 weeks and 57% at 30–31 weeks (EuroNeoStat (2010)).

Knowledge of the normal foetal lung development is central to understanding the pathophysiology of neonatal RDS as it is due to inadequate surfactant activity resulting from lung immaturity.

2.1.3 Pulmonary surfactant

The primary cause of RDS is a deficiency of pulmonary surfactant, which is developmentally regulated. The foetal lung is filled with fluid and provides no respiratory function until birth. In preparation for air breathing, the surfactant is expressed in the lung during the third trimester of pregnancy (Frank and Sosenko (1987)). Surfactant reduces the alveolar surface tension, thereby facilitating alveolar expansion and reducing the likelihood of alveolar collapse atelectasis.

Because of the developmental regulation of surfactant production, the most common cause of surfactant deficiency is preterm delivery. In the preterm infants, both a decrease in the quantity and quality of surfactant contributes to decreased surfactant activity resulting in RDS. In addition to low surfactant production seen with decreasing gestational age, the surfactant produced in preterm infants compared to surfactant from term infants has reduced activity because of differences in lipid and protein composition (Hallman et al. (1976)).

2.1.3.1 Pathophysiology

In the premature lung with inadequate surfactant activity, the resultant higher surface tension leads to instability of the lung at end expiration, low lung volume and decreased compliance. These changes in lung function cause hypoxaemia primarily due to a mismatch between ventilation and perfusion due to a collapse of large por-

tions of the lung (atelectasis) and additional contributions of ventilation/perfusion mismatch from intrapulmonary and extrapulmonary right to left shunts.

Surfactant deficiency also leads to lung inflammation and respiratory epithelial injury, which may result in pulmonary oedema and increased airway resistance. These factors further exacerbate lung injury and worsen lung function. At the same time, abnormal fluid absorption results in the inefficient clearing of lung liquid in the injured, oedematous lung which also impedes gas exchange.

The relationship of the inflating pressure, surface tension and radius of curvature is illustrated by the model of a distal alveolus as a sphere connected to a distal airway described by LaPlace's law. According to LaPlace's law, the pressure (P) necessary to keep the sphere open is proportional to the surface tension (T) and inversely proportional to the radius (R) of the sphere, as shown by the formula:

$P = 2T/R$

If the surface tension is high and the alveolar volume is small (i.e., the radius is low), as occurs at end expiration, the higher pressure is necessary to keep the alveolus open. If this increased pressure cannot be generated, the alveolus collapses. Diffuse atelectasis occurs when an alveolar collapse occurs throughout the lung, which leads to hypoxaemia. Pulmonary surfactant reduces the surface tension, even at low volumes, leading to a decrease in the required pressure, thus maintaining alveolar volume and stability.

Inflammation and lung injury

The role of inflammation in the pathogenesis of RDS is suggested by animal experiments in which surfactant deficiency was also associated with the rapid accumulation of neutrophils in the lung and evidence of pulmonary oedema (Carlton et al. (1997)).

In this model, depletion of neutrophils prevented pulmonary oedema. In addition, as noted above, surfactant deficiency causes atelectasis that may lead to injury of the respiratory epithelium and the alveolar capillary endothelium, which can trigger

a cytokine mediated inflammatory response. A further injury may be caused by positive pressure ventilatory support or excessive oxidant exposure (Clark et al. (2001), Naik et al. (2001)).

The inflammation and lung injury may, in turn, lead to accumulation of protein rich pulmonary fluid that can deactivate any surfactant, which is present, thereby further exacerbating the underlying surfactant deficiency (Nitta and Kobayashi (1994)).

Pulmonary oedema

In infants with RDS, pulmonary oedema often occurs because of the following contributory factors: inflammation and lung injury, reduced pulmonary fluid absorption and low urine output.

Surfactant inactivation

In addition to decreased surfactant production and synthesis of a less active surfactant, surfactant inactivation further reduces the effective surfactant pool size. Factors that contribute to surfactant inactivation are inhaled meconium and blood which can inactivate surfactant activity and increase proteinaceous oedema and inflammatory products.

Its impact on pulmonary function and gas exchange includes low compliance, low lung volume (functional residual capacity) and mildly increased total lung resistance.

Hypoxaemia

It is due primarily to mismatch of ventilation and perfusion with right-to-left shunting of blood past substantial regions of the lung that are poorly ventilated.

Metabolic acidosis

It may be present due to lactic acid production from anaerobic metabolism in response to hypoxaemia and compromised tissue perfusion.

2.1.4 Clinical manifestations and clinical course of RDS

Because RDS is primarily a developmental disorder of deficient surfactant production it presents within the first minutes or hours after birth. If untreated, RDS progressively worsens over the first 48 hours of life. In some cases, infants may not appear ill immediately after delivery but develop respiratory distress and cyanosis within the first few hours of age. The affected infant is almost always premature and exhibits signs of respiratory distress that include: tachypnoea, nasal flaring, expiratory grunting, intercostal and subcostal retractions and cyanosis. On physical examination, auscultated breath sounds are decreased and infants may be pale with diminished peripheral pulses.

Clinical course

Prior to surfactant use, uncomplicated RDS typically progressed for 48 to 72 hours. This was followed by an improvement in respiratory function associated with increased production of endogenous surfactant and resolution of the respiratory disorder by one week of age.

Now we know that the natural history of RDS can be significantly modified by treatment with an exogenous surfactant, which dramatically improves pulmonary function, leading to the resolution of symptoms and shortens the clinical course.

Diagnosis

RDS is diagnosed by the clinical picture of a premature infant with the onset of progressive respiratory failure shortly after birth. A characteristic chest radiograph can also reveal low lung volume and the classic diffuse reticulo-granular ground-glass appearance with air bronchogram.

2.1.5 Prevention of respiratory distress syndrome

Going by the axiom – “prevention is better than cure”, the best way to deal with respiratory distress syndrome would be to avoid premature delivery altogether.

As we know this is easier said than done. However, there are a few strategies successfully employed to prevent the occurrence or reduce the severity of RDS in premature babies.

They include the following:

2.1.5.1 Administration of antenatal corticosteroids

In a systematic review of randomised trials comparing antenatal corticosteroid therapy versus placebo/no treatment by Roberts et al. (Roberts et al. (2017)), the authors found that the use of antenatal steroids led to reduction in RDS (average RR 0.66) as well as moderate/severe RDS (average RR 0.59). They concluded that the evidence from the review supported the use of a single course of antenatal corticosteroids to accelerate foetal lung maturation in women at risk of preterm birth.

In another systematic review by Crowther et al. (Crowther et al. (2011)), the authors reported that the treatment of women who remained at risk of preterm birth seven or more days after an initial course of prenatal corticosteroids with repeat dose(s), compared with no repeat corticosteroid treatment, reduced the risk of their infants experiencing the primary outcomes of respiratory distress syndrome (RR 0.83) and serious infant outcome (RR 0.84).

2.1.5.2 Administration of exogenous surfactant

Several clinical trials have shown the benefit of surfactant administration in preterm infants born of less than 30 weeks' gestation who are at the greatest risk for RDS. The use of surfactant has been further analysed as to whether it is prophylactic or rescue and whether animal-derived or synthetic.

Prophylaxis by synthetic surfactant

In a meta-analysis by Soll et al. (Soll and Ozek (2010)), the authors analysed seven randomised controlled trials which demonstrated a decrease in the risk of pneumothorax, pulmonary interstitial emphysema and neonatal mortality.

The authors concluded that prophylactic use of synthetic surfactant improved clinical outcome whilst increasing the risk of developing patent ductus arteriosus and pulmonary haemorrhage. However, these complications did not overshadow the impact on overall outcome (neonatal mortality or late mortality).

Prophylaxis by natural/animal derived

In another meta-analysis by Soll (Soll (2000)), initially performed in 2000 and updated in 2010, the author concluded that prophylactic intra-tracheal administration of animal-derived surfactant extract to infants judged to be at risk of developing respiratory distress syndrome led to decreased risk of pneumothorax, a reduced risk of PIE, a reduced risk of mortality and a reduced risk of BPD or death. The author also highlighted the lack of long term data in such studies.

Treatment of RDS by synthetic surfactant

In a meta-analysis by Soll (Soll (2000)), the author reported that the use of synthetic surfactant led to a statistically significant decrease in the risk of pneumothorax, pulmonary interstitial emphysema, patent ductus arteriosus, intraventricular haemorrhage, bronchopulmonary dysplasia, neonatal mortality, bronchopulmonary dysplasia or death at 28 days, mortality prior to hospital discharge and a decrease in the risk of mortality during the first year of life.

Treatment of RDS by animal derived surfactant

In a meta-analysis by Seger et al. (Seger and Soll (2009)), the authors reported that the meta-analysis supported a significant decrease in the risk of any air leak, pneumothorax or pulmonary interstitial emphysema. There was a substantial decrease in the risk of neonatal mortality, bronchopulmonary dysplasia (BPD) or death at 28 days of age. No differences were reported in the risk of patent ductus

arteriosus (PDA), necrotising enterocolitis (NEC), intraventricular haemorrhage (IVH), bronchopulmonary dysplasia (BPD) or retinopathy of prematurity (ROP).

Less invasive surfactant administration (LISA) versus intubation for surfactant delivery

In a meta-analysis of six studies, Aldana-Aguirre et al. (Aldana-Aguirre et al. (2017)) reported that LISA technique for surfactant delivery resulted in a lesser need for mechanical ventilation in infants with RDS, reduction in the composite outcome of death or BPD at 36 weeks amongst survivors.

2.1.5.3 Provision of assisted non-invasive ventilation

In a meta-analysis by Rojas-Reyes et al. (Rojas-Reyes et al. (2012)), the authors analysed two studies with a routine application of CPAP, which demonstrated a decrease in the risk of air leak and neonatal mortality associated with prophylactic administration of surfactant. However, the analyses of studies that allowed for routine stabilisation on CPAP demonstrated a decrease in the risk of chronic lung disease or death in infants stabilised on CPAP. When all the studies were evaluated together, the benefits of prophylactic surfactant could no longer be demonstrated. The authors concluded that recent large trials reflecting current practice (including higher utilisation of maternal steroids and routine post-delivery stabilisation on CPAP) did not exhibit any differences in affecting the risk of chronic lung disease or death when using initial stabilisation on CPAP with selective surfactant administration to infants requiring intubation.

2.1.6 Management of respiratory distress syndrome

Surfactant deficiency leads to increased surface tension in the air-liquid interface at the terminal respiratory units resulting in impaired lung expansion. As the disease advances, progressive atelectasis results in both ventilation-perfusion mismatching

and increased intra and extra pulmonary shunting that may lead to respiratory failure.

Intubation and mechanical ventilation have been used to correct atelectasis and provide breathing support till the spontaneous production of surfactant.

This involves the use of supplemental oxygen and maintaining mean airway pressures. These while helpful in improving hypoxaemia, also contribute to the development of bronchopulmonary dysplasia. In particular, the pulmonary injury is caused by volutrauma and barotrauma (associated with intermittent positive pressure ventilation) and oxygen toxicity (due to high concentrations of supplemental oxygen). In addition, other complications associated with intubation and mechanical ventilation include pulmonary air leak and injury due to intubation.

2.1.6.1 Non invasive / Less invasive Ventilation

This involves avoiding using mechanical ventilation either by using CPAP with or without surfactant. Fischer et al. (Fischer and Buhrer (2013)) carried out a meta-analysis of studies comparing the use of mechanical ventilation versus strategies to avoid ventilation and concluded that strategies aimed at avoiding mechanical ventilation in infants <30 weeks' gestation age had a small but significant beneficial impact on preventing bronchopulmonary dysplasia (BPD).

Because of the increased risk of bronchopulmonary dysplasia (BPD) with intermittent positive pressure ventilation, other less invasive modes of ventilation have been evaluated to reduce atelectasis and pulmonary injury in preterm infants at risk of or with established RDS. These include:

- Nasal continuous positive airway pressure (nCPAP)
- Nasal intermittent positive pressure ventilation (NIPPV)
- High flow nasal cannulae

Nasal continuous positive airway pressure (nCPAP)

In premature infants at risk of or with established RDS without respiratory failure, nasal continuous positive airway pressure (nCPAP) is an alternative to endotracheal intubation and mechanical ventilation for prevention of atelectasis (Rojas-Reyes et al. (2012)).

As mentioned earlier, Fischer et al.'s meta-analysis demonstrated that use of CPAP led to less incidence of death or BPD. Vaucher et al. (Vaucher et al. (2012)) carried out a follow-up study of one of the included trials in the review (SUPPORT study) and reported that there were no significant differences in the composite outcome of death or neurodevelopmental impairment among extremely premature infants randomly assigned to early CPAP or early surfactant administration.

Nasal intermittent positive pressure ventilation

Nasal intermittent positive pressure ventilation (NIPPV) augments nCPAP by delivering ventilator breaths via nasal prongs (or nasal mask). Although NIPPV avoids the trauma of endotracheal placement tube, it still is a delivery mode of positive pressure ventilation. However, clinical trials have failed to show that NIPPV is superior to nCPAP as an initial treatment for non-invasive respiratory support.

Meneses et al. (Meneses et al. (2012)) carried out a meta-analysis in 2012 where the authors compared NIPPV with nCPAP and reported that among preterm infants with respiratory distress syndrome, NIPPV decreased the need for invasive ventilation within the first 72 hours of life compared with NCPAP.

The meta-analysis was followed by a multi-centre clinical trial, NIPPV study, led by Kirpalani et al. (Kirpalani et al. (2013)), whereby researchers set out to compare NIPPV versus nasal CPAP in preterm infants. They reported that in extremely low birth weight infants, the rate of survival to 36 weeks of postmenstrual age without bronchopulmonary dysplasia did not differ significantly after non-invasive respiratory support with nasal IPPV as compared with nasal CPAP.

Humidified High flow nasal cannula (HHFNC) therapy

Humidified high flow nasal cannula (HHFNC) therapy is increasingly being used to provide positive distending pressure with or without oxygen instead of traditional nCPAP devices. However, clinical trials have not shown HHFNC to be superior to nCPAP and pressure delivery is highly variable.

In a multicentre, randomised, unblinded study of 432 infants (Yoder et al. (2013)) with gestational age between 28 and 42 weeks (mean gestational age 33 weeks) and birth weight $\geq$ 1000g, there was no difference in failure rate between patients randomly assigned HHFNC versus those who received nCPAP. There were also no differences in the subsequent intubation rates or the risk of adverse outcomes including air leak, bronchopulmonary dysplasia (BPD) and need for oxygen on discharge. However, the median duration of intervention was longer for infants assigned to HHFNC (four versus two days). This study included infants who met criteria for non-invasive respiratory support for either early primary therapy for neonatal respiratory distress or following extubation.

In a multicentre Australian trial (Manley et al. (2013)), Manley et al. compared HHFNC to nCPAP for providing non-invasive support post-extubation. The authors recruited 303 preterm infants (gestational age of less than 32 weeks). They reported that the use of HHFNC was non inferior to the use of CPAP for the primary outcome of treatment failure within seven days after extubation.

Roberts et al. (Roberts et al. (2016)) compared HHFNC with CPAP in their multicentre trial to provide primary support after birth. Researchers recruited 564 preterm infants (gestational age $\geq$ 28 weeks 0 days) with early respiratory distress who had not received surfactant replacement. These were then treated with either nasal high-flow therapy (HHFNC) or nasal CPAP. Authors concluded that when used as primary support for preterm infants with respiratory distress, HHFNC resulted in a significantly higher rate of treatment failure than CPAP.

2.1.6.2 Mechanical ventilation

Along with other technological advancements, such as the administration of antepartum corticosteroids and replacement surfactant therapy, mechanical ventilation has led to improved neonatal survival, especially for premature infants born of less than 30 weeks' gestation with immature lung function. Approximately two-thirds of all infants admitted to the NICU have been reported to receive positive pressure ventilation (McCallion et al. (2005)).

Benefits of mechanical ventilation

The principal benefits of neonatal mechanical ventilation during respiratory failure are as follows:

- Improved gas exchange, primarily by lung recruitment to improve ventilation/perfusion (V/Q) matching.
- Decreased work of breathing.
- Provision of adequate minute ventilation (i.e. carbon dioxide removal) in infants with respiratory depression or apnoea.

Complications of mechanical ventilation

- Barotrauma
- Volutrauma
- Atelectotrauma
- Biotrauma
- Rheotrauma

These are covered in much more detail in a dedicated section (section number 2.3).

Types of ventilation

Ventilators used in the NICU can be divided into two broad categories based on how minute ventilation is provided.

1. Conventional mechanical ventilation (CMV) involves an intermittent exchange of bulk volumes of gas, which are similar in volume to physiologic tidal volume within the airway tree. Delivery of conventional ventilation varies by how the breath is initiated (ventilator or patient triggered), how the delivered tidal volume is regulated (e.g. pressure or volume control), how the breath is terminated (e.g. volume, time, or flow regulated) and the rate of ventilation.

2. High frequency ventilation (HFV) is based upon the delivery of small volumes of respiratory gas, which are equal to or smaller than the anatomic dead space, at an extremely rapid rate (300 to 1,500 breaths per minute).

Conventional mechanical ventilation is further sub-divided into two broad categories: pressure limited and volume limited (Lozano and Newnam (2016)).

Pressure limited ventilation

The continuous flow of circuit gas allows for spontaneous patient breathing, and weaning can occur by progressively reducing PIP and mandatory ventilator rate. PIP and mean airway pressure (MAP) can be adjusted to optimise gas exchange and attempt to minimise the contribution of these factors to chronic lung injury.

The advantage of the pressure limited ventilator is that it is relatively easy to use and less expensive. The main disadvantage of this modality is that there is a variation of tidal volume from breath to breath. Delivered tidal volume varies with alterations in lung compliance and resistance, circuit compressed gas volume and endotracheal (ET) tube leak.

In a study of preterm infants, exhaled tidal volume was greater than the targeted volume for 25% of the breaths and less than the targeted volume for 36% of breaths (Keszler and Abubakar (2004)). These large variations in delivered

volume over time subjected the immature lungs of preterm infants to risks of both over distension and collapse of air spaces resulting in lung injury (volutrauma/atelectotrauma).

Volume targeted ventilation

The development of volume-targeted neonatal ventilation was prompted by evidence that volume distension of the lung rather than peak airway pressure, induced lung injury (Dreyfuss et al. (1988), Hernandez et al. (1989), Dreyfuss and Saumon (1998)).

As discussed above, pressure limited ventilation poorly controls the delivered tidal volume, resulting in a wide range of breath to breath tidal volumes leading to episodes of over distension and atelectasis.

Pressure versus volume ventilation?

Traditionally pressure limited ventilation has been the most frequent mode of ventilation in neonatal units.

The major disadvantage of pressure limited ventilation lies in the variable tidal volume that results from changes in lung compliance. Such changes may occur quite rapidly, especially in the immediate postnatal period, as a result of clearing of lung fluid, optimisation of lung volume and administration of newer, more rapidly acting exogenous surfactant preparations (Keszler (2006)). The consequences of such rapid improvements in compliance are inadvertent hyperventilation and lung damage from excessively large tidal volumes as identified by Bjorklund et al. (Bjorklund et al. (1997)) in their animal model study on lambs. One lamb in each pair was randomly selected to receive six high volume manual inflations of 35-40 mL/kg ("bagging") before the start of mechanical ventilation, a volume roughly corresponding to the inspiratory capacity. Subsequently authors noted that lambs subjected to high volume bagging failed to show the improvement in compliance otherwise observed in control lambs. These lambs were also more difficult to ventilate and tended to have less well expanded alveoli and more widespread lung injury

in histologic sections. The authors concluded that a few large volume inflations in surfactant-deficient lungs immediately at birth compromised the effect of subsequent surfactant rescue treatment.

With recent evidence coming from trials comparing the two, more and more units are preferring to switch to volume targeted ventilation. In a 2011 international survey, volume-targeted ventilation had replaced pressure-limited ventilation in 25 of the 50 neonatal tertiary units surveyed (Klingenberg et al. (2011)).

Several studies have shown that as compared with pressure limited ventilation, volume targeted ventilation was associated with the following additional benefits:

- It maintained tidal volume closer to the targeted goal and reduced the incidence of hypocapnoea (an indicator of overventilation) (Keszler and Abubakar (2004)).
- It provided an effective gas exchange while reducing the number of high volume mechanical breaths (Herrera et al. (2002)).
- It decreased peak inspiratory pressure (PIP) of triggered ventilations while maintaining the targeted tidal volume (McCallion et al. (2008)).
- It has been shown to improve outcome with the reduction in the death or BPD at 36 weeks' gestation (Klingenberg et al. (2017)).
- It was associated with reduced rates of pneumothorax (Klingenberg et al. (2017)), reduced days of mechanical ventilation (Klingenberg et al. (2017)) and reduced rates of hypocarbia (Klingenberg et al. (2017)).
- It was also associated with reduced rates of grade 3 or 4 intraventricular haemorrhage and the combined outcome of periventricular leucomalacia with or without grade 3 or 4 intraventricular haemorrhage (Klingenberg et al. (2017)).

- VTV modes were not associated with any increased adverse outcomes (Klingenberg et al. (2017)).

While these results suggest that volume targeted ventilation improves short term outcome with a reduction in the rate of complications associated with mechanical ventilation, some commentators have voiced the following concerns to suggest further study of this technique in the neonatal intensive care setting is required:

- Randomised studies have been criticised for not being blinded. This has led to quality of evidence considered as moderate to low (Klingenberg et al. (2017)).
- No individual trial demonstrated a reduction in mortality, death or BPD, grade III or IV IVH, or PVL. Only the pooled data from the meta-analysis reached statistical significance (Davis and Morley (2006)).
- Studies were conducted by investigators with expertise in the use of volume targeted ventilation. In general, it was found that these ventilators are more difficult to operate than the simple TCPL (Time Cycled Pressure Limited) ventilators which have been the standard mode of ventilation for many years. As a result, it is unclear whether these results would be replicated in a wider application of this modality by less experienced clinicians. Care providers would need to be fully trained to optimally use volume targeted ventilators in neonates (Davis and Morley 2006).
- Several different ventilatory modalities (VG, PRVC, and VC) and modes (patient-triggered and mandatory ventilation) were used in these studies. At present it is not possible to determine which ventilator or mode of ventilation accounted for the reported differences in outcome (Grover and Field (2008)).
- There were variations in the delivery of tidal volume, inflation time and peak pressures amongst the volume targeted ventilators used in the studies themselves (Sharma et al. (2007)).

- Air leak around non-cuffed endotracheal tubes makes monitoring of expired tidal volume problematic. As a result, if volume triggered ventilation is used, it remains uncertain what the optimal tidal volume is (Lista et al. (2006)).

2.2 Volume Guarantee (VG)

Acknowledgement of superiority of volume targeting and development of microprocessor technology has led to the development of "hybrid modes" which help to deliver precise tidal volume by adopting the most optimum pressure needed for the baby by using a feedback system. This section is devoted to discussion of the various hybrid modes. As we identified volume guarantee (VG) to deliver the tidal volume in our study, we will also review the evidence in support of the use of volume guarantee.

2.2.1 Newer Hybrid Modes

Improvements in technology and increasing acceptance of volume targeting in conventional ventilation has led to the development of "hybrid" modes of ventilation where, with the help of microprocessor technology, the prefixed tidal volume is achieved by using different algorithms. At present three different modalities are in use (Keszler et al. (2009)).

2.2.1.1 Pressure regulated volume control (PRVC)

Pressure-regulated volume control (PRVC) is a pressure-limited, time-cycled mode that when initially activated, adjusts inspiratory pressure to target a set V_t, based on the pressure required to achieve the target V_t of four test breaths. Subsequent adjustments are based on the V_t of the previous breath. Breath to breath increment is limited to 3 cm H_2O or up to 5 cm H_2O below the set upper pressure limit.

2.2.1.2 Volume-assured pressure support (VAPS)

The volume-assured pressure support (VAPS) mode is a hybrid mode designed to ensure that targeted V_t is reached. Each breath starts as a pressure-limited breath, but if the set V_t is not reached, the device converts to the flow-cycled mode by prolonging the Ti with a passive increase in peak pressure. Hence the algorithm in VAPS is based on achieving the set inspiratory tidal volume.

The regulation of delivered volume based on inspiratory V_t has both advantages and disadvantages. It allows the device to respond within the given breath, but it is more susceptible to leakage around the endotracheal tube which is larger during inspiration.

2.2.1.3 Volume Guarantee (VG)

Like PRVC, the VG mode is a volume-targeted, time-cycled, pressure limited form of ventilation. The operator chooses a target V_t (expiratory) with a pressure limit. The microprocessor compares the V_t of the previous breath, using exhaled V_t to minimise possible artefact due to an air leak and adjusts the working pressure up or down to achieve the set V_t. The algorithm limits the amount of pressure increase from one breath to the next to avoid over correction leading to excessive V_t. This, and the fact that the exhaled V_t of the prior breath is used means that with very rapid changes in compliance or patient inspiratory effort, several breaths are needed to reach target V_t.

To overcome the potential disadvantage of using the exhaled V_t of the previous breath and to minimise the risk of excessively large V_t, the microprocessor opens the expiratory valve, terminating any additional pressure delivery if inspiratory V_t exceeds 130% of the target, a volume-limit function. It has a slower adjustment for low V_t and more rapid adjustment for excessive, potentially dangerous V_t. There is a separate algorithm for spontaneous (assisted) and untriggered machine breaths to ensure that the target V_t is more stable when the infant's respiratory drive is

inconsistent. The auto-regulation of inspiratory pressure makes VG a self-weaning mode. Because weaning occurs in real time, rather than intermittently in response to blood gases, VG has been considered to have the potential to achieve faster weaning from mechanical ventilation.

Volume guarantee can be combined with any of the standard ventilator modes, such as assist control, synchronised intermittent mandatory ventilation or pressure support ventilation. The clinician chooses a target tidal volume and a maximum peak inspiratory pressure limit to avoid excessive pressures.

2.2.2 Evidence in favour of volume guarantee

Whilst supremacy of volume targeting was being established, volume guarantee along with other hybrid modes provided the means to use feedback mechanisms to obtain precise expiratory tidal volume.

2.2.2.1 SIMV+VG versus SIMV

In a first-ever attempt to use VG in very low birth weight infants, Harrera et al. (Herrera et al. (1999)) carried out a randomised crossover study comparing VG with SIMV for its short-term safety and efficacy in very low birth weight infants recovering from respiratory failure. In their study, the authors reported that spontaneous minute ventilation (V_E spt) was higher during VG. They concluded that VG allowed VLBW infants to increase their inspiratory effort during the weaning phase.

Dawson et al. (Dawson and Davies (2005)) reported that infants ventilated with VG ventilation had more acceptable pCO_2 levels at the first blood gas measurement and during the first 48h of life.

Polimeni et al. (Polimeni et al. (2006)) reported that VG combined with SIMV could reduce the duration of the hypoxaemic episodes.

Guven et al. (Guven et al. (2013)) reported that when used as the primary mode, infants ventilated with VG mode had a significantly shorter duration of ventilation and needed less amount of total supplemental oxygen. The incidences of oxygen related short-term complications including BPD, ROP and IVH were also significantly lower in these infants. The authors concluded that their data favoured the use of VG ventilation in respiratory support of premature infants.

Erdemir et al. (Erdemir et al. (2014)) compared VG against SIMV for weaning in preterm infants and reported that the VG group had reduced frequency of post extubation atelectasis.

In a crossover study by Harrera et al. (Herrera et al. (2002)), the authors compared VG combined with SIMV with conventional SIMV in stable extreme preterm infants with birth weight from 600-1,200g. They reported that in comparison to conventional SIMV, short-term use of SIMV+VG resulted in the automatic weaning of the mechanical support and enhancement of the spontaneous respiratory effort.

Cheema et al. (Cheema and Ahluwalia (2001)) carried out a crossover randomised cross trial comparing the feasibility and efficacy of the use of VG for utility in the recovery phase of RDS. They reported that VG could achieve equivalent gas exchange using statistically significant lower peak airway pressures and mean airway pressures while using SIMV during the recovery phase of RDS.

In a crossover trial by Scopesi et al. (Scopesi et al. (2007)), researchers set out to compare patient-ventilator interactions and V_t variability in premature infants recovering from respiratory distress syndrome (RDS) who were weaned by various ventilator modes. Each mode combined with VG discharged comparable V_ts, which were very close to the target volume.

2.2.2.2 VG+SIPPV versus SIPPV

Cheema et al. (Cheema and Ahluwalia (2001)) also assessed the utility of VG for the early phase of RDS and reported that VG could achieve equivalent gas exchange using statistically significant lower peak airway pressures and mean airway pressures.

Subsequently moving on from crossover studies, Keszler et al. (Keszler and Abubakar (2004)) carried out an RCT and found that VG significantly reduced hypocarbia and excessively large V_t.

Cheema et al. (Cheema et al. (2007)) also used volume guarantee in their randomised controlled trials and found that VG groups had fewer out-of-range pCO_2 values and fewer instances of hypocarbia.

In their crossover trial, Scopesi et al. (Scopesi et al. (2007)), researchers reported that each mode combined with VG discharged comparable V_ts which was very close to the target volume. They also found that when SIPPV+VG was compared with SIMV+VG, SIPPV+VG showed the greater stability of V_t, fewer large breaths, lower respiratory rate and allowed for lower peak inspiratory pressure. They concluded that with regards to the weaning phase, among combined modes, those in which every breath is supported (SIPPV/PSV) are likely to be the most effective in the delivery of stable V_t using a low working pressure. Therefore, authors believed that at least in the short term, SIPPV+VG was likely to be gentler for the neonatal lung.

2.2.2.3 PSV+ VG versus PSV

In 2004, Lista et al. (Lista et al. (2004)) carried out an RCT on preterm infants born at less than 32 weeks' gestation by evaluating lung inflammatory response in these preterm infants with respiratory distress syndrome. The authors concluded

that volume targeted ventilatory strategy reduced acute inflammatory response in preterm infants with RDS.

2.2.2.4 PSV+VG versus SIMV

Nafday et al. (Nafday et al. (2005)) in their pilot RCT compared PSV+VG to SIMV for the first 24 hours of life. The overall outcomes were not significantly different between the two groups. The authors concluded that PSV-VG did not offer any ventilatory advantage over SIMV in the initial management of surfactant-treated premature newborns with RDS except for minimising the number of times blood gases need to be monitored.

Abd El-Moneim et al. (Abd El-Moneim et al. (2005)) also explored the utility of VG combined with PSV against conventional SIMV in their crossover pilot study of preterm infants in the weaning phase. The authors reported that infants ventilated with PSV-VG required significantly lower ventilation pressures and achieved better infant-ventilator synchrony.

In summary, there has been extensive research to compare VG to conventional modes of ventilation thus has demonstrated that it is a safer mode of ventilation with benefits of better maintaining tidal volume, minute volume and carbon dioxide levels while using lower PIP to achieve the objectives of ventilation.

2.3 Ventilator induced lung injury

Mechanical ventilation is a life-saving therapy that has become the mainstay of management for patients with acute respiratory failure. Since its widespread use was initiated in the mid-1950s for the treatment of paralytic poliomyelitis, the understanding of the impact of mechanical ventilation on gas exchange, pulmonary mechanics and heart-lung interactions concerning mechanical ventilation has in-

creased tremendously. Also, the complications of mechanical ventilation have also become more apparent (Tremblay and Slutsky (2006)).

2.3.1 Definition

Most universally accepted definitions of lung injury due to mechanical ventilation are from international consensus conferences in intensive care medicine. According to these, an acute lung injury that develops during mechanical ventilation is termed ventilator induced lung injury (VILI) if it can be proven that the mechanical ventilation caused the acute lung injury and ventilator-associated lung injury (VALI) if a causative relationship cannot be proven. Many experts consider VALI as the more appropriate term in most clinical situations because it is virtually impossible to prove causation outside of the research laboratory.

2.3.2 Pathology

Ventilator Associated Lung Injury (VALI) is a strain due to excess pressure or volume in the alveoli. The pathological features associated are: high permeability interstitial alveolar oedema, altered surface tension, alveolar haemorrhage, hyaline membranes, loss of functional surfactant and alveolar collapse (Rouby and Brochard (2007)).

Greenfield et al. (Greenfield et al. (1964)) ventilated closed-chest dogs for 2 hours at 26–32 cm H_2O peak inspiratory pressure. The animals underwent thoracotomy after 24 hours. On gross examination, there were zones of atelectasis. Extracts of these lungs had increased surface tension, suggesting altered surfactant properties. In this experiment, the researchers demonstrated one of the mechanisms behind VALI.

Webb et al. (Webb and Tierney (1974)) conducted their study in intact animals which unambiguously demonstrated that mechanical ventilation may produce pulmonary oedema and that the role of positive pressure ventilation (barotrauma) was

a mechanism for ventilation induced lung injury. They subjected rats to positive airway pressure ventilation with peak pressures of 14, 30, and 45 cm H_2O. No abnormality was observed after 1 hour of ventilation with 14 cm H_2O peak pressure. Animals ventilated with higher peak pressures showed not only pulmonary oedema but also, extent of this oedema was directly proportional to the peak pressure which confirmed the role of positive pressure as a means for causing ventilation induced lung injury by barotrauma.

2.3.3 Pathogenesis

The main initiators of ventilator induced lung injuries are - alveolar over distension and cyclic atelectasis

2.3.3.1 Alveolar over distension

Rouby et al. (Rouby et al. (1993)) examined post-mortem histological specimens obtained from young, critically ill patients and found that 86% of them had evidence of airspace enlargement. They also reported that the patients with severe airspace enlargement (2.6-40 mm internal diameter) had a significantly higher incidence of pneumothorax, had been ventilated using higher peak airway pressures and tidal volumes and had been exposed significantly longer to toxic levels of oxygen than patients with mild airspace enlargement (1-2.5 mm internal diameter).

Over distension of the alveoli has been blamed on excess pressure (barotrauma), and excess volume (volutrauma) applied during mechanical ventilation. The studies on ventilator induced lung injury while conclusively establishing over distension as one of the most important modes of injury also led to the question: which of the two, volutrauma or barotrauma is more harmful?

Volutrauma versus Barotrauma

A study by Dreyfuss et al. (Dreyfuss et al. (1988)) compared the consequences of normal and high tidal volume ventilation in mechanically ventilated rats at a

normal and high airway pressure. They created three models: low pressure-low tidal volume; high pressure-high tidal volume and high pressure-low tidal volume. The high pressure-low tidal volume model was obtained by strapping the chest to avoid over expansion of the alveoli while rats were ventilated with high pressures.

The researchers reported that lungs of rats ventilated with high pressure and low tidal volume (strapped chest) were same as controls (rats ventilated with low pressure-low tidal volume). They also reported that the lungs from the rats ventilated with high volume ventilation (due to unstrapped high pressure ventilation) had significant permeability type oedema. This study highlighted the importance of volume as the decisive factor in causing lung injury.

In a similar landmark study, Harnandez et al. (Hernandez et al. (1989)) compared lung injury in mechanically ventilated immature rabbits by subjecting them to high pressure plus high tidal volumes and high pressure plus low tidal volumes.

White rabbits were assigned to one of three protocols with different degrees of inspiratory volume limitation: intact closed chest animals (CC) and closed chest animals with a full body plaster cast (C). The intact animals were ventilated at 15, 30, or 45 cm H_2O PIP for 1 hour, and the lungs of the CC and C groups were placed in an isolated lung perfusion system. The markers were compared against baseline permeability. It was the same as the baseline in the CC group ventilated with 15 cm H_2O PIP. In the CC group permeability increased by 31% after 30 cm H_2O PIP and 43% after 45 cm H_2O PIP. Inspiratory volume limitation by the plaster cast in the C group prevented any significant increase in permeability at the PIP values used. The authors concluded that volume distension of the lung rather than high PIP per se produced microvascular damage.

Dreyfuss et al. (Dreyfuss et al. (1995)) compared the effect of low and high tidal volume and the relation of the severity of the tidal volume to the magnitude of the tidal volume on previously damaged lungs in rats. The authors ventilated these rats by using 7 ml/kg body weight tidal volume mechanical ventilation for

2 minutes with that of 25 (HV25), 33 (HV33), and 45 (HV45) ml/kg body weight high tidal volume in anesthetised rats previously exposed or not exposed to alpha-naphthylthiourea (ANTU). High tidal volume alone resulted in permeability oedema in which severity was proportional to the magnitude of the tidal volume.

These studies demonstrated that it is the high tidal volumes that cause lung injury and not the airway pressure.

2.3.3.2 Alveolar collapse/Atelectotrauma

Subsequently, researchers also demonstrated that ventilation induced lung injury can still occur at low pressures highlighting other modes of injury other than high peak positive pressure. It is proposed that cyclic alveolar expansion (during inspiration) and collapse (during expiration) creates shear forces that distend adjacent alveoli and cause injury in animal models.

2.3.3.3 Rheotrauma

Rheotrauma is a term used for lung injury due to high gas flows delivered by mechanical ventilation.

Rich et al. (Rich et al. (2000)) studied the influence of flow rates in causing ventilator induced lung injury.

They performed their experiment on sheep whereby they ventilated sheep with varying pressure support, respiratory rate and flow rate. They reported that limiting the inspiratory flow rate (LIFR) reduced histologic injury, decreased intra-alveolar neutrophils and reduced wet-dry lung weight. The authors concluded that the experiment showed that the reduction of inspiratory flow provides pulmonary protection.

Bach et al. (Bach et al. (2009)) studied this relatively neglected aspect of role of flow rates in term lambs. Researchers found that there was no difference in ventilatory

parameters until very low flow rates were used. The study, though analysing the therapeutic impact of flow rates, did not assess the lung injury aspect of flow rates.

This study was followed by a study of lung injury by the same researchers. Bach et al. (Bach et al. (2012)) ventilated preterm lambs with bias gas flow of 8 L/min, 18 L/min or 28 L/min. Control lung tissue was collected from unventilated age-matched foetuses. The authors reported that pulmonary mRNA levels of the injury markers, EGR1 and CTGF, were highest in lambs ventilated with bias gas flows of 18 L/min. High bias gas flows also resulted in increased cellular proliferation and abnormal deposition of elastin, collagen, and myofibroblasts in the lung. They concluded that flow rate contributed to ventilator induced lung injury and bronchopulmonay dysplasia.

2.3.3.4 Biotrauma

Mechanical stretch can cause a release of mediators associated with activation of the immune response, further adding to injury and potentially causing remote injury to other organs; this is termed as "biotrauma".

While alveolar over-distension, lung strain and atelectasis are key inciting features of VILI, numerous studies over the past 20 years have demonstrated the possibility of a subtler form of injury, with the release of various mediators into the lung, pulmonary recruitment of leukocytes, and local initiation of inflammatory processes.

The biotrauma hypothesis postulates that the circulating mediators have the potential to cause local lung injury as well as systemic upset (Slutsky and Tremblay (1998)).

In 1985 Kawano et al. (Kawano et al. (1987)) noted that previous studies had shown a large number of granulocytes in the damaged lung. They set out to demonstrate that these cells contributed to lung injury by comparing two lung models in rabbits; one with normal granulocytes, the other depleted of granulocytes by pre-treatment with nitrogen mustard. The non-depleted rabbits had a

poor gas exchange, a substantial protein leak into the lung and extensive hyaline membranes. The depleted animals had a good gas exchange, a tiny protein leak, and no hyaline membranes. Repletion of granulocytes from donor rabbits led to poor gas exchange and hyaline membrane formation. The authors concluded that lung lavage causes prompt margination of granulocytes, which become activated by the on-going epithelial barotrauma of conventional ventilation.

Tremblay et al. (Tremblay et al. (1997)) studied rats by randomising them to receive ventilation with moderate V_t with or without PEEP and ventilation with high V_t without PEEP.

Authors reported maximum levels of cytokines in rats ventilated with Zero PEEP in combination with high volume ventilation (HVZP group). There was a gradual reduction in cytokines levels from HVZP (High Volume Zero PEEP group), MVZP (Moderate Volume Zero PEEP group), MVHP (Moderate Volume High PEEP group) and control (low volume normal PEEP) reinforcing role of cytokines associated with ventilation associated lung injury.

These data suggest that mechanical ventilation plays a role in initiating or propagating a local and possibly systemic inflammatory response.

Study of cytokines in humans was performed by Ranieri et al. (Ranieri et al. (1999)) in which researchers studied the impact of traditional ventilation or lung protective ventilation using inflammatory markers in plasma and alveolar fluid. The authors used lower levels of cytokines to validate the utility of lung protective ventilation.

2.3.4 Prevention of Ventilator induced lung injury

The evidence so far has shown that the following strategies help in preventing the lung injury.

2.3.4.1 High PEEP

Mechanical ventilation using PEEP at inflection point helps in preventing the collapse of lungs even during expiration which not only reduces the work of breathing but also has been shown to reduce ventilation induced lung injury.

Argiras et al. (Argiras et al. (1987)) demonstrated this on surfactant deficient rabbits in whom lung lavage was performed to produce surfactant-deficient lungs. The authors reported that the animals in the high PEEP group had a significantly greater arterial pO_2 than those in the low PEEP group and less incidence of hyaline membranes. However, the mean survival time for each group was similar.

Sandhar et al. (Sandhar et al. (1988)) replicated the findings by comparing low PEEP (1-2cm) versus high PEEP (PEEP at inflection point) keeping mean airway pressure the same in surfactant deficient rabbits. The authors demonstrated that animals with high PEEP showed significantly less hyaline membrane as a marker of lower ventilator induced lung injury.

Muscedere et al. (Muscedere et al. (1994)) assessed the impact of low PEEP (PEEP below inflection pressure, Pinf) in their study on isolated, non-perfused, lavaged rat lungs. Researchers ventilated these lungs with physiologic tidal volumes (5 to 6 ml/kg) at different end-expiratory pressures and studied the effect on compliance and lung injury. Lung injury assessed morphologically was significantly higher in the groups ventilated with a low PEEP, and in these groups, the site of injury was dependent on the level of PEEP. The group ventilated without PEEP had significantly greater respiratory and membranous injury to bronchioles, while the group ventilated with PEEP of 4 cm H_2O had significantly greater alveolar duct injury. It was concluded that in addition to high airway pressures, end-expiratory lung volume is an essential determinant of the degree and site of lung injury during positive-pressure ventilation. The authors speculated that ventilation at very low lung volumes worsens lung injury by repeated opening and closing of the airway and alveolar duct units as ventilation occurs from below to above the inflection

point. This study helped to establish the vital role of PEEP in ventilation to avoid atelectotrauma.

2.3.4.2 High Frequency Ventilation

Sugiura M et al. (Sugiura et al. (1994)) , in their experiments on New Zealand rabbits, used high frequency ventilation with high oxygen as atelectasis minimising measure in surfactant depleted animals. The surfactant replete animals on conventional ventilation acted as controls. The researchers noted that the surfactant depleted animals on conventional ventilation showed a higher degree of neutrophil activation than the ones with measures to minimise atelectasis. The authors concluded that lung injury could be minimised by preventing cyclic alveolar/airway expansion and collapse in the surfactant-deficient lung by use of appropriate ventilator patterns. The study also demonstrated that ventilator patterns selected during such early phases had a powerful impact on the extent of the inflammatory response.

2.3.4.3 Low tidal volume

In adult patients with ARDS, a multicentre randomised trial (Brower et al. (1999)) found that mechanical ventilation using tidal volumes of 6 mL/kg of ideal body weight improved mortality. The trial was stopped midway because in the group treated with lower tidal volumes had significantly lower mortality and more number of days without ventilator use. The authors concluded that using mechanical ventilation with a lower tidal volume than is traditionally used in patients with the acute lung injury and acute respiratory distress syndrome, resulted in decreased mortality and duration on ventilator. This multicentre trial established the utility of low tidal volumes in adults with ARDS as lung protective measure.

2.3.4.4 Lung protection strategies

Use of low tidal volume coupled with moderate PEEP (or PEEP above the low inflection point on the pressure-volume curve) has been proposed as a "lung protective strategy." It is also called “open lung ventilation”. In patients with ALI/ARDS, randomised trials demonstrated that open lung ventilation improved clinical outcomes. One randomised trial (IMPROVE) (Futier et al. (2013)) of patients at high risk of pulmonary complications following major abdominal surgery compared an intraoperative non-protective ventilation strategy (V_t 10 to 12 mL/kg of PBW (patient's body weight); no PEEP; no recruitment) with a lung protective strategy (V_t 6 to 8 mL/kg of PBW; PEEP at 6 to 8 cm of water; recruitment manoeuvres every 30 minutes). At seven days, lung protective ventilation was associated with a reduction in adverse pulmonary and extra pulmonary events, need for mechanical ventilation and a shorter length of stay in the hospital. However, the lung protective strategy did not protect against the development of ARDS nor was it associated with a benefit in mortality rates.

2.3.4.5 Preventing cyclic atelectasis

In a study looking at the impact of spontaneous breathing versus muscular paralysis in an animal model, Yoshida et al. (Yoshida et al. (2013)) reported that in animals with mild lung injury spontaneous breathing was beneficial to lung recruitment; however, in animals with severe lung injury, spontaneous breathing fared worse than recruitment measures.

2.4 Inflammatory markers of lung injury

There is a realisation in the neonatalology community that dependence on traditional clinical and pathological methods to identify lung injuries associated with mechanical ventilation is leading to delayed diagnosis with limited options to inter-

vene. With the development of technology and a better understanding of biochemical molecules, the focus has now shifted to cytokines to help identify the presence and extent of lung injury at an early stage. Their role however, is still very early and in an experimental phase. Once established, cytokines also would have added benefit of a quicker measure to interventions designed to protect against lung injury.

2.4.1 Cytokines

Cytokines are low-molecular-weight soluble proteins that transmit signals between the cells involved in the inflammatory response. (Figure 2.2 (Strieter et al. (2002)).

Cytokines are released in response to external threat, PAMP (Pathogen associated molecular patterns), internal tissue injury and damage, DAMP (Damage Associated molecular Patterns) and alarmins (a subset of DAMP released as a result of unprogrammed cell death). TLR (Toll like receptors) and NLR (Nodd like receptors) are the receptor proteins programmes to release inflammatory cascade in response to external (PAMP, extracellular DAMP and alarmins) and intracellular danger signals (DAMP and alarmins) respectively. (Figure 2.3).

Cytokines are important mediators of immune responses which allow integration of the behaviour of cells in time and geographical location as immune responses are generated. These are hormone-like proteins that enable immune cells to communicate, and they play an integral role in the initiation, perpetuation and subsequent down-regulation of the immune response.

In the lungs, they are produced by bronchial, bronchiolar alveolar epithelial cells, alveolar macrophages, and neutrophils (Pugin et al. (1998), Tremblay et al. (2002)). Whilst ventilator induced lung injury is known to be associated with the presence of polymophonuclear cells in alveoli, the mere presence of polymophonuclear cells has not led to lung injury in experimental studies (Martin et al. (1989)). The activation and attraction of leucocytes are carried out by IL-1, IL-8 and TNF-α.

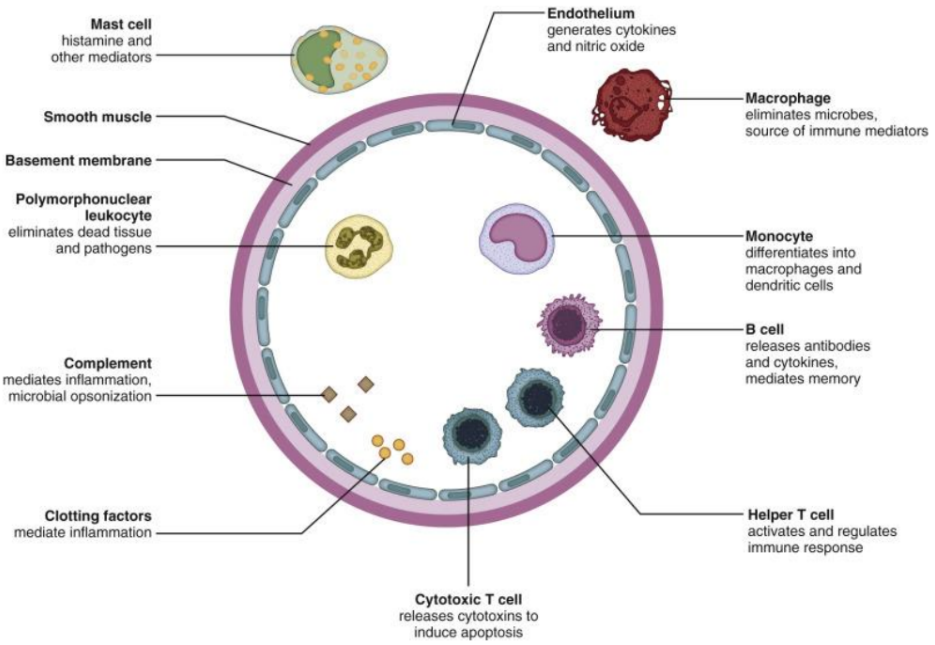


Figure 2.2: Basic components of the immune system and their respective functions (Shah 2017)

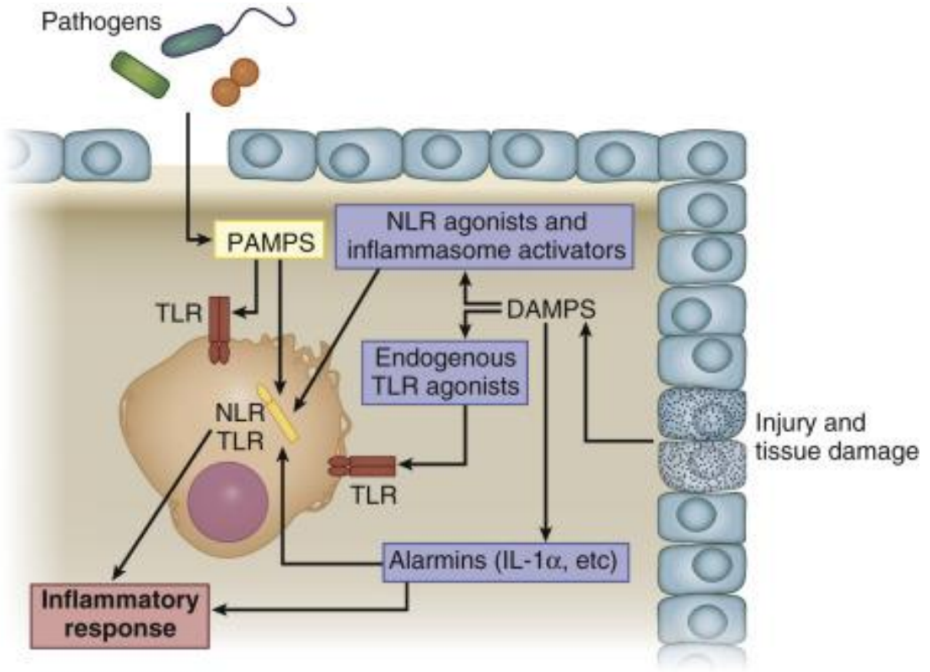


Figure 2.3: Stimulus for release of cytokines causing inflammatory response (Shah 2017)

The inflammatory effect of the cytokines is also exacerbated by reducing apoptosis of leucocytes in alveoli which is mediated by IL-2 and IL-8.

Surfactant dysfunction or deficiency is one of the prominent features of lung injury. Inflammation and more specifically cytokines such as TNF$-\alpha$ and IL-1 are thought to decrease surfactant components either directly or indirectly by inducing alveolar leakage of proteins that subsequently inhibit surfactant function (Kobayashi et al. (1991), Rimensberger (2002)).

Cytokine release can take place due to the following mechanisms (Halbertsma et al. (2005)):

- Mechanotransduction - alterations in the cytoskeletal structure without ultrastructural damage.
- Decompartmentalisation - stress failure of the alveolar barrier.
- Necrosis - stress failure of the plasma membrane.
- Effects on the vasculature independent of stretch or rupture.

Mechanotransduction

It is mediated by transmembrane receptors such as integrins, which are stretch-activated ion channels and start various intracellular processes. Most alveolar cells can produce pro- and anti-inflammatory mediators such as TNF-α, IL-1, IL-6, IL-8, and IL-10 when stretched in vitro or when ventilated with a large tidal volume (V_t) in ex-vivo and in-vivo experiments.

Translocation and decompartmentalisation

Direct trauma to the plasma membrane of alveolar cells and loss of cell integrity also leads to the release of intracellular cytokines to the interstitium resulting into decompartmentalisation to both the alveolar space and the systemic circulation.

2.4.1.1 Cytokines in VILI

Whilst the role of the cytokines is still being studied, they offer one explanation for the role of antenatal and postnatal steroids in preventing the incidence of chronic lung disease. Three major cytokines have been studied in the field of lung injury – Interleukin - 6 (IL-6) Interleukin - 8 (IL-8) Tumour Necrosis Factor - alpha (TNFα)

2.4.1.2 Interleukin 6 (IL-6)

Human IL-6 is made up of 212 amino acids, including a 28-amino-acid signal peptide.

Process of the release of IL-6

IL-6 functions as a mediator to notify the occurrence of an emerging inflammatory event. It is generated in an infectious lesion and has an impact in all parts of the body. Monocytes and macrophages recognise the signature of exogenous pathogens, known as pathogen associated molecular patterns (PAMPs). PAMPs are sensed by evolutionarily conserved, host sensors known as pathogen recognition receptors (PRRs) (Kumar et al. (2011)). These PRRs comprise toll like receptors, retinoic acid inducible gene 1 like receptors, nucleotide binding oligomerisation domain like receptors, and DNA receptors. They stimulate a range of signalling pathways including NF-κB and enhance the transcription of the mRNA of inflammatory cytokines such as interleukin 6, tumour necrosis factor α and IL-1β. TNF-α and IL-1β also help in increasing the production of IL-6 by activating transcription factors.

Functions

IL-6 performs a variety of functions. This can be identified by the fact that historically few proteins were deemed to be responsible for these functions. However, it later transpired that it is one protein in itself. IL-6 is involved in many steps of the activated immune system, and its function helps to explain many of the

physiological effects of chronic inflammation. Because of the activity at so many levels, dysregulated continual production of IL-6 leads to the onset or development of various diseases (Figure 2.4).

- After its local synthesis in the initial stage of inflammation, IL-6 moves to the liver and plays a vital role in the rapid induction of acute phase proteins such as C-reactive protein (CRP), serum amyloid A, fibrinogen, haptoglobin and α1-antichymotrypsin (Heinrich et al. (1990)). It also reduces the production of fibronectin, albumin, and transferrin.

- IL-6 promotes megakaryocyte maturation leading to the release of platelets (Ishibashi et al. (1989)). Of note, these haematological and biochemical changes are used for the evaluation of inflammatory severity in routine clinical laboratory examinations.

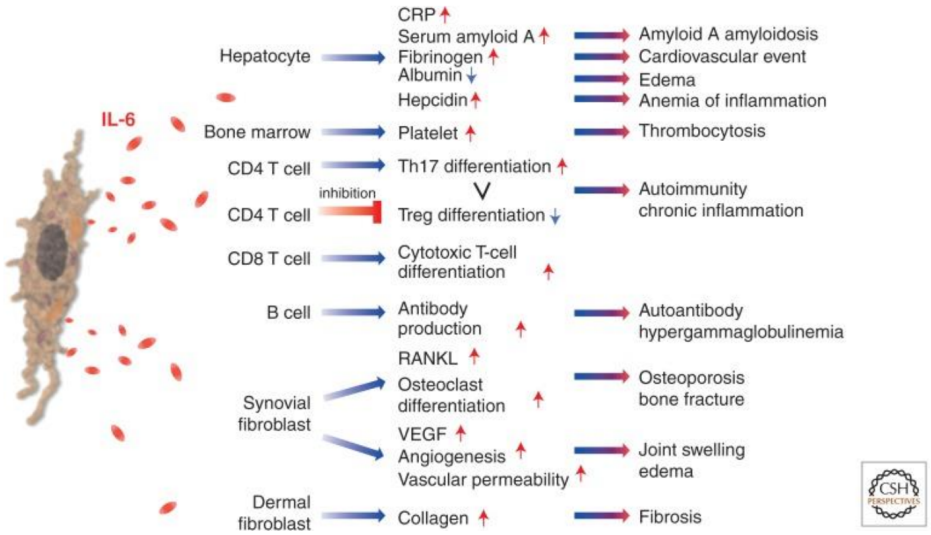
- IL-6 performs an important function in the linking of innate to acquired immune response by promoting specific differentiation of naive CD4+ T cells.

- IL-6 promotes T-follicular helper cell differentiation as well as the production of IL-21 (Ma et al. (2012)), which regulates immunoglobulin (Ig) synthesis and IgG4 production in particular.

- IL-6 induces the differentiation of CD8+ T cells into cytotoxic T cells (Okada et al. (1988)).

- IL-6 induces the differentiation of activated B cells into antibody-producing plasma cells, so that continuous over synthesis of IL-6 results in hypergammaglobulinemia and autoantibody production.

- IL-6 is responsible for enhanced angiogenesis and increased vascular permeability by inducing production of Vascular Endothelial Growth Factor, leading to some of the common pathological features of inflammatory lesions.



Treg - regulatory T cell; RANKL - recepter activator of nuclear factor κB(NF-κB) ligand; VEGF - vascular endothelial growth factor

Figure 2.4: IL-6 in inflammation, immunity and disease (Tanaka et al. (2014))

- IL-6 reduces serum iron levels (Hashizume et al. (2009) by inducing hepcidin production, which blocks the action of iron transporter ferroportin on gut and hence is also responsible for hypoferremia (Nemeth et al. (2004)) and anaemia associated with chronic inflammation (Liuzzi et al. (2005)).
- IL-6 stimulates the RANKL which is indispensable for the differentiation and activation of osteoclasts and leads to bone resorption and osteoporosis (Poli et al. (1994)).

2.4.1.3 Interleukin 8 (IL-8)

The protein, consisting of 72 amino acids in its mature form, is identified as a basic and heparin-binding protein (Matsushima et al. (1988)).

Source

IL-8 production has been observed in vitro monocytes, T lymphocytes, neutrophils, vascular endothelial cells, dermal fibroblasts, keratinocytes, hepatocytes (Strieter

et al. (1989),Thornton et al. (1990)).

Process of the release of IL-8 cytokines

Levels of IL-8 increase rapidly following an appropriate stimulus and have been demonstrated to correlate with important clinical parameters in patients with sepsis.

IL-8 production is stimulated by numerous stimuli viz., TNF, Pathogen associated molecular patterns (PAMPs) such as bacterial and viral products, and cellular stress.

Functions

- IL-8 is a powerful attractant and stimulator of neutrophils and plays an important role in the body's inflammatory response (Harada et al. (1994)).
- IL-8 stimulates neutrophil degranulation (Walz et al. (1987)), upregulates expression of adhesion molecules, and increases production of reactive oxygen species (ROS). It has a vital role in stimulating chemotaxis and regulates transvenular traffic during acute inflammatory responses (Huber et al. (1991)).
- In particular, IL-8 is thought to play a key role in the epithelial and physiologic dysfunction observed in acute lung injury and acute respiratory distress syndrome.

2.4.1.4 Tumour Necrosis Factor alpha (TNF-α)

Tumour necrosis factor (TNF) is a cytokine involved in systemic inflammation (Idriss and Naismith (2000)). It is a member of a group of cytokines that participates in the acute phase reaction. TNF-α has been dubbed the "master regulator" of inflammatory cytokine production because of its early and broad role in mediating downstream cytokine production. TNF's central role is in the regulation of immune

cells. In studies using injections of TNF-α, animal and human subjects manifest a clinical response resembling systemic inflammatory response syndrome or septic shock. Dysregulation of TNF has been implicated in a variety of human diseases - autoimmune diseases, insulin resistance, and cancer.

Source

TNF-α is produced by a wide variety of cells, most notably monocytes and macrophages.

Process of release and mode of action

It is initially synthesised as a membrane-bound 26 kDa molecule that is subsequently cleaved by a TNF converting enzyme to form a soluble 17 kDa protein. TNF-α binds to two distinct receptors, TNFR1 and TNFR2, which initiate a broad cascade of pro-inflammatory events leading to the production and release of downstream inflammatory mediators. TNF-α is rapidly transcribed, translated and released within 30 minutes of an inciting event. Bacterial endotoxin is a powerful stimulus for TNF-α release, along with the IL-1 family of cytokines.

TNF exerts its effect(s) by binding to, as a trimer and clustering high-affinity receptors present in great numbers on most cell membranes. The ligand/receptor complex is rapidly internalised via clathrin-coated pits and ends up in secondary lysosomes where it is degraded. TNF exerts its effects by activating a number of secondary proteins that provoke a variety of responses within the cell such as activation of gene transcription and/or production of reactive oxygen or nitrogen radicals (e.g., NO). Activated proteins include G-Protein, transcription factors (e.g., NF- B, AP-1), protein kinases (e.g. CK II, erk-1, erk-2, and MAP2), phospholipases (e.g. PLA2, PLC, PLD and sphingomyelinase) mitochondrial proteins (e.g. manganese superoxide dismutase) and serine and cysteine proteases, known as caspases.

Although TNF-α and IL-1 are structurally distinct, their biologic functions in the inflammatory response overlap considerably. In animal and human models, IL-1 and TNF-α have been shown to act synergistically to mediate the early inflammat-

ory response and induce a shock-like state characterised by vascular permeability, loss of vascular tone, pulmonary oedema, and haemorrhage.

Functions

- Like IL-1, TNF-α acts on macrophages, neutrophils, and endothelial cells. TNF-α causes increased production of macrophages, stimulates macrophage activity and prolongs macrophage survival.
- In endothelial cells, TNF-α increases the expression of adhesion molecules, including intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and chemokines.
- TNF-α promotes extravasation of neutrophils into the tissue by increasing adhesion via integrins.
- Along with IL-1, TNF-α is a main mediator of a hypercoagulable state in sepsis, in part by upregulating endothelial expression of procoagulant.
- Together with IL-1, TNF-α activates macrophages to secrete additional inflammatory cytokines such as IL-6 and IL-8 and other mediators such as nitric oxide, which contribute to vascular instability and may depress myocardial function in sepsis.
- TNF binding mediates alterations in cell volume associated with necrosis/apoptosis by inducing regulation of the activities of several anion/cation channels.

2.4.2 Experimental studies

Experimental studies consist of both in-vitro, ex-vivo and in-vivo models, using different species and applying various techniques, which probably explains some of the observed inconsistencies in cytokine response. The involvement of TNF-α in the pathogenesis of VILI is still under debate. Increased TNF-α levels after mechanical ventilation were found in most but not all uninjured lung models, surfactant

depletion and acute lung injury (ALI) models and sepsis models. Endotracheal instillation of anti-TNF-α antibody attenuates VILI in both the previously uninjured and injured lung, suggesting a role for TNF-α. However, lack of TNF-α signalling (TNF-α receptor -/- mice) does not show diminished VILI.

In a randomised controlled trial on lambs, Hillman et al. ((Hillman et al. (2012)) demonstrated that ventilation at moderate levels could lead to inflammation in lungs. The authors found that ventilation increased pro-inflammatory cytokines as compared with controls and lambs on continuous positive airway pressure, with the recruitment of primarily monocytes to bronchoalveolar lavage fluid. They also noted that brief exposure to 95% oxygen did not alter lung inflammation. The researchers used cytokines in their study to conclude that it was the ventilation and not the oxygen which was responsible for lung injury.

Imai et al. ((Imai et al. (1994)) isolated and studied polymophonuclear cells and Inflammatory chemical mediators and reported that the numbers of polymophonuclear cells and the levels of PAF and TXB2 in lung lavage fluid were significantly greater during conventional mechanical ventilation than during high frequency oscillation (HFOV). This led them to conclude that the high frequency oscillation (HFOV) was superior to conventional ventilation. Brew et al. ((Brew et al. (2011))used assessment of cytokines as a mean to study the injury and repair in the premature lung. Authors reported that even a brief period (two hours in their study) of mechanical ventilation induced severe injury in the very immature lung and that these lungs could repair spontaneously in the absence of further ventilation.

In a critical review of the relationship between cytokines and lung injury by Halbertsma et al. ((Halbertsma et al. (2005)), the authors reviewed 57 articles on cytokines in in-vitro settings (n=5), ex-vivo models in-vitro models and clinical trials. The reviewers concluded that there was strong circumstantial evidence that the release of cytokines into the systemic circulation contributed to the pathogenesis of multiple organ dysfunction syndromes and hence cytokines were good surrogate

endpoints in exploring the pathogenesis and pathophysiology of ventilator induced lung injury in both experimental and clinical studies.

In general, most of the animal studies showed a more pronounced increase in cytokine levels with larger tidal volumes or absent PEEP or when animals are concomitantly subjected to other injurious strategies such as hyperoxia. It is to be noted that the observed pro-inflammatory response has been demonstrated to be parallel to the observed histopathology. The injured lung appears to be far more susceptible for VILI than the healthy lung (two-hit model).

Stuber et al. ((Stuber et al. (2002)) showed that increasing V_t from 6 to 12 ml/kg in ARDS patients increased cytokine levels in both BAL fluid and plasma within one hour. These findings were consistent with both the results demonstrated by and ARDSnet trial ((Parsons et al. (2005)). In this study by Parsons et al., authors found that the baseline plasma levels of interleukin-6 and interleukin-8, were each associated with an increased risk of death, significant decrease in ventilator free and organ failure free days. They also reported that lower tidal volume strategy (6 mL/kg) on day 3 was associated with a greater decrease in interleukin-6 and interleukin-8 levels. Authors concluded that the low tidal volume ventilation was associated with a more rapid attenuation of the inflammatory response.

Wrigge et al. (Wrigge et al. (2004)) reported that while cytokine levels were elevated after elective surgery in patients with normal lungs, there was no difference between patients ventilated with V_t 15 ml/kg and those with V_t 6 ml/kg.

Encouraged by the animal and in-vivo studies in adults, these inflammatory markers have also been used by neonatal researchers as surrogate markers to compare ventilatory modalities.

Lista et al. (Lista et al. (2008)) studied IL-6, IL-8, and TNF-α on day 1, 3 and 7 in tracheal aspirates of preterm infants ventilated with either volume guarantee (VG) or high frequency oscillation (HFO). They found that in the HFOV group IL-6 levels were significantly higher on day 3. The authors concluded that VG ventilation is

an effective lung-protective strategy to be used in acute RDS, inducing a lower expression of early inflammation markers than HFOV.

In another study explained in more detail in the next section, the same group of researchers (Lista et al. (2006)) also used cytokines assessment in tracheal aspirate to compare two different tidal volumes (3 ml/kg versus 5 ml/kg) and demonstrated that raised cytokines levels were well correlated with longer duration of mechanical ventilation observed in babies ventilated with 3ml/kg tidal volume.

Whether these inflammatory markers are of only short-term significance or if they can help predict medium to long-term clinical issues like chronic lung disease is not answered. Bose et al. addressed this question (Bose et al. (2008)) in their review of biomarkers and their relationship to chronic lung disease. The authors highlighted that:

- Increased concentrations of IL-1β in tracheal aspirates predict the requirement for mechanical ventilation and oxygen supplementation.
- Increased concentrations of IL-1, TNF-α, IL-6, and IL-8 correlate with the duration of supplemental oxygen and mechanical ventilation and are increased in infants who develop bronchopulmonary dysplasia (BPD) compared with infants of similar gestational age who do not develop bronchopulmonary dysplasia (BPD).
- Increased IL-1β concentrations and IL-1β/IL-6 ratios are also associated with risk factors for bronchopulmonary dysplasia (BPD), specifically colonisation with Ureaplasma urealyticum.
- TNF-α, which appears during the early phases of an inflammatory response, is raised in BAL samples from ventilated preterm infants with poor pulmonary outcomes.

Finally, in longitudinal studies in neonates, elevated pro-inflammatory cytokine levels are associated with more severe lung injury and worse outcomes, supporting

the concept that lung injury is partly the result of a massive pro-inflammatory response (Groneck et al. (1994), Hitti et al. (1997),Jonsson et al. (1997),Munshi et al. (1997)).

All the evidence so far suggests that the cytokines are indeed very useful markers of ventilator associated lung injury. Currently, they are used in experimental settings, but in future with better availability and economy, they have the potential to guide management in both respiratory distress syndrome as well as established chronic lung disease.

2.5 Tidal volume

This is the final section of the chapter where evidence so far has been explored from all possible sources – animal, adult, paediatric and neonatal studies. This provides insight into the enormous efforts of researchers in dealing with the question of ideal tidal volume as well as the lack of evidence in helping to manage preterm and term, newborn infants.

What is tidal volume?

Tidal volume is the volume of air delivered by the ventilator per breath.

Why is it important?

Physiological studies in animal models cited in the previous section demonstrated that it has a stronger association with lung injury as compared to pressures. Also, while high tidal volume can potentially lead to volutrauma and hypocarbia, which in turn increases the risk of cerebral injury, a low tidal volume can cause atelectotrauma and hypercarbia.

2.5.1 Lessons from animal studies

In a randomised trial by Kozian et al. (Kozian et al. (2011)), it was found that ventilation with high V_t increased aeration and tidal recruitment in animal models

of one lung ventilation (OLV).

Hillman et al. (Hillman et al. (2012)) studied the impact of normal and high tidal volume ventilation at birth in causing lung injury. Researchers studied the extent of lung injury using pro-inflammatory cytokines and bronchoalveolar lavage fluid for inflammatory cells and concluded that degree of lung injury was less with low V_t (6 mL/kg) than with higher V_t ventilation (15 mL/kg). Ratner et al. (Ratner et al. (2013)) explored the impact of large tidal volume and small tidal volume in altering bioenergetics. The authors concluded that their data suggested that the failure of bioenergetics to support normal lung development was caused by large tidal volume and prolonged ventilation.

Zick et al. (Zick et al. (2013)) found that high tidal volumes increased regional respiratory system compliance in a porcine model of ALI and better lung recruitment in healthy animals. In a study on infant rats with lung injury induced by acid, Sly et al. (Sly et al. (2013)) showed that high tidal volume (21 mL/kg) compared with low tidal volume (7 mL/kg) did not exacerbate lung injury. The authors also found that tissue elastance and airway resistance were less deteriorated and there were no differences in histologic lung scores or the concentration of IL-6 in bronchoalveolar lavage fluid in the high tidal volume group. In this study, in contrast to experimental studies with adult rats, short-term mechanical ventilation with high V_t - low PEEP was not deleterious when compared to low V_t - high PEEP in both healthy and pre-injured infant rat lungs. In this study, the authors challenged the use of the conventional lung protective model of low PIP/V_t and high PEEP in children based on adult studies.

2.5.2 Lessons from studies in adults

Tidal volumes have been compared in adults in different clinical scenarios like ARDS (Adult Respiratory Distress Syndrome) (Amato et al. (1998), Gajic et al. (2004), Brower et al. (2000), Petrucci and Iacovelli (2004), Wheeler et al. (2006),

Villar et al. (2006), Futier et al. (2013)); without ARDS (Determann et al. (2010), Gajic et al. (2004), Serpa Neto et al. (2012), Neto et al. (2015)) and single lung ventilation (Licker et al. (2009), Michelet et al. (2006), Yang et al. (2011), Maslow et al. (2013), El Tahan et al. (2017)).

These studies have been summarised as follows.

The studies reported that low tidal volume was associated with favourable outcome as it was associated with

- Decreased mortality (Amato et al. (1998), Brower et al. (2000), Petrucci and Iacovelli (2004), Wheeler et al. (2006), Villar et al. (2006), Serpa Neto et al. (2012)).
- Decreased duration of mechanical ventilation (Amato et al. (1998), Brower et al. (2000), Villar et al. (2006)).
- Lower development of lung injury (Amato et al. (1998), Determann et al. (2010), Gajic et al. (2004), Serpa Neto et al. (2012)).
- Decreased risk of acute lung injury (Gajic et al. (2004), Licker et al. (2009)).
- Lower rates of pulmonary complications (Futier et al. (2013), Neto et al. (2015).
- Lower rates of extrapulmonary complications (e.g., sepsis) (Futier et al. (2013)).
- Less intensive care unit (ICU) and hospital days ((Licker et al. (2009)).
- Greater paO2/FiO2 ratio (Michelet et al. (2006), Yang et al. (2011)).
- Better preservation of postoperative oxygenation (El Tahan et al. (2017)).
- Reduction in airway pressures and lung infiltrates (El Tahan et al. (2017)).

High tidal volume was found to be better in being associated with

- Lower alveolar atelectasis and higher dynamic pulmonary compliance (Maslow et al. (2013)).
- Better gas exchange (El Tahan et al. (2017)).

Studies also reported that both were comparable in their effect on

- Short term or long-term mortality (Petrucci and Iacovelli (2004), Futier et al. (2013), Licker et al. (2009), Michelet et al. (2006), Yang et al. (2011)).
- Development of ARDS (Futier et al. (2013)).
- Hospital or ITU stay (Michelet et al. (2006), Yang et al. (2011), Maslow et al. (2013), El Tahan et al. (2017)).
- Pulmonary or extra pulmonary morbidity (Michelet et al. (2006), Yang et al. (2011), Maslow et al. (2013), El Tahan et al. (2017)).

2.5.3 Lessons from Paediatric studies

Sousse et al. (Sousse et al. (2015)) carried out a comparison of high versus low tidal volume in children with inhalation injury in a prospective cross-over study. Researchers reported that the high tidal volume was associated with significantly decreased ventilator days and maximum positive end-expiratory pressure and significantly increased maximum peak inspiratory pressure compared with those in patients with low tidal volume. The incidence of atelectasis and ARDS was significantly decreased with high V_t compared with low V_t. However, the incidence of pneumothorax was significantly increased in the high V_t group compared with the low V_t group.

In a recent meta-analysis by de Jager et al. (de Jager et al. (2014)), the authors aimed to identify an association of tidal volume with mortality in critically ill, mechanically ventilated patients. The authors identified eight studies which included 1,765 patients.

In a recent meta-analysis by de Jager et al. (de Jager et al. (2014)), the authors aimed to identify an association of tidal volume with mortality in critically ill, mechanically ventilated patients. The authors identified eight studies which included 1,765 patients. The authors reported that there was no association between tidal volume and mortality when the tidal volume was dichotomised at 7, 8, 10, or 12 mL/kg. The authors concluded that a relationship between tidal volume and mortality in mechanically ventilated children could not be identified, irrespective of the severity of the disease. They suggested that the significant heterogeneity observed in the pooled analyses necessitated future studies in well-defined patient populations to understand the effects of tidal volume on patient outcome.

The meta-analysis identified the difficulty in interpretations when the criteria of low and conventional (high) tidal volume varied. The population included did not meet uniform criteria as well. The study, therefore also highlighted lack of consensus as to what can be constituted as ideal low or high tidal volume in children.

2.5.4 Lessons from Neonatal studies

The tidal volume range has been suggested to be 4-8 mL/kg. Previous studies in newborn infants targeted this successfully (Piotrowski et al. (1997), Sinha et al. (1997), Courtney et al. (2002)). However, there is limited data to guide clinicians whether low or high normal tidal volumes should be targeted (van Kaam (2011)).

Historically studies using volume targeted ventilation as performed by Piotrowski et al. (Piotrowski et al. (1997)) and Sinha et al. (Sinha et al. (1997)) have used a tidal volume of 5-8 mL/kg.

The lower end of tidal volume has been extrapolated from studies in children, but as shown by Sly et al. (Sly et al. (2013)) in their study on infant rats, this strategy of employing low tidal volume is not without its shortfalls.

Some uncontrolled studies using volume targeted ventilation have reported that using high V_t (5–6mL/kg) is more appropriate in the acute phase of RDS (Abubakar

and Keszler (2001),Keszler and Abubakar (2004)).

As discussed earlier; historically, pressure targeting has been the mainstay of providing support for children and young infants. While volume targeting was increasingly recognised in adult breathing support, it could not be tried because of a lack of technology. In 1983, Cote et al. (Cote et al. (1983)), in their in vivo study demonstrated that the use of adult circuits was very unreliable in providing the precise level of tidal volume as required in the paediatric population due to their highly compliant nature.

In one of the very first studies using "volume targeting," Courtney et al. (Courtney et al. (2002)), in their study compared high-frequency ventilation versus conventional SIMV using the tidal volume of 4-7 mL/kg. The study was remarkable in that researchers paid strict attention to expiratory tidal volume. The authors justified their insistence on maintaining a close watch and control based on evidence at that time in maintaining minute volume.

In a prospective interventional study on preterm infants <32 weeks and <2,000 grams by Mishra et al. (Mishra et al. (2003)), the authors reduced PIP from 20 to 10 while monitoring arterial pCO_2. The authors showed that despite the decrease in PIP leading to reduced V_t, pCO_2 was maintained due to increase in respiratory rate leading to maintenance of MV. They concluded that this demonstrated that given an opportunity, preterm infants have the aptitude to modulate their respiratory rate in response to changes in tidal volume.

In the crossover study by Herrera et al. (Herrera et al. (2002)), quoted earlier in VG section, authors also compared VG + SIMV(3 mL/kg) with SIMV+VG (4.5 mL/kg). In this study, peak inspiratory pressure and mean airway pressures were significantly lower during SIMV+VG 3.0 when compared to both SIMV and SIMV+VG 4.5. The authors noted that both groups maintained similar minute volume which could be attributed to an increase in spontaneous minute volume during SIMV+VG 3.0. Although there was no difference in arterial oxygen satura-

tion by pulse oximetry and transcutaneous carbon dioxide tension, transcutaneous carbon dioxide tension increased slightly during SIMV VG 3.0.

In this study, carbon dioxide tension increased by the end of the period babies were ventilated using lower V_t and rise was found to be non-significant. It could have resulted in statistically significant hypercarbia if the period of observation was longer or the study recruited more babies than in the trial. Also, it was noted that though the minute volume remained similar, with babies on 3mL/kg, spontaneous breaths contributed a higher proportion of total minute volume giving rise to concerns about whether a smaller tidal volume meant the baby had to work harder to achieve an acceptable tidal volume.

These studies brought the question of normal tidal volume of the term and preterm infants to the fore of ventilatory world.

Williams et al. (Williams et al. (2011)), in their prospective observational study aimed to find out the normal range of tidal volumes in term and preterm infants in 43 infants between 23-41 weeks gestation, chronological age 2-111 days. Out of 43, 23 infants were receiving CPAP and 20 were breathing spontaneously. The authors found that V_t ranged from 2.3–10.4 mL/kg.

Van Kaam et al. (van Kaam et al. (2010)) conducted a prospective international cross-sectional survey between April 2007 and May 2008 which included 535 infants (mean gestational age 28 weeks and birth weight 1,024 grams) in 173 European NICUs in 21 countries. This was a live collection of data over two predetermined days. The authors aimed to assess contemporary ventilation practices in newborn infants. To make sure that the results provided an accurate reflection of daily clinical practice, this study collected data on neonatal ventilation "live" at the bedside and not by questionnaire. The authors reported that tidal volumes were measured in 84% of the patients on conventional ventilation, with a mean tidal volume of 5.7 mL/kg (Figure 2.5). In 66% of the patients, the tidal volume was between 4-7 mL/kg and in 18% of the patients, the tidal volume was above 7mL/kg.

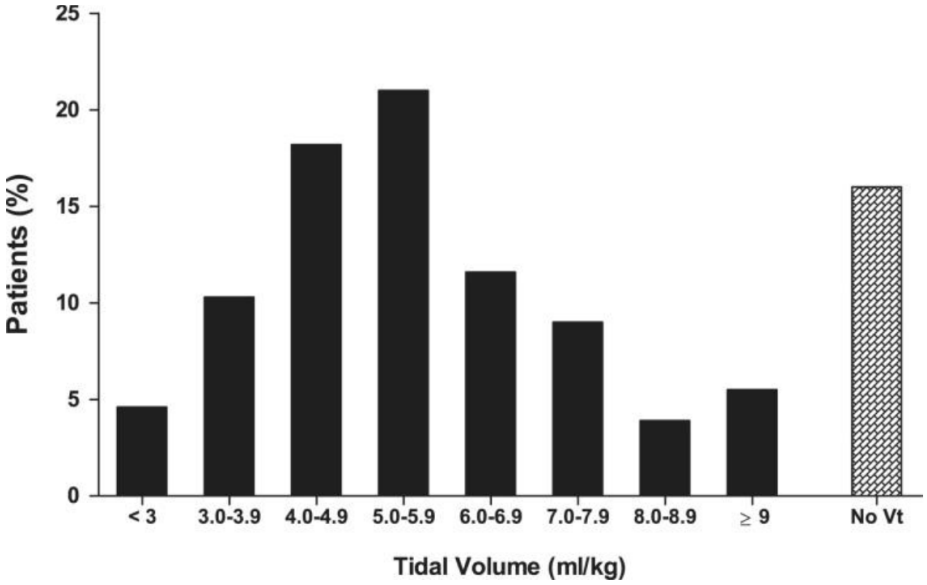


Figure 2.5: Distribution of the expiratory tidal volumes in patients on conventional mechanical ventilation (Adapted from van Kaam et al.) (van Kaam et al. (2010))

Mian et al. (Mian et al. (2015)) examined the temporal course of lung aeration at birth in preterm infants. The researchers observed tidal volume of the first 100 breaths of preterm infants on CPAP born at less than 33 weeks' gestation. The researchers found that babies' tidal volume for first 30 breaths, 20 breaths, and 50 final breaths was 5-6 mL/kg, 7-8 mL/kg and 4-6 mL/kg respectively. The authors suggested that targeting tidal volume to these normal values could be an effective means of regulating the administration of PPV to high-risk newborns that require respiratory assistance. Researchers excluded any breaths with mask leak $> 30\%$ as they would have underestimated the expired tidal volume.

Though the researchers aimed to examine the first 100 breaths, they did not include the initial breath before mask application which has the potential to limit its interpretation. Also, the criteria to apply CPAP rather than provide mask ventilation was arbitrary as well.

Whereas some authors have also observed variation in tidal volume requirement based on the age of preterm infants, Keszler et al. (Keszler (2009)) in their pro-

spective observational study showed an evolution of tidal volume requirement during the first three weeks of life in infants <800 grams ventilated with volume-guarantee. The authors measured tidal volumes matched with normocapnoea from birth until three weeks of life in preterm infants less than 800 grams and found that mean expiratory tidal volume rose from 5.15 mL/kg at birth to 6.07 mL/kg at three weeks of age. The increase in tidal volume occurred despite accounting for hypercapnoea.

Sharma et al. (Sharma et al. (2015)) in their retrospective, case-controlled, study compared tidal volume requirement of successfully ventilated newborn infants with meconium aspiration syndrome. To find out the tidal volume requirement, they used only those V_t values with the corresponding partial pressure of carbon dioxide between 35-60 mm Hg (4.6-8 kPa) were included. Mean V_t/kg and MV/kg were calculated for each patient. The control patients were the term and late preterm infants without meconium aspiration and were ventilated during the same period for severe lung diseases such as respiratory distress syndrome (RDS) or pneumonia.

Authors found that infants required V_t/kg (mean, SD) 6.11, 1.05 and 4.86, 0.77 mL/kg for MAS and controls respectively (p = < 0.0001). Similarly, the MV was different between the two groups as it was found to be 371, 110 (mean, SD) and 262, 53 (mean, SD) mL/kg/minute for MAS and controls respectively as well (p = <0.0001). The authors concluded that infants with MAS required 26% higher V_t and 42% higher MV compared with controls in order to maintain equal $paCO_2$. They felt that the need for larger V_t and higher total MV to achieve similar alveolar ventilation was consistent with the pathophysiology of MAS.

This study involved babies with meconium aspiration syndrome who have a different pathology to preterm infants with respiratory distress syndrome. This was also a single centre, a retrospective study based on written observations rather than electronic records. The MV was a calculated value rather than actual minute volume.

The same group (Sharma et al. (2015)) also reviewed tidal volumes of babies with the congenital diaphragmatic hernia and found no difference between the two groups.

Patel et al. (Patel et al. (2009)) carried out a prospective cross-over study on preterm infants to determine the impact of different tidal volumes on the work of breathing. The researchers recruited 20 preterm infants with a median gestation age of 28 weeks who were being weaned from respiratory support. They measured the transdiaphragmatic pressure-time product as an estimate of the work of breathing. They did this by measuring transdiaphragmatic pressure-time product first without volume targeting (baseline) and then at targeting tidal volumes of 4, 5, and 6mL/kg, delivered in random order. They found that work of breathing was least at a higher tidal volume of 6 mL/kg. The work of breathing at 6mL/kg was less than baseline itself.

The authors concluded that low volume-targeted levels increased the work of breathing during volume-targeted ventilation. They speculated that their results suggested that, during weaning, a higher volume-targeted level of 6 mL/kg, rather than a lower level, could be used to avoid an increase in the work of breathing.

In this study, the authors noted that at all tidal volumes, minute volume was maintained which was due to increased respiratory rates at lower tidal volumes which further explained the higher work of breathing at lower tidal volumes. As researchers did not go beyond 6mL/kg, they also speculated whether the work of breathing could be lower at tidal volumes higher than 6mL/kg.

Chowdhury et al. (Chowdhury et al. (2012)) evaluated work of breathing using tidal volumes of 4-6mL/kg in term or near-term babies (>34 weeks in gestation at birth) in their prospective crossover study. These infants were also studied first without V_t (baseline) and then at V_t levels of 4, 5 and 6mL/kg delivered in a random order. The researchers had similar findings as observed by Patel et al. in their study of premature infants, i.e., low V_t levels (4 mL/kg) during volume

targeted ventilation increased the work of breathing in ventilated infants born at term or near term.

The infants in this study behaved similarly to preterm infants in the previous study including maintenance of minute volume across the tidal volumes. The researchers did note that rather than compensations for minute volume by increasing respiratory rate, the infants in this study compensated by increasing the depth of their respiratory efforts and tidal volume exchange and hence their work of breathing was increased at the lower V_t level.

Lista et al. (Lista et al. (2006)) used the measurement of cytokine levels to compare the tidal volumes – 3 mL/kg to 5 mL/kg in their prospective RCT in preterm infants (25-32 weeks) in the acute phase of RDS. Researchers measured pro-inflammatory cytokines (interleukin-6 (IL-6), interleukin-8 (IL-8) and tumour necrosis factor alpha (TNF-α) in the tracheal aspirate on 1, 3, and 7 days' of life. They reported that IL-8 and TNF-α levels collected on day 7 were significantly higher in the low tidal volume group (tidal volume 3mL/kg). This group also had a longer duration of mechanical ventilation. The authors concluded that ventilation with V_t = 5mL/kg when compared with 3mL/kg, reduced inflammatory response as well as the length of ventilation. The study, however, did not assess whether the difference was related to the incidence of bronchopulmonary dysplasia in these infants.

Hence while the lower tidal volumes could limit volutrauma, the studies mentioned above by Patel, Chowdhury and Lista et al. suggest that such low tidal volumes, in turn, can lead to increased work of breathing or raised inflammatory markers.

In summary, while volume targeting appears to be superior to pressure targeting, it is not clear what the optimal V_t set is on the volume guarantee hybrid modes widely used across the world. This remains a vital question that needs investigation.

Chapter 3

Aims and Objectives

The invention of a mechanical ventilator, while helping to improve survival in babies, also gave rise to debate about ventilator associated lung injury in the form of barotrauma or volutrauma (Tremblay and Slutsky (2006)). Since then, there have been debates about which of two were more harmful. Legendary studies by Dreyfuss and Hernandez (Dreyfuss et al. (1988), Hernandez et al. (1989), Dreyfuss and Saumon (1998)) demonstrated the superiority of volume targeting in animal models. This triggered the debate of this being clinically applicable in the area of ventilation. Their research inspired studies in babies with respiratory distress syndrome and in these, volume targeted ventilation came out as a better mode of ventilation than a pressure targeted. This was also supported by the studies as well as meta-analyses (Herrera et al. (2002), Keszler and Abubakar (2004), McCallion et al. (2008), Klingenberg et al. (2017)).

The development of microprocessor technology and the establishment of volume targeting as the better mode helped to develop the next generation of hybrid modes of ventilation. These help to deliver precise tidal volume to very small babies. Arguably, volume guarantee is the most studied and commonly used hybrid mode in ventilating extremely preterm infants with respiratory lung disease. In view of this overwhelming evidence in support of volume guarantee, its uptake has been rapid. This has led to the question of ideal tidal volume for babies which has been

addressed by different studies in animals, adults and children.

In view of this overwhelming evidence in support of volume guarantee, its uptake has been rapid. This has led to the question of ideal tidal volume for babies which has been addressed by different studies in animals, adults and children.

Historically, studies using volume targeted ventilation as performed by Piotrowski et al. (Piotrowski et al. (1997)) and Sinha et al. (Sinha et al. (1997)) have used a tidal volume of 5-8 mL/kg. Some researchers have tried to establish the normal tidal volume for term and preterm infants. Williams et al. (Williams et al. (2011)) in their prospective observational study of non-ventilated term and preterm infants found the normal range of tidal volumes in term and preterm infants to be 2.3-10.4 mL/kg. Whereas in their cross sectional study of ventilated preterm infants, van Kaam et al. (van Kaam et al. (2010)) found that in 66% of the patients the tidal volume was between 4-7 mL/kg.

As demonstrated by studies in adults and children, the efforts to compare the tidal volumes have also disclosed varied ranges under the definition of low and high tidal volumes. Most of these studies were also limited by their design in being either cross sectional or focussed on very limited clinical aspects.

Understanding these limitations, studies in newborns so far have shown that

- Low tidal volume fared better in providing breathing support with lower airway pressures (Herrera et al. (2002)).
- There were no differences in maintained minute volume, arterial oxygen saturation by pulse oximetry, transcutaneous carbon dioxide tension and fraction of inspired oxygen (Herrera et al. (2002)).
- High tidal volume fared better in providing support with lower work of breathing (Patel et al. (2009), Chowdhury et al. (2012)), reduced inflammatory response and length of ventilation (Lista et al. (2006)).

In the absence of clear evidence, experts have suggested the tidal volume range to be 4-8 mL/kg but there is limited data to guide clinicians as to whether low or high normal tidal volumes should be targeted (van Kaam (2011)).

In summary, while volume targeting appears to be superior to pressure targeting, it is not clear what the optimal V_t set is on the volume guarantee hybrid modes widely used across the world. This remains a vital question that needs investigation.

Aims

Through this study, we aimed to ascertain an improved way to provide respiratory support to premature babies. By targeting the ideal tidal volume, we believed that we would be able to maximise the efficacy of ventilation while minimising the adverse effects due to ventilation induced lung injury

Objectives

In order to compare high normal versus low normal tidal volumes we set out to study the differences between: the time to achieve 25% reduction in peak pressure (the primary outcome).

Respiratory distress syndrome in very low birth preterm infants is characterised by poorly compliant lungs. Preterm infants with significant respiratory distress syndrome therefore need mechanical ventilation support with high peak pressures reflective of poor compliance at time of birth. As the lungs improve, the compliance gradually increases enough for them to be able to breathe with no or non-invasive support.

Peak inspiratory pressure is a quantitative marker of compliance in presence of a constant tidal volumes. In traditional pressure targeted ventilation, with improvement in compliance, a baby's ventilatory support is gradually reduced by manual reduction of peak inspiratory pressure. As volume guarantee works by using a feedback mechanism, the peak inspiratory pressure is tailored to the set tidal volume. Similar to pressure targeted ventilation, this peak inspiratory pressure is initially high to be able to deliver the required tidal volume. But contrary to pressure tar-

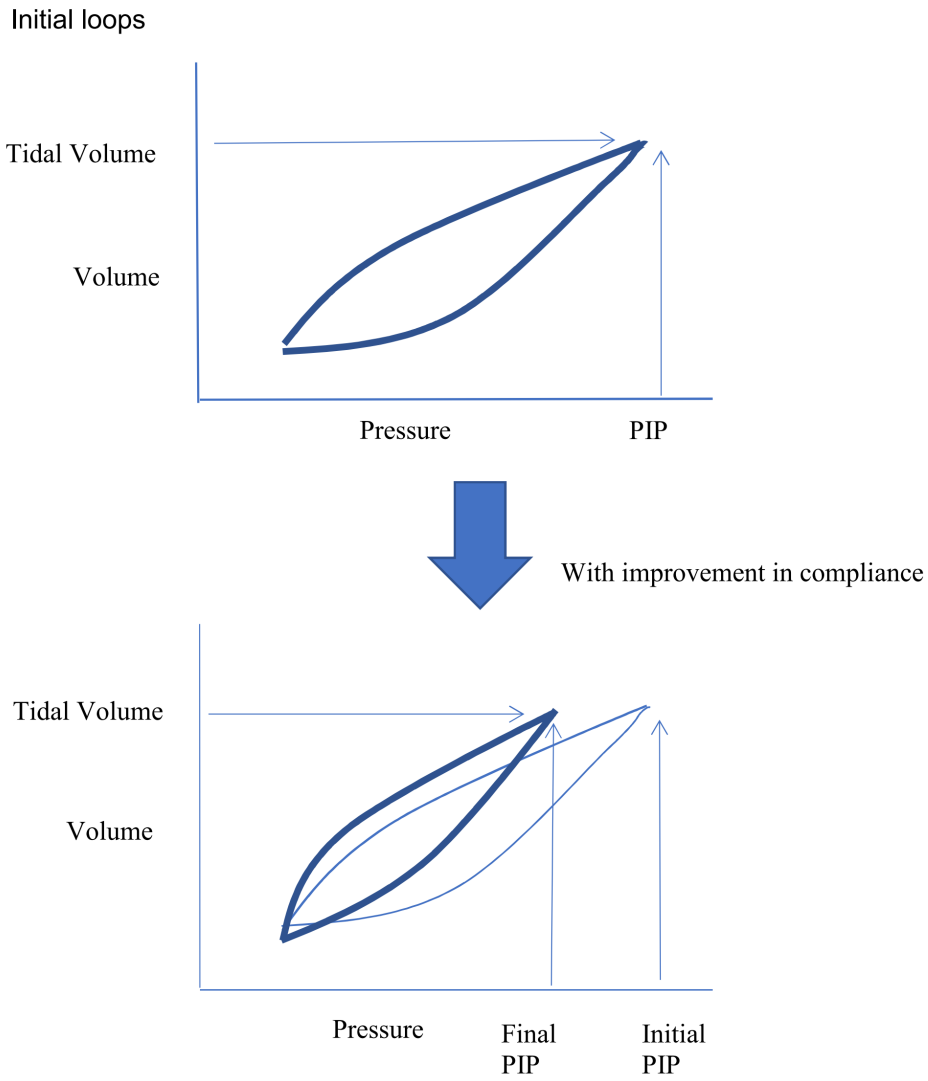


Figure 3.1: Pressure volume loops

geted ventilation, in volume guarantee, with improvement in compliance, peak inspiratory pressure reduces gradually, a phenomena also dubbed as "auto-weaning". Pressure and volume loops demonstrate this phenomena very well(Figure 3.1).

As the study was conducted in a real life clinical setting, it was necessary for us to have an objective parameter to assess the impact of the randomisation arm. Due to lack of any previous studies, we chose a 25% drop in peak inspiratory pressure as the primary outcome, as in our experience, peak inspiratory pressure at extubation

was 75% of initial PIP. Primary outcome was achieved when initial PIP dropped to final PIP by 25% hence it can be mathematically explained as

End point PIP => Final PIP = 3/4(Initial PIP)

We also set out to study the following clinically relevant parameters as our secondary outcomes.

- Duration of mechanical ventilation
- Incidence of pulmonary and non-pulmonary complications
- Survival to discharge without significant bronchopulmonary dysplasia

As this was a pilot project, we also aimed to evaluate the operational feasibility of such a study for potential roll out at a larger, multicentre level.

Research Question (with null hypothesis): Null hypothesis:

There is no difference in the time needed to achieve 25% reduction in peak pressure using either 7-8 mL/kg or 4-5 mL/kg tidal volume.

Alternative hypothesis:

The time needed to achieve a 25% reduction in peak pressure in very premature babies receiving mechanical breathing support using volume-targeted ventilation is less using high normal tidal volume (7-8 mL/kg) as compared to low normal tidal volume (4-5 mL/kg).

Chapter 4

Methodology

4.1 Setting

This study was conducted at the neonatal unit of the University Hospital of North Tees, Stockton-On-Tees in the United Kingdom. It was one of four level 3 neonatal units in North-East England. It had 3500 deliveries per year and 80-100 admissions to the intensive care unit for ventilator support. While most of these admissions were from in-house deliveries, 10-20 babies per year were admitted after ex-utero transfer having been born in one of the regional level-1 district general hospitals or other level 3 units if they surpass their capacity. The majority (80%) of these babies were born premature and needed intensive care and breathing support for immature lungs. These babies were born to mothers who either went into early labour (maternal indication) or had to be delivered for poor growth (foetal growth restriction also known as intra-uterine growth retardation). For this pilot study we aimed to enrol 70 babies from an expected cohort of 90-100 eligible babies admitted during the study period.

4.1.1 Subjects

We enrolled extremely low birth weight babies between 500 to 1500 grams or less than 32 weeks completed weeks' gestation at birth.

4.1.2 Intervention

4.1.2.1 Identification of potential recruits

To study the impact of intervention on respiratory distress syndrome, we randomised the babies when they were commenced on ventilation. All of these babies were ventilated soon after birth or in the first 12 hours of life. As a standard practice, when eligible mothers arrived in labour, they received information on premature delivery from the obstetric team. The neonatal team was also informed who in turn counselled mothers and their families about what to expect after a preterm delivery. Depending on the mother's condition, information about research on the unit and this trial was provided and they were invited to consider if their baby could be included in the studies.

For our study, we provided verbal information to prospective mothers along with a patient information leaflet. (appendix 1). Once born the preterm babies were either intubated from birth depending on the condition or managed on non-invasive respiratory support as per standard practice. If babies on non-invasive respiratory support were deemed to be struggling with spontaneous breathing, they were intubated with an appropriate sized tracheal tube. The tracheal aspirate in all babies was collected before ventilation (after intubation).

4.1.2.2 Inclusion Criteria

1. Preterm babies weighing 500-1500g
2. Requirement of intubation and mechanical ventilation

4.1.2.3 Exclusion Criteria

1. Serious underlying congenital anomaly
    - Congenital diaphragmatic hernia

- Cyanotic congenital heart disease
- Airway anomalies
- Abdominal wall defects

2. Babies initiated on ventilation after 12 hours of life or transferred from other centres.

For babies born of multiple pregnancies – only the first-born was randomised; the others received the same strategy.

4.1.2.4 Collection of tracheal aspirate

Tracheal aspirate was collected by following method: Materials used – 2.5 ml syringe, needle, trachea suction set (unomedical), 5 ml saline vial and suction catheter (Figure 4.2)

Steps:

1. 1.0 ml saline was aspirated into the syringe using needle.
2. This saline then was instilled through the endotracheal tube.
3. The baby received intermittent positive pressure ventilation for 30 seconds.
4. Using the suction catheter size 5-6, the endotracheal aspirate was sucked from the endotracheal tube. The aspirate by this procedure was collected in the suction bottle of the trachea suction set.
5. As there was aspirate in the catheter as well, this was flushed into the suction bottle by using the remaining saline in the ampoule (4.0 ml) which helped to standardise the volume of the aspirate.
6. This suction bottle was then transferred to hospital research freezer and stored at -80 degree C temperature. The sample was stored in the freezer until it was processed.

7. These samples were then assessed by the research scientist for TNF-α, IL-6 and IL-8.

The baby then received 200 mg/kg surfactant (poractant alpha®) through the endotracheal tube.

4.1.2.5 Randomisation

The baby was then brought to the neonatal unit and weighed by the nursing team. Once the team confirmed that the baby had fulfilled the criteria for the trial, baby was randomised by opening the envelope before commencing the ventilation.

Assignment of the ventilation mode (low tidal volume or high tidal volume) was done by computer generated block randomisation using opaque sealed envelopes. This randomisation sequence was created by a member of research team not directly involved in the trial and was kept hidden from the clinical and researchers directly involved in the trial. Aim of the block randomisation was to keep the numbers of the subjects in the two groups closely balanced at all times. Block randomisation was done with a fixed block design of four per block. Study babies were stratified into two groups – Babies weighing $\leq$1000 grams and >1000 gram.

Block sizes of four with the following sequences for the two stratification groups were used. 'L' for 'Low TV' and 'H' for 'High TV' $\leq$1000 grams birth weight HHLL LHHA HLHL LLHH LHLH HLLH > 1000 grams birth weight LHHL LHLH HHLL HLHL LLHH HLLH

Two sets of envelopes (one for each birth weight strata) within opaque, sealed, serially numbered envelopes in the above sequence were created. Babies were weighed just after admission to the unit and when they were ready for randomisation, they were allocated to the trial group according to the above sequence. The randomisation envelopes were not drawn or opened by the principle researcher. The envelope was drawn by the clinician caring for the baby or a member of the clinical team. No

attempts were made to blind the clinicians to the assigned group of tidal volume group as it was not practically feasible.

The baby's ventilator was then set as per defined tidal volume as follows ≤1000 gram – low – 4ml/kg; high 7 ml/kg >1000 gram = low – 5 ml/kg; high 8 ml/kg

The baby was then ventilated as per the protocol as in appendix D.

4.1.2.6 Initial ventilation strategy

The attending senior doctor (registrar or consultant) set the ventilator (Avea® or Dräger®) (Figure 4.1) as follows

- Mode: Pressure Assist control (Pr AC) with volume guarantee (VG)
- Rate – 40/min
- PEEP – 5cm H2O
- Ti - ≤1000 gram – 0.26 second; >1000 gram – 0.30 seconds
- FiO_2 – as per the requirement
- Pmax – initially 50 and then readjusted to 10 cm above the required peak inspiratory pressure.

4.1.2.7 Management of baby in the unit (appendix D)

Then baby was assessed by the attending doctor for respiratory and cardiovascular status by checking saturations, heart rate, blood pressure, capillary refill time, air entry, heart sounds and peripheral pulses. Following assessment, peripheral intravenous access was obtained and a blood sample sent for full blood count, culture and group and save and blood gas. As these babies were going to require a stable central access, the attending doctor secured umbilical venous and arterial access. If arterial access was not possible or not attempted then blood gas analysis

was performed on capillary blood samples obtained from heel prick. A chest x-ray for obtained to assess lung condition, position of tracheal tube and position of umbilical catheter tips.

A blood gas (capillary or arterial) sample was obtained in first two hours to gauge the status of ventilation by ascertaining pCO_2. If the pCO_2 was within normal limits (i.e., between 4.5-6.5 kPa) the ventilation was continued unchanged. If the pCO_2 was low the mode of ventilation changed to Pressure SIMV with volume guarantee with rate of 40/min. If the pCO_2 was high then respiratory rate was increased in steps of 10 until a rate of 60 was achieved. If the baby continued to have raised pCO_2 the baby was taken out of the trial and switched to high frequency oscillation.

The oxygenation was optimised by adjusting PEEP. The level of oxygenation was ascertained by maintaining a saturation of 90-95% and watching the required oxygen content (FIO_2) to deliver that. If the oxygen requirement was more than 0.4 then PEEP could be increased to 6 cm H2O and if the oxygen requirement was less than 0.3, PEEP could be brought down to 4 cm H2O.

4.1.2.8 Criteria for extubation

With the progress of time, the ventilator requirements of the baby reduced. This was ascertained by pCO_2 on blood gases. Once we had established hyperventilated blood gases, the baby was deemed to be weanable and switched onto Pressure SIMV (with VG with original tidal volume). With continuing improvement, the rate on SIMV reduced in steps of 10 until the baby was deemed to eligible for extubation if they met extubation readiness criteria -

(a) Mean airway pressure was less than 8.

(b) Baby was initiating more than 50% of his breaths (good respiratory drive).

(c) Baby was not on any sedation and had received a loading dose of caffeine.

4.1.2.9 Minute ventilation test (appendix E)

Once the baby met extubation readiness criteria (as above), the minute ventilation test (appendix E) was carried out to predict the success of extubation.

Whilst on SIMV, data was recorded each minute for five minutes for heart rate, respiratory rate, oxygen requirement (FIO_2) to maintain normal saturation (90 to 95%) and minute ventilation VE (SIMV mode). Then whilst on SIMV mode, control rate and pressure support was turned to zero (i.e., CPAP mode) and data was recorded for every one minute for five minutes for same parameters (i.e., heart rate, respiratory rate, oxygen requirement (FIO_2) to maintain normal saturation (90 to 95%) and minute ventilation VE) Baby was considered to have 'passed' the test if

(a) The CPAP minute ventilation was >50% of SIMV mode.

(b) The rise in CPAP mode FIO_2 was less than <10

(c) There was no significant episode of bradycardia.

If the baby did not meet the pass criteria, he/she continued SIMV ventilation and the test was repeated every 6-12 hours till he/she passed the test.

4.1.2.10 Consent

Despite waiver of consent allowing us to randomise baby when considered eligible, we obtained written informed consent from parents or a person with parental responsibility.

Every effort was made to approach parents before delivery and provide them patient information leaflet (appendix B).

In the trial run before commencement of the study, we realised that it was not practically feasible to obtain consent because baby would only meet the second essential

criteria of needing intubation and ventilation after birth. Hence, unless parents had expressed a desire against the trial before baby's birth, we decided to approach for consent in postnatal period once we were certain of the eligibility. Parents were approached within 12 hours of randomisation. They received at least 24 hours to read and understand the patient information leaflet. Once they expressed desire to provide consent, it was obtained within 72 hours of randomisation.

Postnatally after randomisation, at an appropriate time, parents were approached by myself or another Good Clinical Practice (GCP) competent member of staff for written consent. Parents had opportunity to ask questions or clarifications about any aspect of the study before signing the consent form (appendix C). Reasons for non-enrolment of the eligible babies were included in the enrolment log. (Figure 5.2)

4.1.2.11 Exit Criteria

Babies were discontinued on volume guarantee ventilation and switched to high frequency oscillatory ventilation (HFOV) if any of the following occurred

- Inadequate ventilation (pH <7.25 and pCO_2 >8 kPa)
- Inadequate oxygenation (FIO_2 >0.6 to maintain SpO_2 >91%)
- Clinical deterioration: Significant pulmonary haemorrhage or air leak

4.1.3 Primary outcome

Time to reach 25% reduction in initial PIP.
As our primary outcome was on 25% reduction in initial peak inspiratory pressure (PIP), I noted the initial PIP by averaging PIP from first four hours and deduced the time when average of four hours was about 75% of the initial PIP.

4.1.4 Secondary outcome

As part of the study we also collected data for secondary outcomes as follows

- Time taken for extubation (duration of mechanical ventilation)
- Efficacy of ventilation - Maintenance of stable pCO_2 till primary outcome (range 4.5-6.5 kPa)
- Pulmonary complication –
  - Immediate - air leak (Pneumothorax or pulmonary interstitial emphysema)
  - Delayed - Bronchopulmonary dysplasia
- Non-pulmonary complications –
  - Sepsis
  - Necrotising Enterocolitis (NEC)
  - Patent ductus Arteriosus (PDA) requiring medical/ surgical treatment
  - Intraventricular Haemorrhage (IVH)
  - Periventricular leukomalacia (PVL)
  - Retinopathy of prematurity (RoP)
  - Weight gain
  - Survival to discharge including survival to discharge without BPD

Though not part of secondary outcome, we also analysed the total duration of respiratory support and duration of admission and ventilatory parameters to cover all possible relevant clinical parameters for clinicians.

4.2 Definitions of Secondary outcomes measured in this study

4.2.1 Time taken for extubation (duration of mechanical ventilation)

This was calculated as the period of invasive breathing support on mechanical ventilator until extubation. To standardise the criteria for extubation, it was considered only after meeting pre-defined criteria and once criteria were met, minute ventilation test (appendix E) was used to determine whether baby could be extubated.

4.2.2 Efficacy of ventilation –

Maintenance of stable pCO_2 till primary outcome (range 4.5-6.5 kPa)

The babies were monitored for efficacy of ventilation by performing pCO_2 assessment on a regular basis. To mimic clinical conditions, the frequency of pCO_2 assessment was determined by the attending team. After admission to the unit, an arterial line was attempted. When it was not possible, capillary and venous bloods were used for blood gas assessment. These values were then corrected to make them equivalent to arterial result (arterialised). The method to arterialise was used from the articles by Zavorsky and Byrne (Zavorsky et al. (2007), Byrne et al. (2014)).

4.2.3 Pulmonary complications

4.2.3.1 Immediate: significant air leak (Pneumothorax or pulmonary interstitial emphysema)

Significant Pneumothorax was defined as radiologically conformed intrathoracic, extra pulmonary air needing intervention in the form of needle thoracocentasis

or chest drain. Pulmonary interstitial emphysema was considered significant if it warranted change in ventilatory strategy to high frequency ventilation. All the x-rays were reported by a Paediatric radiologist who was blinded to the category of randomisation.

4.2.3.2 Delayed:Bronchopulmonary dysplasia (Ryan (2006)

)

- Mild BPD - supplemental oxygen for ≥28 days and on room air at 36 weeks' postmenstrual age (PMA) or at discharge (for infants <32 weeks at birth) or at 56 days or at discharge (for infants ≥32 weeks at birth).
- Moderate BPD - supplemental oxygen for ≥28 days and a need for supplemental oxygen <30% at 36 weeks PMA/discharge (for <32 weeks) or at 56 days/discharge (for infants ≥32 weeks)
- Severe BPD - supplemental oxygen for ≥28 days and a need for ≥30% oxygen or on nasal CPAP or mechanical ventilation at 36 weeks PMA/discharge (<32 weeks) or at 56 days/discharge (≥32 weeks).

4.2.4 Non pulmonary complications

4.2.4.1 Sepsis

Sepsis was subdivided into early onset (<72 hours) and late onset sepsis (>72 hours). An early onset sepsis was considered significant when either the blood culture was positive or baby was symptomatic with signs of sepsis or there were significantly raised inflammatory markers (CRP/WBC). A late onset sepsis was considered when there was a true nosocomial infection.

4.2.4.2 Intraventricular Haemorrhage and periventricular leucomalacia

All babies underwent routine ultrasound scan of head at 1st and 6th week of life. This was performed by Paediatric radiologist in the unit. The ultrasound images consisted of images in coronal plane from frontal lobe to occipital lobe and in sagittal plane from left to right including midline.

Intraventricular haemorrhages were scored according to the Papile classification (Papile et al. (1978)).
Grade 1 – Sub-ependymal haemorrhage only
Grade 2 – Intraventricular haemorrhage without ventricular dilatation
Grade 3 - Intraventricular haemorrhage with ventricular dilatation
Grade 4 - Intraventricular haemorrhage with parenchymal extension
Periventricular leukomalacia (PVL)- It was scored as no PVL or cystic periventricular leukomalacia

4.2.4.3 Patent ductus Arteriosus (PDA)

Patent ductus arteriosus was diagnosed on the basis of clinical signs (murmer and hyperdynamic circulation) and colour Doppler echocardiography performed by personnel trained in neonatal echocardiography using Philips HD11TM , 5-12 MHz probe.

A significant PDA (Kluckow and Evans (1995)) was defined as any duct requiring medical or surgical closure based on findings of echocardiography of

- Left atrium: aortic root ratio > 1.4
- Pulse wave Doppler showing turbulent flow in the main pulmonary artery with retrograde diastolic flow in mesenteric blood vessels

- Duct diameter of > 1.5 mm measured at its attachment with the pulmonary artery

Baby's clinical condition was also taken into consideration while determining whether the baby would benefit from treatment for significant PDA.

4.2.4.4 Necrotising Enterocolitis

A significant necrotising enterocolitis was recorded as one meeting criteria of Bell stage 2 or worse (Walsh and Kliegman (1986)) on clinical presentation, radiological appearance with or without surgical or autopsy confirmation.

4.2.4.5 Retinopathy of prematurity

Significant retinopathy of prematurity was defined as "threshold disease" defined as stage III ROP present in eight cumulative clock hours or five contiguous clock hours with plus disease (presence of tortuous vessels) in zone I or II (International Committee for the Classification of Retinopathy of (2005)). Ophthalmologic examination were performed by designated paediatric ophthalmologist as per British Association of Perinatal Medicine (BAPM) recommendations.

4.2.4.6 Weight gain

The weight gain from admission to discharge was obtained and weight gain per kg/day was calculated. In addition, birth weight z scores were obtained using Fenton charts (Fenton and Sauve (2007)). Fenton's z scores were also calculated for discharge weight and a difference in birth and discharge score was also calculated.

4.2.4.7 Sample Size

We aimed to recruit 70 babies – 35 in each arm. As this was a pilot study, the sample size was calculated on the clinically important difference between the two

study groups of 50%.

In addition to the above protocol following procedures were applied as well.

4.3 Adjunctive treatment

4.3.1 Surfactant

Babies received an additional dose of surfactant (poractant alpha®) at twelve hours after the first dose, if the baby was still requiring mechanical ventilation, and had an oxygen requirement >30% and/or mean airway pressure was >10 cm H_2O. Some of the babies received an extra dose on top of the additional dose at the discretion of the attending consultant. The total dose of surfactant was recoded on the proforma and used for multiple regression analysis.

4.3.2 Caffeine

All babies were commenced on caffeine immediately after intubation and stabilisation. The babies received a loading dose of 20 mg/kg caffeine citrate followed by a maintenance dose of 5-10 mg/kg/day until 34 weeks of corrected gestation.

4.3.3 Ibuprofen

If baby was diagnosed to have clinically significant PDA as per pre-defined criteria and there was no contraindication for medication then baby received ibuprofen for closure of duct.

4.3.4 Dexamethasone

Dexamethasone was used when a baby was considered “ventilator dependant” i.e., a baby who was ventilated for at least two weeks of life and/or had failed trial of extubations. These babies were considered high risk of developing bronchopulmonary

dysplasia. The dose and duration of dexamethasone in this situation was according to DART regime (Doyle et al. (2006)): twice daily dose of a 10-day tapering course of dexamethasone sodium phosphate (150 microgram/kg /day for three days. 100 microgram/kg/day for three days, 50 microgram/kg/day for two days and 20 microgram/kg/day for 2 days and discontinue. Dexamethasone was also used when a baby was suspected of having "laryngeal oedema". In this situation baby received three doses of 250 microgram/kg at 12 hour intervals. This could be started before or after the extubation based on the clinical circumstances.

4.3.5 Bronchodialators and long term diuretics (>7 days)

These were not used in enrolled babies.

4.4 Routine clinical care and monitoring

Routine care and monitoring was done according to unit protocols. The baby was formally reviewed at least twice a day by a senior neonatal doctor. The baby was monitored continuously and his/her observations were recorded on an ITU chart. These observations included vital parameters, ventilatory settings, blood gases and pain chart. Any changes in the clinical management were formally recorded on the charts as well. The regular monitoring continued even after extubation but the frequency of observations reduced to four hours while on non-invasive support. When baby graduated to special care, concentration was more focussed on apnoea or desaturation episodes along with feeds and growth.

The babies also underwent mandatory screenings as per unit policy. This included cranial ultrasound scan at 1st and 6th week of life. Babies had echocardiography performed if there was suspicion of poor cardiac function or signs and symptoms suggestive of PDA. The babies also ROP screening as per BAPM guidelines and had hearing screen and formal newborn examination before discharge home.

4.5 Duration of study

39 months – From July 2013 to September 2016

4.6 Data collection

The data for the study was collected from direct observation, regular observations as performed by the staff recording regular observations and from case notes, for demographic information. It was initially entered into paper proformas (appendix F) which were then transferred over to spreadsheets on password protected computers complying with data protection guidelines. The relevant data then were stored in Excel on NHS research computers. The computers were used for research purposes only and are password protected. The research office, where the computer was stored, could only be accessed by a member of research team. Accuracy of the transcription of the case report forms onto the electronic database was checked by reviewing all the records before final analysis.

4.7 Data Analysis

All analyses were conducted on data from the intention-to-treat population, which included all patients who underwent randomisation. The normality of data was assessed using the Kolmogorov-Smirnov statistic. For time dependant variables, survival statistic was used. Log rank test using Kaplan Meier survival curve was used for the primary outcome. This was also used for other time to event analysis like duration of mechanical ventilation and duration of hospital stay (admission). Multiple logistic-regression analysis using cox-regression was used to identify relevant baseline covariates associated with the primary outcome, in addition to the stratification variables. Variables tested in the model were selected if the p value was less than 0.20 and if they were clinically relevant.

A chi-square test (or Fisher's exact test, when appropriate) was used for secondary binary outcomes.

Continuous variables were compared with the use of an unpaired t-test or the Mann–Whitney U (Wilcoxon rank sum) test. Adjusted analyses were performed with the use of the same adjustment variables that were used in the linear-regression model.

All analyses were conducted with the use of spss® software, version 20 (IBM). A two-sided p value of less than 0.05 was considered to indicate statistical significance. The statistical analysis was supervised by Mr Kasim Adetayo, research statistician, Durham University.

4.8 Ethics approval

REC Reference No: 13/NE/0110 (appendix A)
The study was approved by the NHS research ethics committee, the hospital research and development peer review committee and Durham University. Though the "waiver of consent" allowed us to randomise babies before formal consent, the baby was formally included and data collected only after a written informed consent from the parents or a person with parental responsibility was obtained. All parents received patient information leaflet (appendix B) along with verbal explanation and had at least 12 hours to read and discuss with partners if they wished so. A complete confidentiality was maintained. Apart from data being stored in locked research computers, all patients received a study number to obviate the need to use name or other identity parameters of both baby as well as parents.

4.9 Roles

With the help of other team members, I identified the potential mothers whose babies could be recruited in the trial. I then approached the mums with information

of the trial with patient information leaflet. After baby' birth, I obtained tracheal aspirate before intubation. I ensured that baby was randomised after admission to the unit by one of the members of clinical team. If the consent was not obtained so far, I approached parents for written informed consent. I collected data on paper proforma. In my absence, the attending neonatal registrar and consultant were responsible to identify the potential recruits, speak to parents about the trial and obtain consent. In my absence they were also responsible for collection of tracheal aspirate, randomisation procedure and for the clinical monitoring of the baby.

4.10 Equipment

The study needed use of mechanical ventilation which was provided by Drager™ Babylog C500 and Avea™ Viasys Carefusion ventilators (Figure 4.1). These were machines owned by the neonatal department, hence no additional funding or any financial support was received with regards to ventilators. We did, however, successfully apply for a local research and development department grant to be able to process the tracheal samples for inflammatory markers.

Measurements of Tidal Flow and Volume Direct measurement of tidal by respiratory flow signals have most commonly been obtained using a pneumotachograph (PNT) connected in series to a silicon mask encompassing the infant's nose, alone or together with the mouth.

We ensured minimisation of technical dead space (ideally, <2 ml/kg) (Frey et al. (2000)) is critical, since elevation of CO_2 consequent to dead space or apparatus resistance may alter control of breathing and affect the tidal flow waveform.

The flow through technique enables acquisition of tidal flow data through an apparatus with minimum dead space. Differential flow occurring between a pneumotachograph placed before and after the airway opening is measured and is ideally suited to studies in small (<2 kg) infants.

4.11 Co-intervention and contamination

Contamination was avoided by not enrolling the baby in any other respiratory trial until the primary outcome was reached. According to protocol, no cross over was allowed during the study.

4.12 Pictures of equipment

The picture of the ventilatory equipments used in the study is shown in the Figure 4.1 (a) and Figure 4.1 (b). The equipment used to collect tracheal aspirate has been depicted in Figure 4.2.

Protocol - Please refer to appendix D

(a):Dräger C500 (b): Avea ventilator

Figure 4.1: The Ventilators

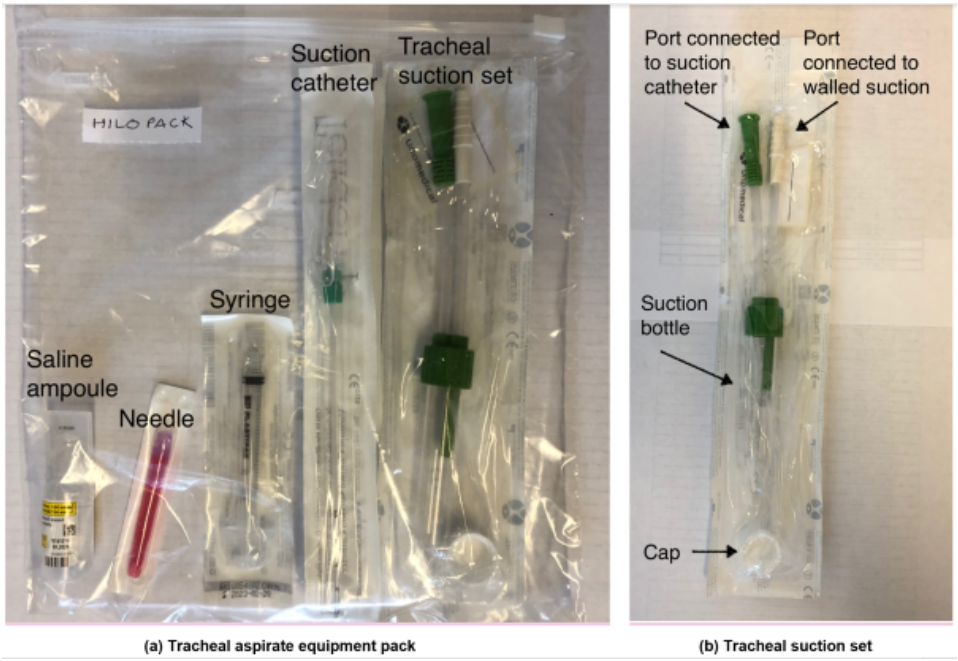


Figure 4.2: Tracheal aspiration equipment

Chapter 5

Results

5.1 Introduction

The study was carried out between July 2013 and September 2016. During this period, a total of 262 babies were screened of which 97 were eligible for enrolment into the study. Of these, 70 babies were randomised (Figure 5.1). The remaining 27 babies could not be randomised for the reasons highlighted in Figure 5.3. All the babies randomised to the study were confirmed to be eligible and their complete data was available for analysis.

The enrolment commenced in July 2013 and was completed in September 2016. During the time of the study, there were 265 babies in the eligible group (born at less than 32 weeks or birth weight less than 1500 grams) who were admitted to the unit. Out of these, 152 had required intubation and ventilation within first 12 hours of life. As 55 of these were ex-utero transfers whereby either the neonatal team of the referral unit or the transport team had already intubated the babies and commenced them on mechanical ventilation. That left 97 babies for possible recruitment. Out of these 70 babies were randomised and consented by parents. Out of these 70, 55 achieved primary outcome. Eleven babies died before discharge. 9 babies died when still ventilated

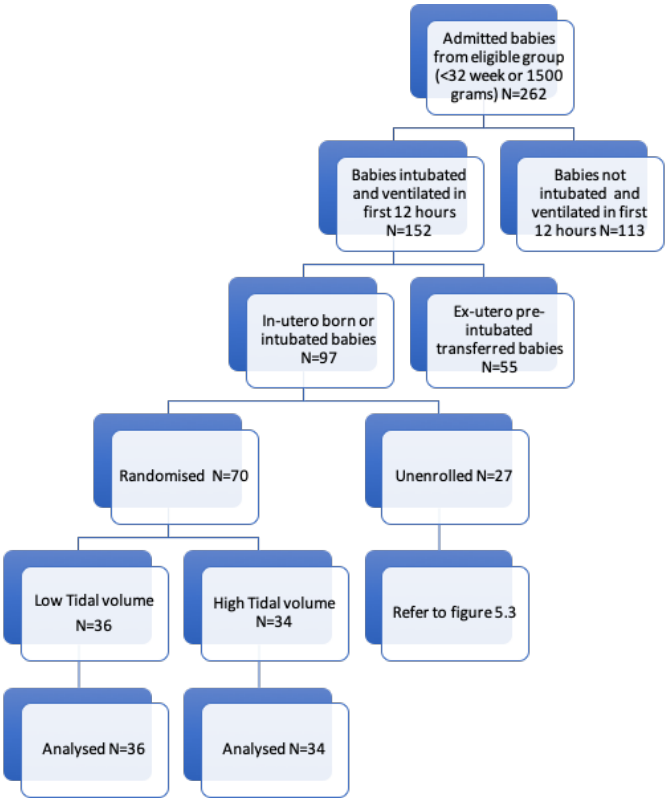


Figure 5.1: CONSORT diagram showing recruitment and randomisation of babies

5.1.1 Projection versus enrolment

The study was commenced on 18th July 2013. Experience of the department over the previous 12 months indicated that there were 90 babies admitted from the eligible cohort of babies born at less than 32 weeks or birth weight between 500-1500 grams. Out of these 45 babies were admitted for respiratory distress syndrome within 12 hours of age. As 6 of them were ex-utero transfers who arrived intubated and ventilated this made 39 babies eligible for the trial. As we set off for the numbers required for the trial and time frame, it became apparent that about 26 months were required to complete the enrolment keeping in mind various exclusions and consent refusals. To achieve a sample target of 70 babies, the recruitment was projected to be at 3 babies per month. This was based on trends in the rate of admission to the unit over preceding 12 months. The target sample size was

reached over a period of 38 months.

Over the period of 26 months, we recruited 55 babies. Therefore we needed to continue the trial until September 2016 whereby we managed to enrol 70 babies. The graph of projection versus enrolment is shown in Figure 5.2.

To further explain this, we collated the figures for each category every year and plotted them for the duration of preparation and performance of the study. These figures combined with the total admissions of babies with low birth weight in the neonatal unit presents a further interesting observation as in Table 5.1 Figure 5.4. On close scrutiny, there was a trend of the number of extremely low birth weight infants going down with less number of babies getting intubated hence being eligible for the trial.

A further breakdown of missed babies (Figure 5.3) was also carried out. As severe congenital anomaly was one of the excluding criteria, two babies were not randomised because of their antenatal history. In 10 instances, the admitting team used their own judgement and as there was a possibility, in view of pre-existing requirement of oxygen or difficulty in ventilating the baby, the team decided to not to recruit the baby. Such a clinical condition in most cases led to use of high frequency mode of ventilation. This was our rescue mode for this trial which again vindicated their decision. On some occasions, as baby was very unwell and died in first 48 hours, though the baby was randomised at the time of birth, we did not feel it appropriate to approach grieving parents for consent.

We are grateful to have been considered for "waiver of consent". Without this a robust methodology for keeping the tidal volume constant from the birth, whilst waiting for consent from parents, who are already stressed because of untimely delivery, would have made it extremely difficult to recruit many babies. This is well documented in randomised controlled trials where teams struggled to recruit extremely unwell babies. Having said that, the parents still had the right to not participate or if agreed, to withdraw from the study. Fortunately for us, we man-

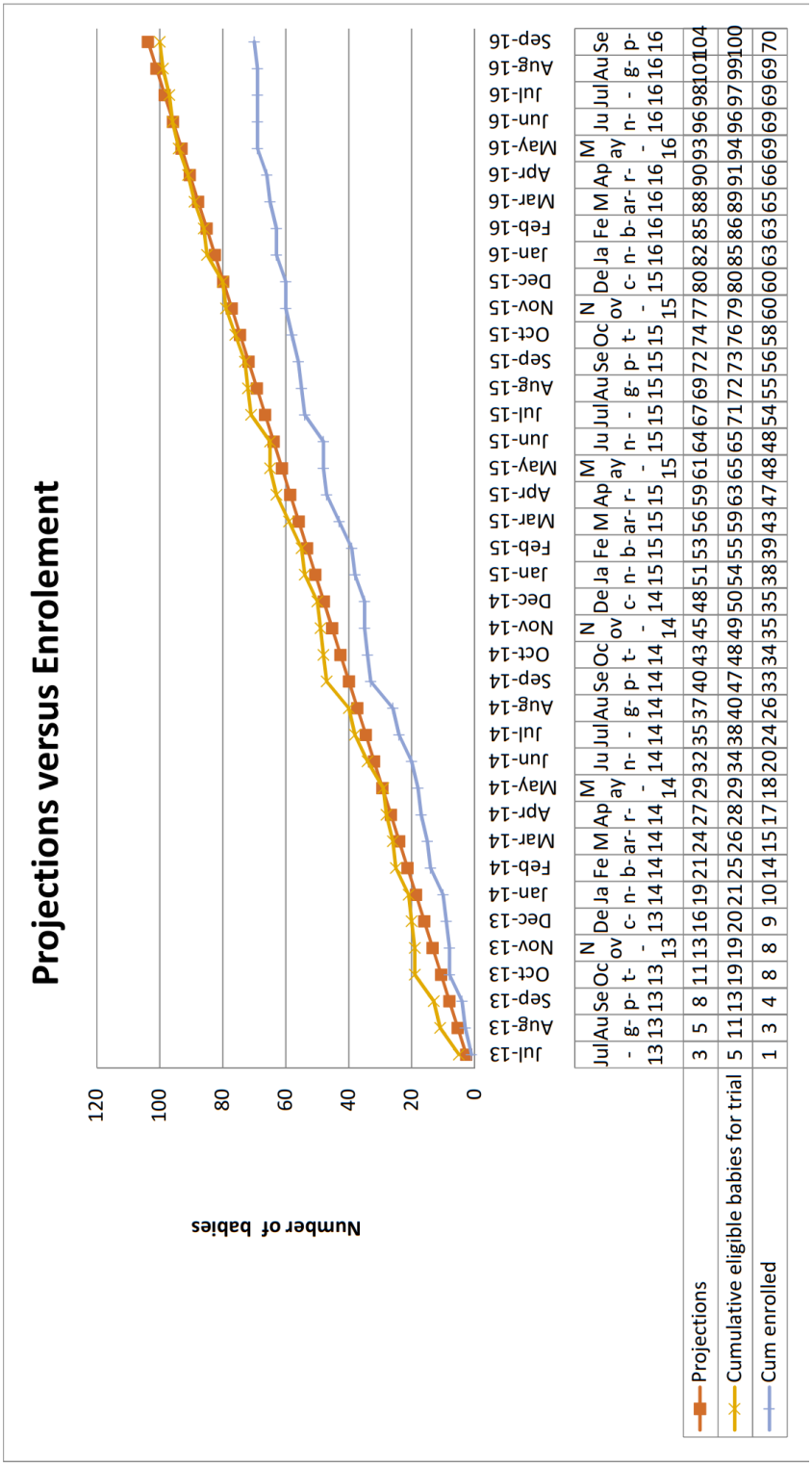


Figure 5.2: Graph of projections versus enrolment

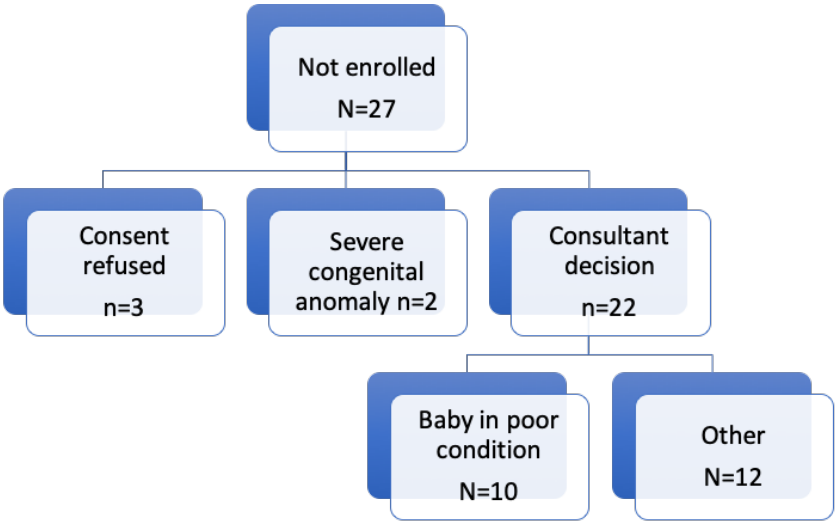


Figure 5.3: Flow diagram explaining non-recruitment for eligible babies

aged to get the consent in most cases, except for three babies. That left 12 babies where the cause of not randomising the baby was not entirely clear.

In summary, The reasons for extending recruitment period were:

1. Overall number of eligible babies had come down.

2. Team/consultant decided not to randomise baby if baby was very unwell and had imminent risk of escalating treatment or death.

3. On some occasions, as baby was very unwell and died in first 48 hours, though the baby was randomised at the time of birth, we did not feel it appropriate to approach grieving parents for consent.

Table 5.1: Breakdown of recruitment as per the category

Year	**Cumulative Eligible group**	**Cumulative intubated**	**Cumulative eligible for trial**	**Cumulative enrolled**	**Cumulative missed**
2013*	41	24	18	9	9
2014	80	45	30	26	4
2015	71	45	29	25	4
2016**	70	37	20	10	10

*From 18th July to 31st December 2013
**From 1st January to 31st September 2016

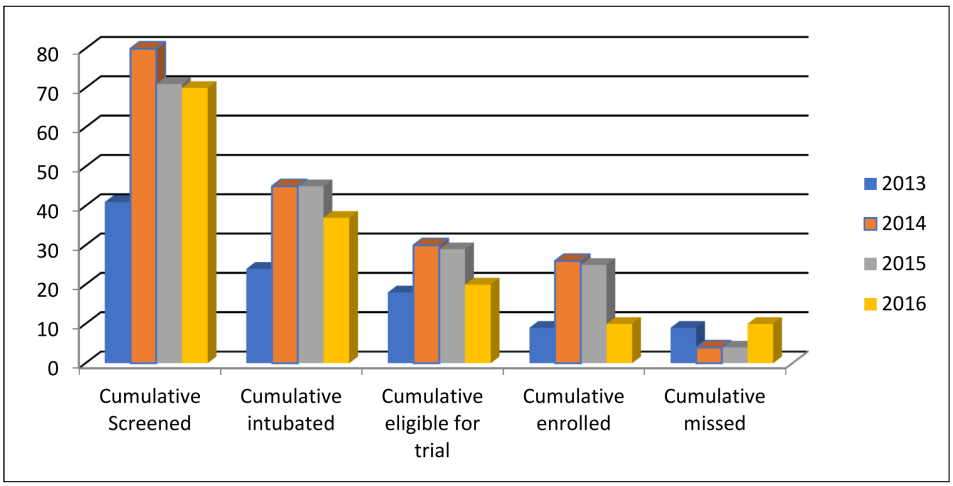


Figure 5.4: Breakdown of recruitment as per the category

5.1.2 Study population

The characteristic of whole study population is provided in the Table 5.2. The mean birth weight was 1044.23 gram (Figure 5.5) and median gestation of the babies at birth was 27+2 (Figure 5.6). 91.4% Percent of babies had received at least one dose of antenatal steroids. All the babies received at least one dose of surfactant and caffeine. Mean severity of illness at birth assessed by CRIB II score was 9.14.

5.2 Maternal characteristics

The babies in the two groups were compared for maternal demographics and clinical characteristics. There were no differences between any of the baseline maternal characteristics between the two study groups (Table 5.3).

Table 5.2: Profile of study population

Characteristic	**Study infants N=70**
Gestational age in weeks, mean (SD)	27.4 (2.14)
Birth weight in grams, mean (SD)	1044.23 (334.18)
Birth weight z score, mean (SD)	0.2 (0.9)
Birth weight Fenton's centile, mean (SD)	55.57 (26.67)
Antenatal steroids	
At least one dose, number (%)	64 (91.4%)
2 doses, count (%)	46 (65.7%)
Male sex, count (%)	44 (62.9%)
Multiple births, count (%)	19 (27.1%)
Born in study population, count (%)	69 (98.6%)
Postnatal transfer, count (%)	1 (1.4%)
Intubated at birth, count (%)	65 (92.8%)
CPAP before ventilation, count (%)	5 (7.1%)
Surfactant mg/kg, mean (SD)	237 (178)
CRIB II Score*, mean (SD)	9.14 (3.5)
Apgar Score at 1 min– median (IQR)	5(3-6)
Required chest compression, count (%)	5 (7.1%)
Temperature on admission in ^{o}C, mean (SD)	35.56 (0.96)
Oxygenation Index on admission, mean (SD)	5.71 (4.63)
A-a DO_2 on admission, mean (SD)	17.04 (17.98)

CPAP – Continuous Positive Airway Pressure; CRIB II – Clinical Risk Index for babies – II; A-a DO_2 – Alveolar arterial Oxygen Gradient

5.3 Baseline infants' characteristics

We compared the babies in the two groups by both demographic and clinical characteristics.

5.3.1 Comparison of two study groups according to birth weight and gestational age strata

There was no difference between the two groups with when the groups were stratified whether the birth weight was less than or more than 1000 grams (Table 5.4). Further subdivision of the babies based on birth weights into four groups did not

Table 5.3: Baseline Maternal characteristics

Categories	**High TV N=34**	**Low TV N=36**	**p-value**
Maternal Age in years, Mean (SD)	29.3 (6.19)	27.7 (7.16)	0.321
White ethnicity, Count (%)	31 (91.2%)	36 (100%)	0.109
Rubella immune, Count (%)	30 (88.2%)	29 (80.66%)	0.672
Normal Anomaly Scan, Count (%)	29 (85.36%)	33 (91.76%)	0.421
Maternal Pregnancy induced Hypertension*, Count (%)	4 (11.86%)	4 (11.16%)	1
Maternal diabetes, Count (%)	4 (11.86%)	2 (5.66%)	0.422
Multiple pregnancy, Count (%)	10 (29.4%)	9 (25%)	0.79
Two doses of Antenatal steroids, Count (%)	24 (70.6%)	22 (61.1%)	0.259
Maternal chorioamnionitis**, Count (%)	7 (20.66%)	3 (8.36%)	0.182
Pre-labour rupture of membranes, Count (%)	13 (38.2%)	14 (38.8%)	1
Rupture of membranes >18 hr, Count (%)	12 (35.36%)	11 (30.66%)	0.8
Antepartum Haemorrhage***, Count (%)	4 (11.86%)	5 (13.96%)	1
Spontaneous onset delivery, Count (%)	22 (64.76%)	25 (69.46%)	0.8
Vaginal Mode of delivery, Count (%)	19 (55.96%)	22 (61.16%)	0.81

*Pregnancy induced hypertension – significant enough to need intervention
**Chorioaminionitis – defined as fever in the mother with raised C-reactive protein or white cell count or histopathological findings of chorioamnionitis on examination of placenta
***Antepartum haemorrhage – significant enough to need intervention

Table 5.4: Breakdown of groups when stratified by birth weight

	High TV N=34 Count (%)	**Low TV N=36 Count (%)**	**p-value**
≤1000 gram (%)	16 (47.1%)	18 (50.0%)	0.497
>1000 gram (%)	18 (52.9%)	18 (50.0%)	

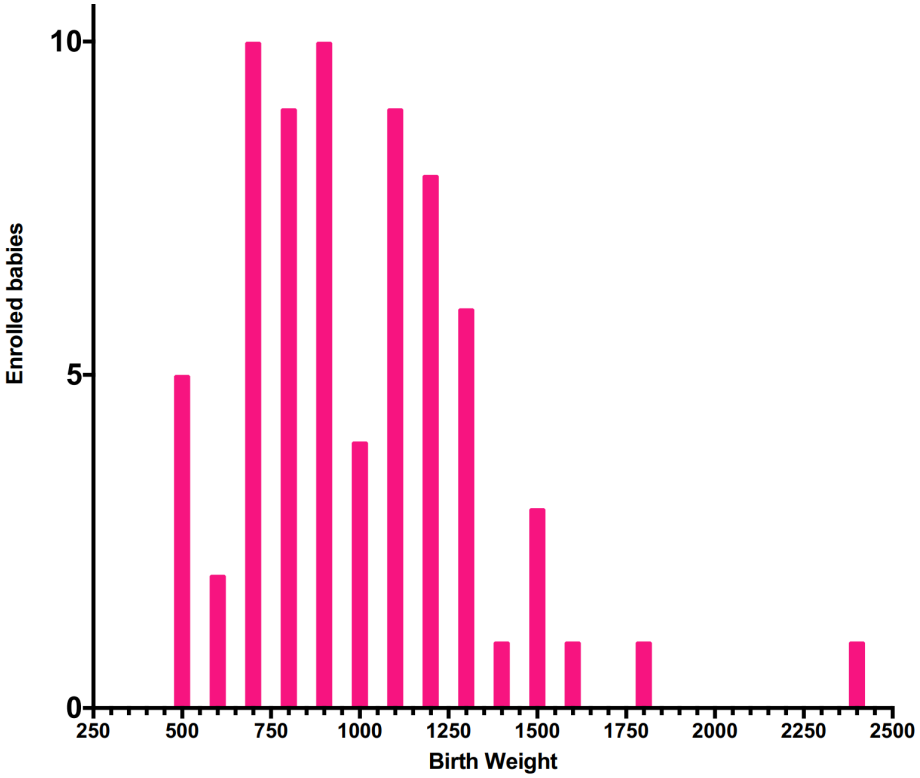


Figure 5.5: Distribution of birth weight

reveal any statistically significant difference (Table 5.5).

Table 5.5: Proportion of babies in study group according to birth weight

Weight Groups	**High TV N=34 Count (%)**	**Low TV N=36 Count (%)**
<750 gram	7 (20.6%)	4 (11.1%)
751-1000 grams	11 (32.4%)	14 (38.9%)
1001-1250 grams	7 (20.6%)	10 (27.8%)
>1250 grams	9 9(26.5%)	8 (22.2%)

Similarly groups were not different when stratified based on birth gestation 28 weeks (Table 5.6).

Further subdivision of the babies based on gestation into four groups did not reveal any statistically significant difference (Table 5.7)

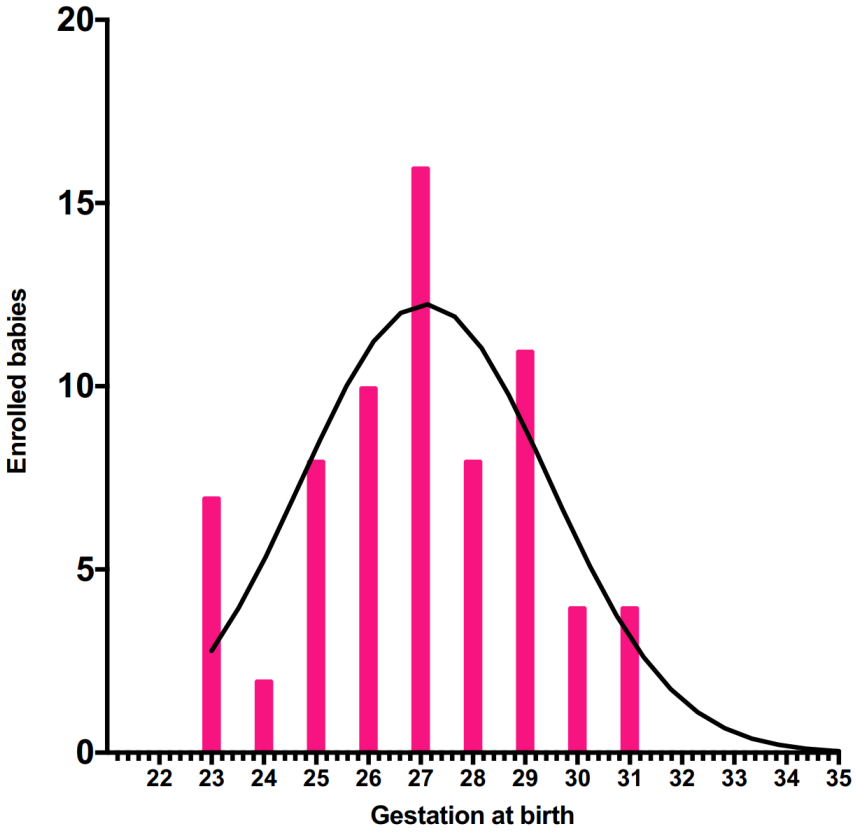


Figure 5.6: Distribution of birth gestation

Table 5.6: Breakdown of groups when stratified by birth gestation

	High TV N=34 Count (%)	**Low TV N=36 Count (%)**	**p-value**
≤28w (%)	20 (58.8%)	23 (63.9%)	0.425
>28 w (%)	14 (41.2%)	13 (36.1%)	

Table 5.7: Proportion of babies in study group according to gestation at birth

Gestation at birth	**High TV N=34 Count (%)**	**Low TV N=36 Count (%)**
≤25 weeks	7 (20.6%)	3 (8.3%)
25-26+6	9 (26.5%)	12 (33.3%)
27-28+6	8 (23.5%)	14 (38.9%)
>29	10 (29.7%)	7 (19.4%)

5.3.2 Comparison of baseline clinical characteristics

The important demographic and clinical characteristic details of the two study groups are shown in the Table 5.8. There were no differences between the two groups except baseline oxygenation index. This difference was further explored by comparing various parameters of baseline severity of initial lung disease.

Table 5.8: Comparison of infant characteristics in the two study groups

Parameter	**High TV N=34**	**Low TV N=36**	**p-value**
Gestational age in weeks, median (IQR)	27.29 (25.46-29.53)	27.43 (26.43-28.78)	0.892
Birth weight in grams, mean (SD)	1064.15 (393.56)	1025.42 (270.89)	0.63
Birth weight Fenton's z score, mean (SD)	0.26 (0.96)	0.14 (0.86)	0.58
Birth weight Fenton's centile, mean (SD)	56.56 (26.5)	54.64 (27.1)	0.77
CRIB II Score, median (IQR)	9 (6-12)	9 (7-11)	0.836
Male sex, number (%)	20 (45.5%)	24 (54.5%)	0.333
Apgar score at 1 min, median (IQR)	5 (3-6)	4.5 (3.25-7)	0.943
Apgar score at 5 min, median (IQR)	7 (6-8)	8 (6.25-9)	0.559
Temperature on admission in ^{o}C, mean (SD)	36.62 (1,02)	36.5 (0.91)	0.61
Male sex, count (%)	20/34 (58.8%)	24/36 (66.7%)	0.333
AGA, count (%)	30/34 (88.2%)	30/36 (83.3%)	0.736
inborn in study population, count (%)	33 (97.1%)	36 (100%)	0.486
Age at intubation, mins, mean (SD)	32.76 (123.2)	25.61 (70.5)	0.76
Required chest compression, count (%)	3 (8.8%)	2 (5.6%)	0.472
CPAP before ventilation, count (%)	2 (5.9%)	3 (8.3%)	0.528

CRIB II – Clinical Risk Index for babies – II; CPAP – Continuous Positive Airway Pressure; AGA – Appropriate for Gestation Age

5.3.3 Comparison of babies based on baseline severity of initial lung disease at the time of initial ventilation

The severity of lung disease at the time of initial ventilation was comparable between the two groups as assessed by FIO_2 and (Alveolar arterial oxygen gradient A-aDO_2) (Table 5.9).

On the contrary, there was a significant difference between the two groups in other baseline parameters like oxygenation index (OI), peak inspiratory pressure (PIP), mean airway pressure (MAP), minute volume (MV) and partial pressure of CO_2 (pCO_2). Difference in OI could be explained based on increased pressure required to deliver the higher tidal volume which also altered oxygenation index. This is reinforced by the fact that FIO_2 and paO_2 were no different in two groups.

The difference in PIP and MAP can be explained on the basis of need for higher pressure to generate high TV. Likewise, due to lack of control of RR initially, MV was more in babies with high TV. High MV also correlated with higher initial $paCO_2$.

Table 5.9: Severity of initial lung disease at the time of initial ventilation in two study groups

Severity of Initial lung disease	High TV N=34 Mean (SD)	Low TV N=36 Mean (SD)	p-value
Initial A-aDO_2	18.53 (18.68)	15.63 (17.46)	0.5
Initial FIO_2	0.35 (0.18)	0.37 (0.23)	0.7
Initial OI	7.00 (5.44)	4.50 (3.30)	<0.05*
Initial MAP	10.70 (2.38)	8.64 (1.94)	<0.005*
Initial PIP	29.22 (4.94)	19.99 (8.62)	<0.0001*
Initial paO_2	7.95 (2.96)	9.04 (2.42)	0.096
Initial $paCO_2$	4.45 (2.15)	5.47 (1.79)	<0.05*

A-aDO_2 - Alveolar to arterial oxygen gradient, PIP – Peak Inspiratory Pressure, MAP – Mean Airway Pressure, FIO_2 – Fractional inspired Oxygen; pO_2 – Partial pressure of Oxygen, pCO_2 – partial pressure of Carbon dioxide

5.4 Primary outcome measures

The primary outcome for this study was chosen as the time to achieve 25% reduction in peak inspiratory pressure. Out of 70 babies enrolled, 15 babies exited from trial for various reasons outlined in Table 5.11. Out of the remaining 55 babies, 50 babies survived to discharge while 5 babies died before discharge.

5.4.1 Success record of babies in the trial

On comparing the two groups for their success in achieving primary outcome, 29/34 and 26/36 babies in high and low TV group achieved primary outcome. This was not statistically significant (p=.247) (Table 5.10).

Table 5.10: Primary outcome success

Successful in achieving primary outcome	**High TV**	**Low TV**	**Total**	**p-value**
Yes	29	26	55	0.247
No	5	10	15	
Total	34	36	70	

When the babies exited the trial, of note, hypercapnoea was responsible for 7 (19.4%) babies in low TV groups as compared to 1 (2.9%) baby in high TV group, the difference was not statistically significant. Comparison of other reasons behind the exit did not reveal any clinical or statistically significant difference. Majority of the exited babies received high frequency ventilation, the pattern between them was not statistically significant (Table 5.11).

5.4.2 Primary outcome after randomisation

The median duration of 25% drop in peak inspiratory pressure (PIP) in high and low TV group was 13.8 hours (95% CI, 6.4-21.3) and 18.1 hours (95% CI, 10.4-

Table 5.11: Record of exited babies in two study groups

	High TV N=34 Count (%)	**Low TV N=36 Count (%)**	**p-value**
Met trial exit criteria?	5 (14.7%)	10 (27.8%)	0.15
If yes, which one,			
High pCO_2	1 (2.9%)	7 (19.4%)	0.23
High FIO_2	2 (5.8%)	2 (5.6%)	
Pulmonary haemorrhage	1 (2.9%)	1 (2.8%)	
Air leak	1 (2.9%)	0 (0%)	
If yes, then switched to which mode of ventilation			
HFO	5 (100%)	6 (60%)	0.38
Pr AC	0 (0%)	1 (10%)	
Pr AC + VG with different TV	0 (0%)	2 (20%)	
Vol AC	0 (0%)	1 (10%)	

pCO_2 – partial pressure of CO_2, FIO_2 – Fractional concentration of inspired oxygen, HFO – High frequency oscillation ventilation, Pr AC – Pressure Assist Control Ventilation, VG – Volume Guarantee, TV – Tidal Volume, Vol AC – Volume assist control Ventilation

25.8) respectively. The log rank statistic for equality of survival distribution for randomisation arm was not significant (p=0.931) (Table 5.12, Figure 5.7).

Table 5.12: Comparison of primary outcome in the two study groups

	High TV N=34	Low TV N=36	p-value
Primary outcome, Median (95% CI)	13.8 (6.4-21.3)	18.1 (10.4-25.8)	0.931

5.4.3 Univariate analysis of primary outcome measure

We performed regression analysis for the continuous outcome measures. This considered effects of variables which could influence primary outcome. On univariate analysis, a number of factors such as birth weight less than 1000 gram, gestation less than 28 weeks, fluid prescription in first week of life, were significantly associated with primary outcome of 25% reduction in peak pressures (Table 5.13).

Table 5.13: Univariate analysis of primary outcome measure

Variable	**Hazard ratio (95% CI)**	**p-value**
Randomised to High TV	1.024 (.598 – 1.754)	0.931
Stratified to ≤1000 g birth weight	1.592 (1.199 -2.114)	0.001
Gestation age ≤28 weeks at birth	1.889 (1.406 - 2.537)	<0.001
Birth weight <10th centile	.790 (.439 - 1.421)	0.431
Male Gender	.992 (.752 - 1.309)	0.957
Singleton	.994 (.741 - 1.3330)	0.968
Antepartum Haemorrhage	1.254 (.816 - 1.926)	0.302
Chorioamnionitis	1.199 (.802 - 1.793)	0.376
Maternal age	1.023 (.981 - 1.066)	0.296
Vaginal delivery	956 ((.728 - 1.254))	0.744
Antenatal steroids – 2 doses	827 (.617- 1.110)	0.206
Apgars at 1 min	1.095 (.969 - 1.238)	0.147
Surfactant (x100mg/Kg)	.708 (.478 - 1.048)	0.084
Temperature on admission	1.123 (.866 - 1.456)	0.381
Temp <36	1.184 (.871 - 1.609)	0.281
Severe lung disease based on OI	1.99 (0.98-4.1)	0.059
Severe lung disease based on A-aDO_2	1.21 (0.92 – 1.6)	0.181
Base deficit on admission	1.041 (.972 - 1.115)	0.253
Metabolic acidosis, BD >5	1.184 (903 - 1.551)	0.221
Fluid (x10ml/kg)	1.099 (1.017 - 1.188)	<0.05
CPAP before ventilation	.778 (.465 - 1.302)	0.339
Mean Airway Pressure initial	1.069 (.977 - 1.170)	0.148
Peak airway pressure initial	1.016 (.984 - 1.049)	0.32
Fractional inspired oxygen initial	.235 (.045 -1.222)	0.085
Chest expansion >8 ribs (L)	1.099 (813 - 1.484)	0.54
Chest expansion >8 ribs (R)	1.058 (.807 - 1.386)	0.684
Initial pCO_2 (<4.5)	.864 (.705 - 1.060)	0.162

OI – Oxygenation Index, A-aDO_2 – Alveolar Arterial oxygen gradient, BE – Base Excess, CPAP – Continuous Positive Airway Pressure, pCO_2 – partial pressure of CO_2

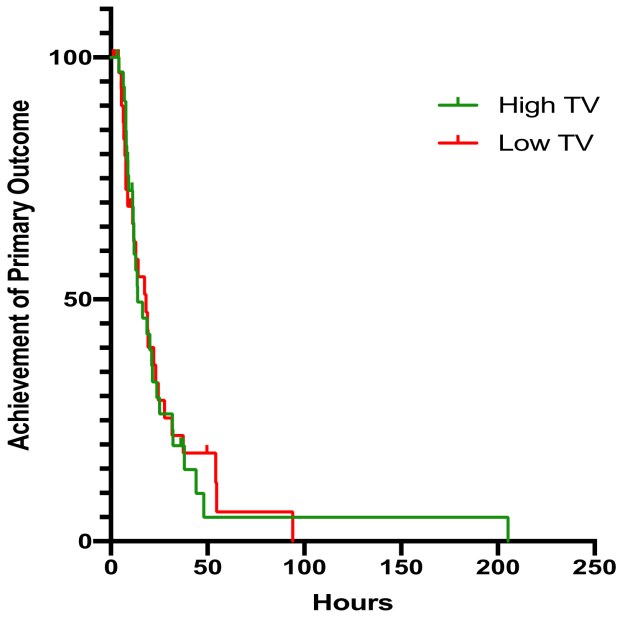


Figure 5.7: Kaplan-Meier curve for 25% reduction in PIP for babies randomised to high and low TV

5.4.4 Multiple logistic regression analysis of primary outcome measure

For multiple linear regression analysis, only variables at significance of $p<0.2$ were included from univariate analysis. These were: the gestation age less than 28 weeks, Apgars at 1 min, Surfactant, initial PCO_2, fractional inspired oxygen (FIO_2), mean airway pressure (MAP), fluids in first week. Out of these, gestation age less than 28 weeks, fractional inspired oxygen (FIO_2) and fluids in first week turned out to be significantly associated with primary outcome (Table 5.14).

5.4.5 Birth weight and primary outcome

Babies in the study were stratified at randomisation into two groups based on birth weight: $\leq$1000 gram or >1000 gram. Out of total 70 babies randomised, 36 and 34 babies were stratified into $\leq$1000 grams and >1000 gram respectively. Out of these, 15 babies (13 and 2 babies in $\leq$1000 gram or >1000 gram respectively) met exit

Table 5.14: Multiple logistic regression analysis of primary outcome measure

Variable	**Hazard ratio (95% CI)**	**p-value**
Gestation age <28 weeks	.30 (.14 - .64)	**0.002**
Apgars at 1 min	1.07 (.93 - 1.23)	0.35
Surfactant (x100mg/Kg)	.99 (.99 - 1.004)	0.69
Fractional inspired oxygen Initial	.18 (.03 - .94)	**0.04**
Mean Airway Pressure initial	.93 (.84 - 1.03)	0.18
Initial pCO_2 (<4.5)	.94 (.76 - 1.16)	0.55
Fluid (x10ml/kg)	1.10 (1.02 - 1.19)	**0.018**

criteria. The difference between the groups with regards to providing successful ventilation was statistically significant (Table 5.15).

Table 5.15: Effect of birth weight on primary outcome achieved

Primary outcome achieved	**High TV N= 34 Count (%)**	**Low TV N= 36 Count (%)**	**p-value**
≤1000 gram birth weight	13 (72.2%)	10 (55.6%)	0.78
>1000 gram birth weight	16 (100%)	16 (88.9%)	

Post birth weight stratification, comparison between the two groups was not statistically significant (Table 5.16, Figure 5.8, Figure 5.9).

Table 5.16: Comparison of the Primary outcome based on birth weight

Primary outcome	**High TV Median (95% CI)**	**Low TV Median (95% CI)**	**p-value**
≤1000 gram birth weight	21.1 (1.9-40.1)	23.2 (19.1 – 27.4)	0.612
>1000 gram birth weight	11.2 (5.8-16.7)	14.1 (6.5 – 21.8)	

5.4.6 Effect of gestational age on primary outcome

The effect of gestation age on primary outcome was studied by post-hoc analysis. The study infants were subdivided into two subgroups, ≤28 weeks' gestation and

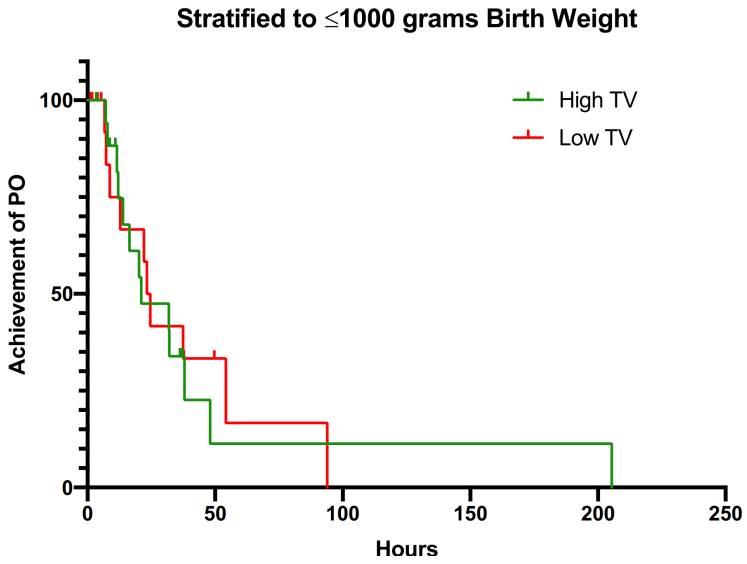


Figure 5.8: Primary outcome stratified according to birth weight ≤1000 grams

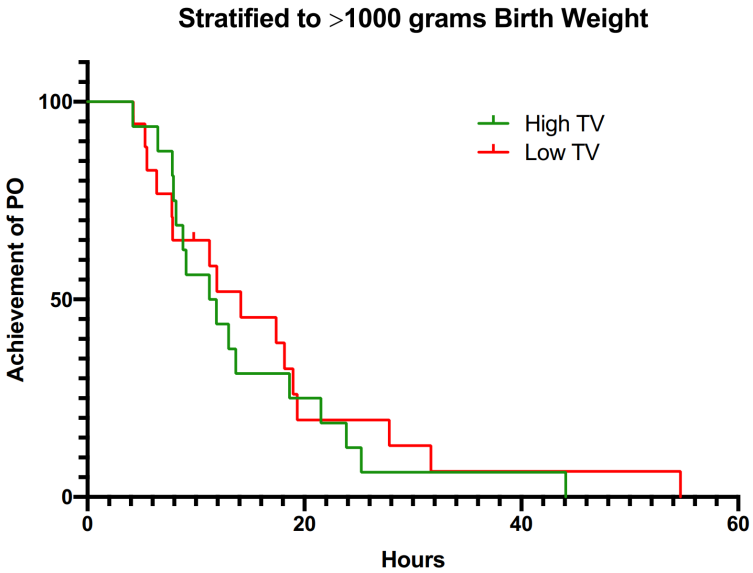


Figure 5.9: Primary outcome stratified according to birth weight >1000 grams

>28 weeks' gestation at birth.

The proportion of babies of ≤28 weeks' gestation who met the exit criteria were 13/44 (29.5%) as compared to babies >28 weeks' gestation who had failure rate of 2/26 (7.7%). Hence, babies in less than 28 weeks' group were 3.83 times likely to not to achieve the primary outcome as compared to the babies in >28 weeks group (Table 5.17).

Table 5.17: Effect of gestation age at birth on primary outcome achieved

Primary outcome achieved	**High TV N= 34 Count (%)**	**Low TV N= 34 Count (%)**	**p-value**
≤28 weeks	16 (76.2%)	15 (65.2%)	1
>28 weeks	13 (100%)	11 (84.6%)	

When stratified, babies in two groups were assessed for time to reach 25% drop in peak inspiratory pressure. In either gestation strata, the difference in both groups was not statistically significant (Table 5.18, Figure 5.10, Figure 5.11).

Table 5.18: Comparison of the Primary outcome based on gestation age

Primary outcome	**High TV Median (95% CI)**	**Low TV Median (95% CI)**	**p-value**
≤28 weeks	23.9 (16.3-31.5)	24.6 (12.7-36.4)	0.928
>28 weeks	8.8 (7.4 – 10.2)	11.2 (5.1 – 17.4)	

5.4.7 Effect of severity of lung disease at birth on primary outcome

We also carried out subgroup analysis based on severity of respiratory illness assessed by alveolar arterial oxygen gradient (A-aDO_2) and oxygenation index (OI) at initial ventilation. This did not return a clinically or statistically significant result.

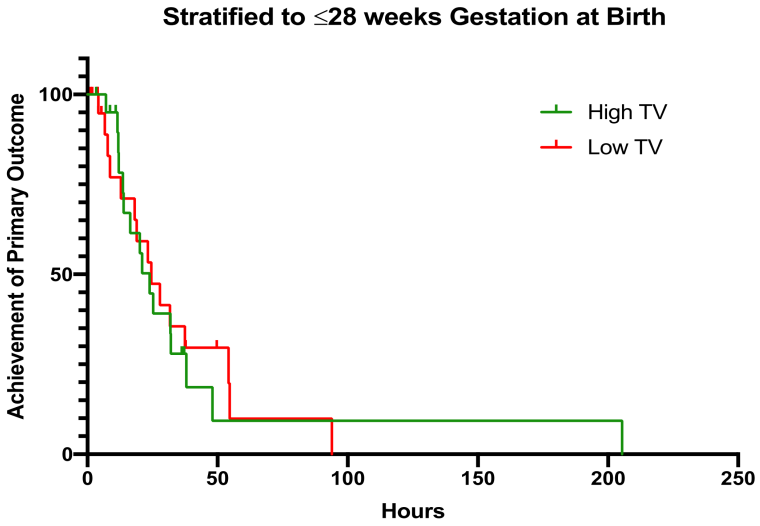


Figure 5.10: Primary outcome stratified according to ≤28 weeks' gestation at birth

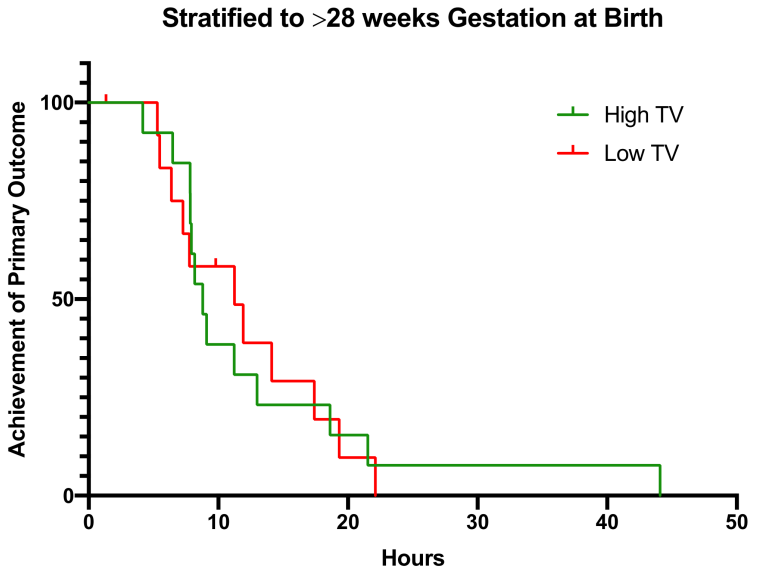


Figure 5.11: Primary outcome stratified according to >28 weeks' gestation at birth

5.5 Total duration of mechanical ventilation in two study groups

Out of 70 babies, 9 (12.8%) died before they could be extubated. 6/36 (16.6%) and 3/34 (8.8%) babies could not be extubated in low and high group respectively. When this was broken down as per birth weight stratification, 8/36 and 1/34 babies born at ≤1000g and >1000g died before extubation respectively. 8/44 and 1/26 babies born at ≤28 weeks and >28 weeks gestation died before extubation respectively).

The median duration of ventilation in the high TV group was 35 hours (95% CI (9.8-60.2 hrs)) as compared to 61.8 hours (95% CI 14.4 – 109.2 hrs) in low TV group. The log rank statistic for equality of survival distribution for randomisation arm was not significant (p=0.928) (Table 5.19, Figure 5.12).

Table 5.19: A Comparison of two groups for duration of mechanical ventilation

	High TV Median (CI)	**Low TV Median (CI)**	**p-value**
Mechanical Ventilation duration, hrs	35 (9.8-60.2)	61.8 (14.4 – 109.2)	0.928

5.5.1 Effect of birth weight on duration of mechanical ventilation

To assess the effect of birth on duration of mechanical ventilation, babies were analysed in two groups, birth weight ≤1000 grams and >1000 grams. In the subgroup of babies ≤1000 grams birth weight, babies in the high tidal volume group received mechanical ventilation support for median duration of 368.9 hrs (95% CI 69.7-668 hrs) as compared to those on low tidal volume group who remained on mechanical ventilation for a median duration of 137.9 hrs (95% CI 77.6 – 198.3 hrs). (Table 5.20, Figure 5.13).

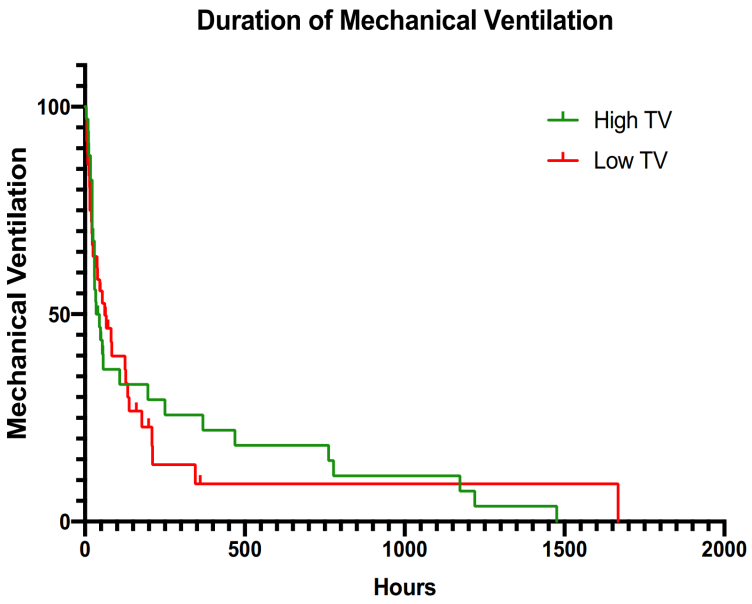


Figure 5.12: Kaplan Meier survival curves for the duration of ventilation support in two study groups

In the group of babies >1000 grams birth weight, babies in high tidal volume group received mechanical ventilation support for median duration of 29.1 hrs (95% CI 21.2 – 36.9 hrs) as compared to those on low tidal volume group who remained on mechanical ventilation for a median duration of 21.6 hrs (95% CI 0-61.6 hrs). The difference between the two study groups was not significant and the log rank statistic for equality of survival distribution for randomisation arm was p=0.516 (Table 5.20, Figure 5.14).

Table 5.20: Comparison of duration of mechanical ventilation by birth weight

Birth weight	**High TV Median (95% CI)**	**Low TV Median (95% CI)**	**p-value**
≤1000	368.9 (69.7- 668)	137.9 (77.6 – 198.3)	0.516
>1000	29.1 (21.2 – 36.9)	21.6 (0-61.6)	

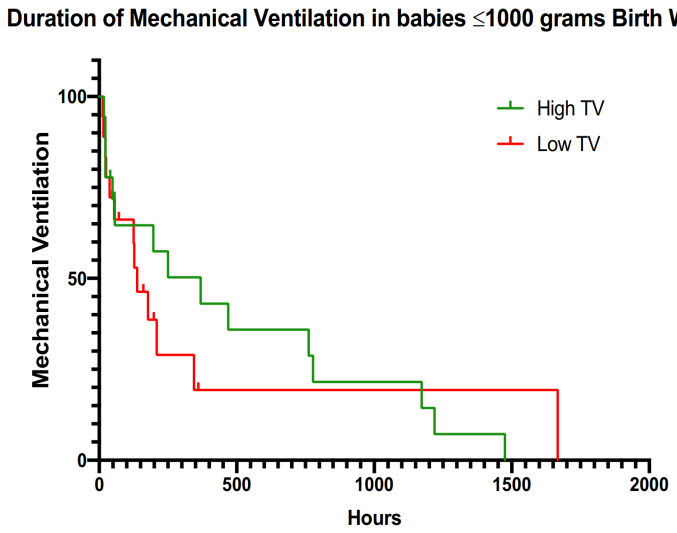


Figure 5.13: Kaplan Meier survival curves for the duration of mechanical ventilation in two birth weight groups of ≤1000 gram

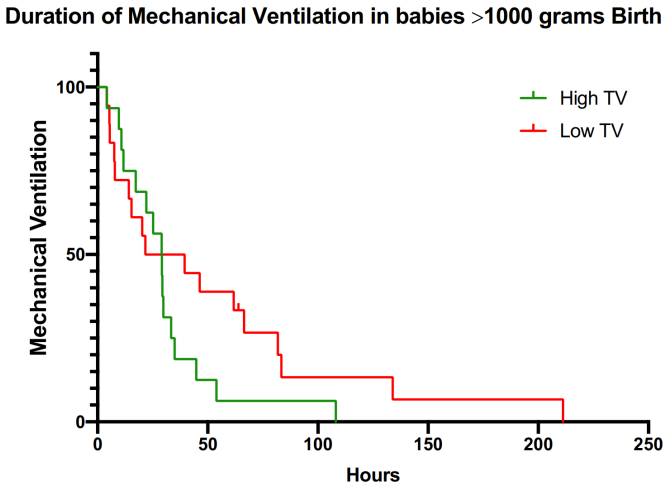


Figure 5.14: Duration of mechanical ventilation stratified according to birth weight >1000 grams

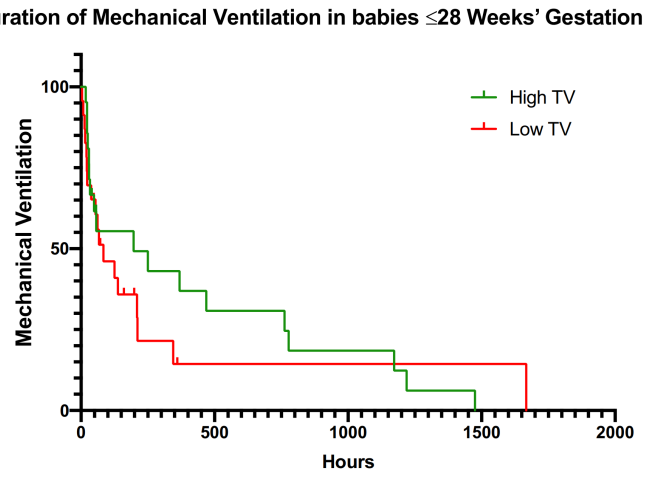


Figure 5.15: Kaplan Meier survival curves for the duration of mechanical ventilation in babies with ≤28 weeks of gestation age at birth

5.5.2 Effect of gestation age on duration of mechanical ventilation

To asses the effect of gestational age at birth on the duration of mechanical ventilation on the whole, babies were analysed into two groups, those with gestation age equal to or less than 28 weeks and more than 28 weeks.

In the group of babies less than or equal to 28 weeks' gestation, babies in high tidal volume group received mechanical ventilation support for median duration of 196.3 hrs (95% CI 0- 545.2 hrs) as compared to those on low tidal volume group who remained on mechanical ventilation for a median duration of 83.4 hrs (95% CI 0-173.1 hrs). The difference between the two study groups when considered their birth gestation was not significant and the log rank statistic for equality of survival distribution for randomisation arm was p=0.595 (Table 5.21, Figure 5.15).

Table 5.21: Comparison of duration of mechanical ventilation by gestation age

Gestation at birth	**High TV Median (95% CI)**	**Low TV Median (95% CI)**	**p-value**
≤28w	196.3 (0 – 545.2)	83.4 (0-173.1)	0.595
>28w	22 (9.9 – 34.1)	39.5 (3.2 – 75.8)	0.88

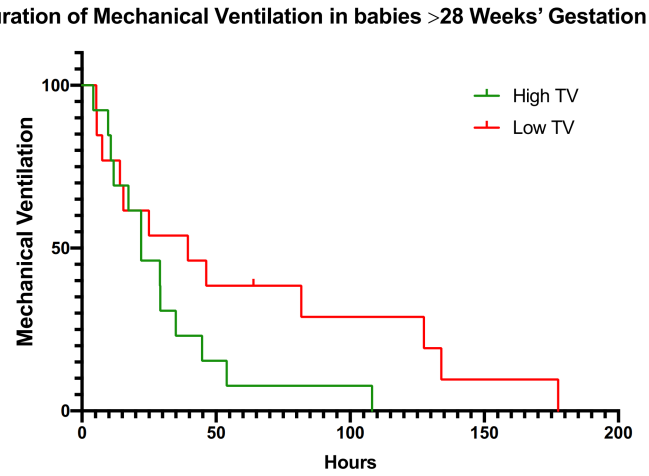


Figure 5.16: Kaplan Meier survival curves for the duration of mechanical ventilation in >28 weeks gestation group – comparison of low versus high tidal volume groups

In the group of babies more than 28 weeks of gestation, babies on high tidal volume received mechanical ventilation support for a median duration of 22 hrs (95% CI 9.9-34.1 hrs) as compared to those on low tidal volume who received mechanical ventilation support for 39.5 hrs (95% CI 3.2-75.8 hrs). This difference in the two study groups was not significant and log rank statistic (using Kaplan meier survival statistics) for equality of survival distribution for randomisation arm was p= 0.88 (Table 5.21 Figure 5.16).

5.5.3 Effect of severity of lung disease at birth on duration of mechanical ventilation

We carried out subgroup analysis based on severity of respiratory illness assessed by alveolar arterial oxygen gradient (A-aDO_2) and oxygenation index (OI) at initial ventilation. The analysis did not reveal any clinical or statistically significant difference between the two groups.

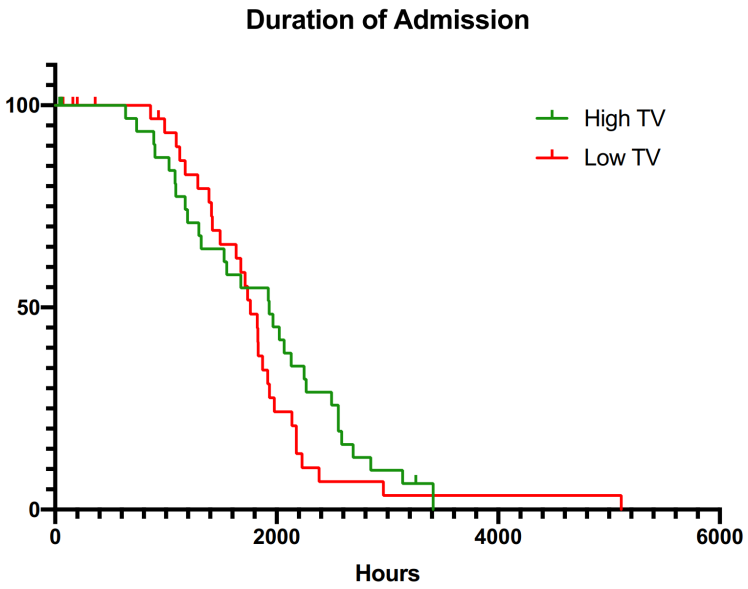


Figure 5.17: Comparison of duration of admission between the two randomised groups

5.6 Duration of admission

Out of 70 babies enrolled, 30/34 babies and 29/36 babies from high and low TV group were discharged home. The duration of admission was compared between the two study groups. The median duration of admission in high tidal volume group was 1932.7 hrs (95% CI 1416.1 – 2449.2 hrs) as compared to 1762.3 hrs (95% CI 1576.7 – 1957 hrs). The long rank statistic for equality of survival distribution for randomisation arm was p= 0.304 (Table 5.22, Figure 5.17)

Table 5.22: Duration of admission

	High TV Median (95% CI)	**Low TV Median (95% CI)**	**p-value**
Duration of admission, hrs	1932.7 (1416.1 – 2449.2)	1762.3 (1576.7 – 1957)	0.304

5.7 Efficacy of the ventilation

The preterm infants enrolled in the study were monitored for their ventilator parameters. We also wanted to find out the efficiency and effectiveness of the maintaining the tidal volume and whether it had any correlation with the volume being low or high.

5.7.1 Ventilatory parameters

The babies in both groups had blood gases monitored at regular intervals. The normal limit of $paCO_2$ was deemed to be 4.5-6.5 kPa in arterial blood gas. If the source of blood gas was other than arterial, it was adjusted to match. The blood gasses then were analysed to find out how many of these values, babies had pCO_2 in normal limits, higher than normal and lower than normal. One baby in high tidal volume group exited because of either persistently high or low pCO_2 as compared to 7 babies in low tidal volume group.

We also compared the mean pCO_2 between the two groups by independent t test.

While mean (SD) tidal volume were different between the two groups – 6 (1.6) and 4.4 (1.4) in high and low tidal volume group respectively (p=<.0001), there was no difference in minute volumes of the two groups. This could be explained on the difference in set respiratory rate (RR) which was adjusted to maintain the $paCO_2$ in desired limit, as per protocol. Mean (SD) set RR for high and low TV group was 33.7 (6.7) and 38.5 (10.2). This was statistically significant (p=<0.05). There was also difference in two groups for mean PIP which was proportional to the difference in the tidal volume in both statistical and clinical significance (p=<0.0001, Table 5.23, Figure 5.18).

While mean $paCO_2$ was not different between the two groups, the number of low $paCO_2$ values were higher in high tidal volume group (61.3% versus 32.6%, p=<.005, Table 5.23, Figure 5.19).

Table 5.23: Ventilatory Parameters

Ventilatory Parameters	**High TV Mean (SD)**	**Low TV Mean (SD)**	**p-value**
PIP, cm H_2O	26.27 (4.94)	18.81 (7.88)	**<0.0001***
TV, mL/Kg	6.06 (1.63)	4.40(1.44)	**<0.0001***
MV, mL/Kg/min	291.18 (104.1)	252.40 (108.25)	0.132
$paCO_2$, kPa	4.55 (1.89)	5.17(1.9)	0.174
RR, count	52.66 (12.35)	54.57 (13.78)	0.544
Set RR, count	33.71 (6.77)	38.49 (10.22)	**<0.05***
Spontaneous RR, count	19.93 (15.66)	15.6 (13.18)	0.214
Mean pH	7.38 (0.1)	7.14 (1.23)	0.255
Normal $paCO_2$ values, %	27.63% (30.78%)	43.52% (40.26%)	0.067
Low $paCO_2$ values, %	61.63% (38.19%)	32.65% (39.44%)	**<0.005***
High $paCO_2$ values, %	10.74% (22.89%)	21.05% (36.41%)	0.159

PIP – Peak Inspiratory Pressure, TV – Tidal Volume, MV – Minute Volume, pCO_2 – partial pressure of CO_2, RR – Respiratory Rate

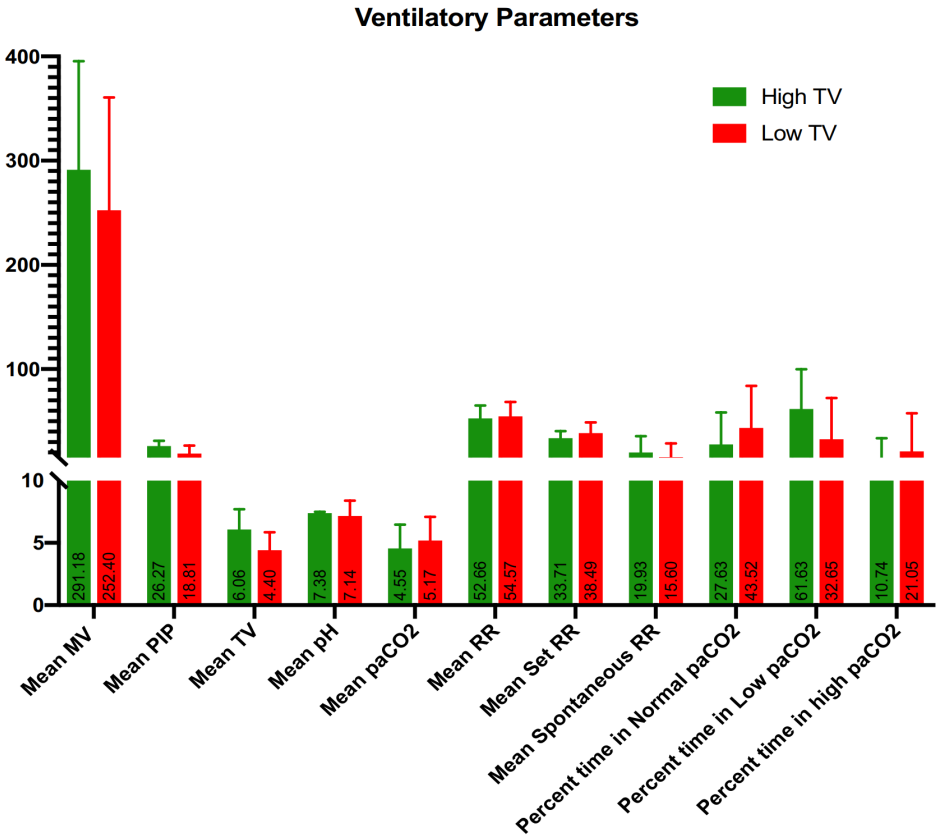


Figure 5.18: Ventilatory Parameters

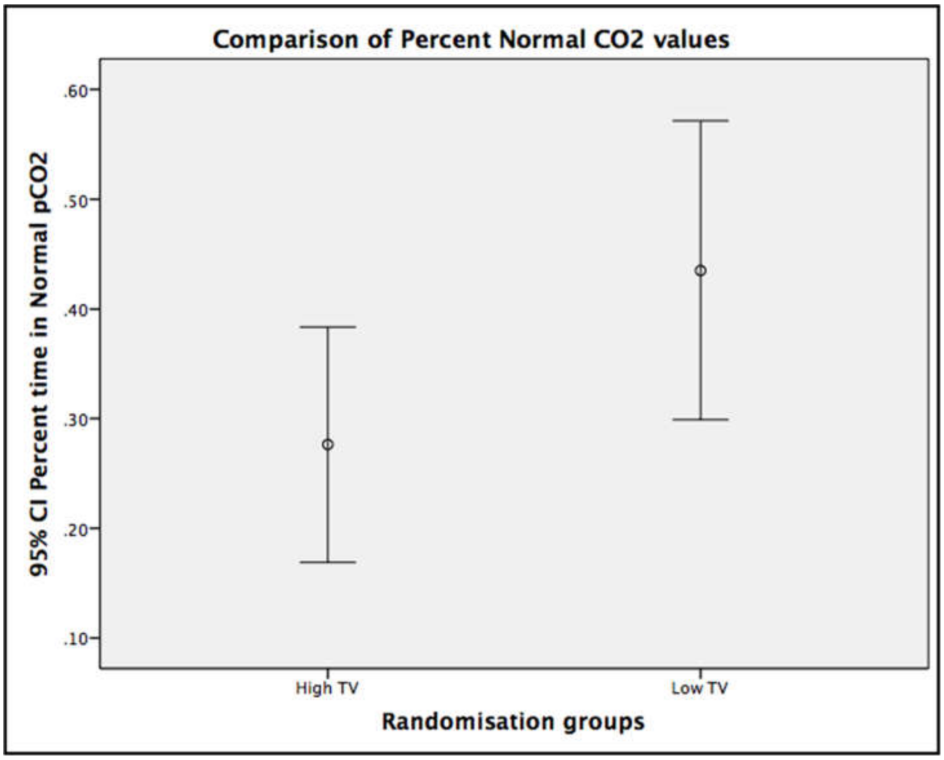


Figure 5.19: Comparison of normal $paCO_2$ values between two groups

5.7.2 Oxygenation parameters

As per protocol, we tried to maintain saturation within the current acceptable limits i.e., 90-95%. The fractional inspired oxygen (FIO_2) was altered to achieve these acceptable limits. As FIO_2 was marker for oxygen requirement, this was monitored on hourly basis in both groups. Two babies in high tidal volume group exited because of excessive oxygen requirement as did 2 babies in low tidal volume group. The data were collected as to what was the maximum requirement of FIO_2 in each baby. The data led to median FIO_2 in each group and described in the table 5.24.

Babies in both groups were also monitored for mean airway pressure generated to provide optimum oxygenation. The maximum mean airway pressure values were then analysed to ascertain the difference between the two groups. The mean max-

imum mean airway pressure in high tidal volume group was 12.3 as compared to 10.1 cm H_2O in low tidal volume group. This was statistically significant (p=<0.0001) probably because of increased peak inspiratory component in achieving the tidal volume (Table 5.24, Figure 5.20).

Table 5.24: Oxygenation parameters

Oxygenation parameters	**High TV Mean (SD)**	**Low TV Mean (SD)**	**p-value**
Initial VG FIO_2	0.35 (0.18)	0.37 (.23)	0.725
Max VG FIO_2	0.40 (0.19)	0.41 (0.19)	0.837
Extubation VG FIO_2	0.27 (0.1)	0.24 (0.05)	0.168
Max CPAP FIO_2	0.35 (0.12)	0.36 (0.14)	0.8
Initial VG MAP, cm H_2O	10.7 (2.4)	8.6 (1.9)	**<0.0001**
Max VG MAP, cm H_2O	12.3 (2.2)	10.1 (2)	**<0.0001**
Max CPAP MAP, cm H_2O	7.5 (1.3)	7 (1)	0.123

VG – Volume Guarantee, FIO_2 – Fractional inspired Oxygen, CPAP – Continuous Positive Airway Pressure, MAP – Mean Airway Pressure

5.8 Duration of respiratory support in two groups

We also monitored the time spent by each baby while receiving mechanical ventilation, CPAP, high flow nasal cannula therapy and low flow oxygen therapy. This was then compared separately and together between the two groups. There was no difference between the two groups (Table 5.25, Figure 5.21).

Table 5.25: Comparison of duration of respiratory support between the two groups

Duration of Respiratory support	**High TV mean (SD)**	**Low TV mean (SD)**	**p-value**
Mechanical ventilation Duration, hrs	400.1 (467.8)	208.4 (401.42)	0.09
CPAP Duration, hrs	258.5 (289.6)	286.5 (318.4)	0.725
HHFNC Duration, hrs	228.9 (268.1)	317.7 (403.8)	0.322
Non-Invasive support Duration (CPAP+HFC), hrs	487.5 (452.5)	604.2 (531.9)	0.367
Total respiratory support duration (MV+CPAP+HF), hrs	887.6 (802.5)	812.6 (838.5)	0.727

CPAP – Continuous Positive Airway Pressure, HFNC – High Flow Nasal Cannula, MV – Mechanical Ventilation

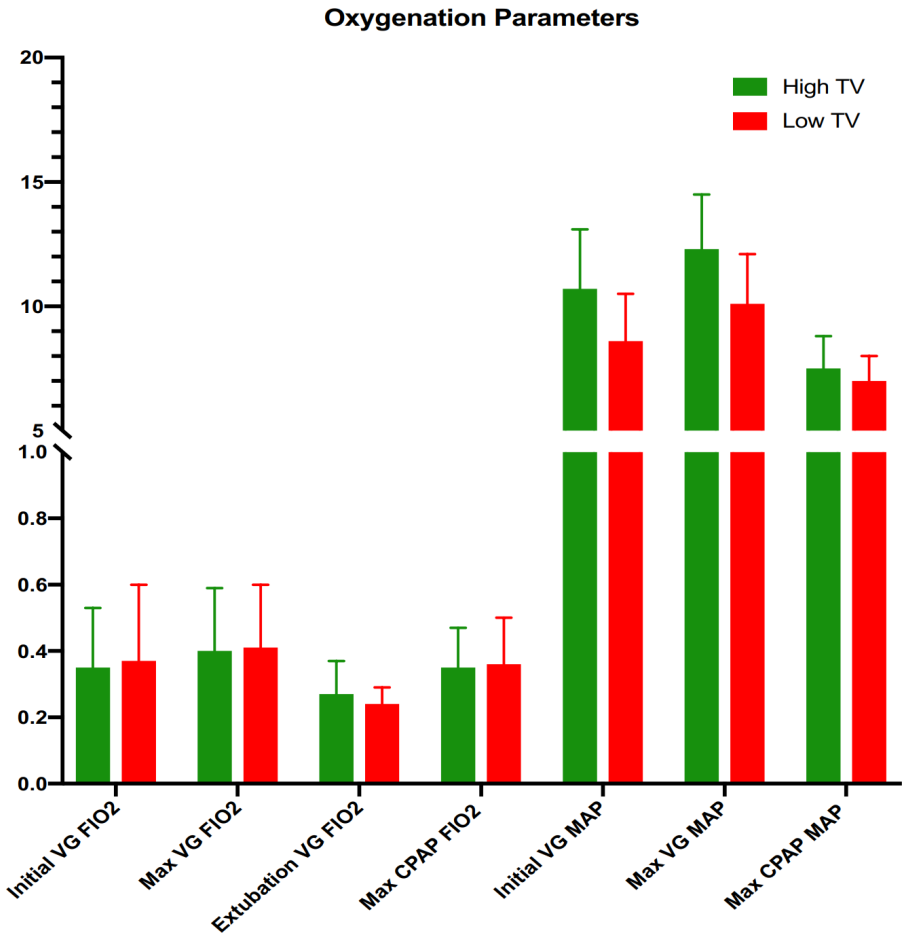


Figure 5.20: Oxygenation Parameters

5.9 Other outcome measures

5.9.1 Pulmonary complications

Premature infants are known to be prone to various pulmonary complications which are contributed by many factors, most important of them are prematurity and mode of respiratory support.

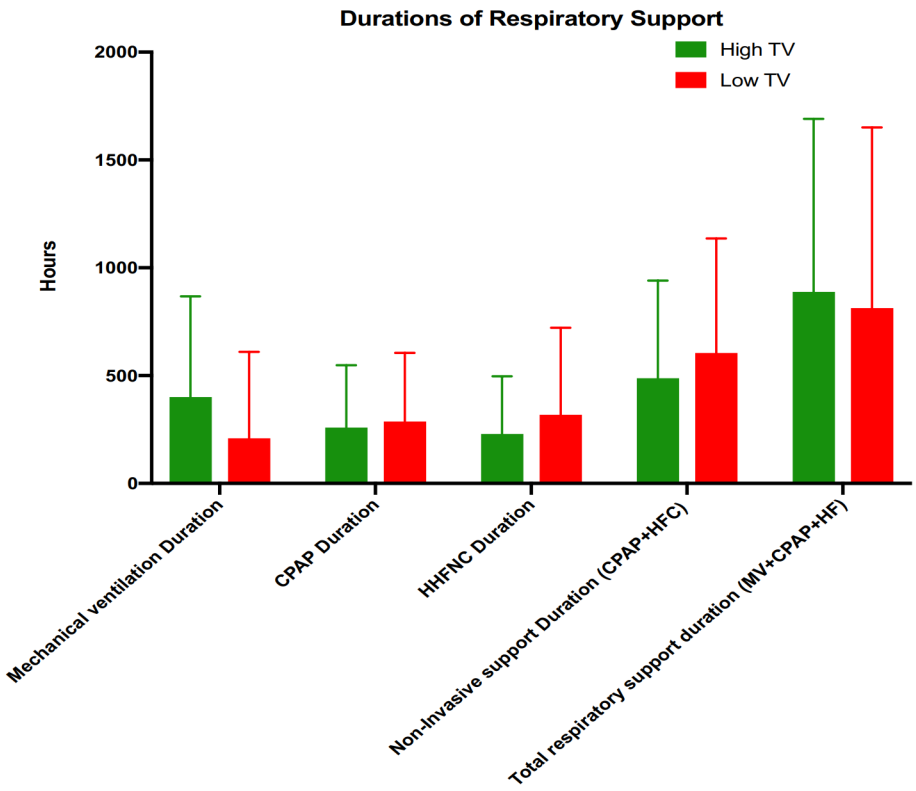


Figure 5.21: Duration of respiratory support between the two groups

5.9.1.1 Short term pulmonary complication - air leaks and pulmonary haemorrhage

The babies in two groups were compared for incidence of air leak and whether it was severe enough to warrant any intervention during the period of mechanical ventilation using volume guarantee. One baby in high tidal volume group exited because of air leak (pulmonary interstitial emphysema) as compared to none in low tidal volume group (Table 5.11) . The total number of significant incidences (3 and 0 in high and low TV group respectively) in two groups were not significantly different (p=0.10, Table 5.26, Figure 5.22).

The babies in two groups were also compared for incidence of pulmonary haemorrhage and whether it was severe enough to warrant any intervention during the

period of mechanical ventilation using volume guarantee. One baby each in high tidal volume group and low tidal volume group exited because of pulmonary haemorrhage (Table 5.11). The total number of significant incidences (5 and 7 in high and low TV group respectively) in two groups was not significant either (p=0.754, Table 5.26, Figure 5.22).

Table 5.26: Comparison of incidence of air leaks and pulmonary haemorrhage in two groups

	High TV N= 34 count (%)	**Low TV N= 36 count (%)**	**p-value**
Number of episodes of air leaks	3 (8.8%)	1 (2.8%)	0.35
Number of episodes of significant air leaks	3 (8.8%)	0 (0%)	0.109
Number of episodes of pulmonary haemorrhage	5 (14.7%)	8 (22.2%)	0.543
Number of significant pulmonary haemorrhage	5 (14.7%)	7 (19.4%)	0.754

5.9.1.2 Incidence of bronchopulmonary dysplasia

We defined bronchopulmonary dysplasia as requirement of oxygen or respiratory care at 36 weeks post menstrual age. To get a full perspective on its comparison between the two groups, we also identified the babies needing oxygen or respiratory care at 28 days of age and categorised the severity of chronic lung disease, whether babies received postnatal steroids, required home oxygen and if yes, how much. On analysis, there was no statistical significance between the two groups. (Table 5.27, Figure 5.22)

5.9.2 Non-pulmonary complications related to prematurity

Premature babies are prone to non-pulmonary complications like sepsis, intraventricular haemorrhage (IVH), periventricular leukomalacia (PVL), Patent duc-

Table 5.27: Comparison of incidence of Bronchopulmonary Dysplasia between the two groups

	High TV N=34 Count (%)	**Low TV N=36 Count (%)**	**p-value**
Received postnatal steroids, count (%)	10 (29.4%)	5 (13.9%)	0.238
Respiratory support at 28 days, count (%)	23 (67.6%)	25 (69.4%)	0.534
BPD at 36 PMA, count (%)	20 (58.8%)	16 (44.4%)	0.599
Severity of BPD, count (%)			0.054
Mild	1 (2.9%)	5 (13.9%)	
Moderate	11 (32.4%)	13 (36.1%)	
Severe	9 (26.5%)	3 (8.3%)	
Home Oxygen, count (%)	18 (52.9%)	16 (44.4%)	0.795
Amount of home oxygen, mean	0.17	0.21	0.617

BPD – Bronchopulmonary Dysplasia

tus arteriosus (PDA), hypotension (requiring inotropes), necrotising enterocolitis (NEC) and retinopathy of prematurity (ROP).

There were no significant differences in the incidence of the complications related to prematurity in the two study groups. (Table 5.28, Table 5.29,Figure 5.23)

5.10 Use of therapeutic agents

All babies received surfactant before intubation and caffeine before extubation. There was no difference in use of the dose of surfactant, postnatal steroids, medically treated PDA between the two groups (Table 5.30).

5.11 Weight gain/postnatal growth

As shown in the section of baseline characteristics, the birth weights of the two groups were similar. The mean total weight gain from admission to discharge in babies ventilated initially with high and low tidal volume was 1466.1 and 1566.1 gram respectively. This weight gain when taken into consideration of the number

Table 5.28: Comparison of non-pulmonary complications of prematurity in two groups

Outcome	**High TV N=34**	**Low TV N=36**	**p-value**
Sepsis suspected in first 72 hours, Count (%)	13 (38.2%)	14 (38.9%)	0.575
Sepsis proven in first 72 hours, Count (%)	0 (0%)	1 (2.78%)	0.514
Courses of antibiotics in >72 hours, Median (IQR)	1 (1-3)	2 (0.5-3)	0.947
BC positive sepsis in >72 hours, Count (%)	14 (41.2%)	13 (36.1%)	0.942
Any IVH, Count (%)	14 (41.2%)	12 (33.3%)	0.588
Severe IVH (grade 3 or 4), Count (%)	2 (5.9%)	7 (19.4%)	0.32
PVL, Count (%)	2 (5.9%)	6 (16.7%)	0.438
Significant PDA, Count (%)	13 (38.2%)	14 (38.9%)	0.901
Inotropes required?, Count (%)	10 (29.4%)	15 (41.7%)	0.206
Inotrope score ≤20, Count (%)	8 (23.5%)	8 (22.2%)	0.561
NEC, Count (%)	9 (26.5%)	17 (47.2%)	0.173
NEC ≥bell stage II, Count (%)	4 (11.8%)	8 (22.2%)	0.667
ROP, Count (%)	16 (47.1%)	11 (30.6%)	0.236
ROP ≥ Stage 3, Count (%)	7 (20.6%)	5 (13.9%)	0.445

BC – Blood Culture, IVH – Intraventricular Haemorrhage, PVL – Periventricular leukomalacia, PDA – Patent Ductus Arteriosus, NEC – Necrotising Enterocolitis, ROP – Retinopathy of Prematurity

Table 5.29: Comparison of bacterial growth between the two groups

Bacteria	**High TV N=34 Count (%)**	**Low TV N=36 Count (%)**	**p-value**
Negative	17 (50%)	19 (52.8%)	0.354
CONS	13 (38.2%)	10 (27.8%)	
Staph aureus	0 (0%)	3 (8.3%)	
E coli	1 (2.9%)	0 (0%)	
Enterococcus*	1 (2.9%)	0 (0%)	
Strep porcinus*	1 (2.9%)	0 (0%)	

CONS – Coagulase Negative Staphylococcus Aureus
*1 baby had more than bacteria positive blood culture

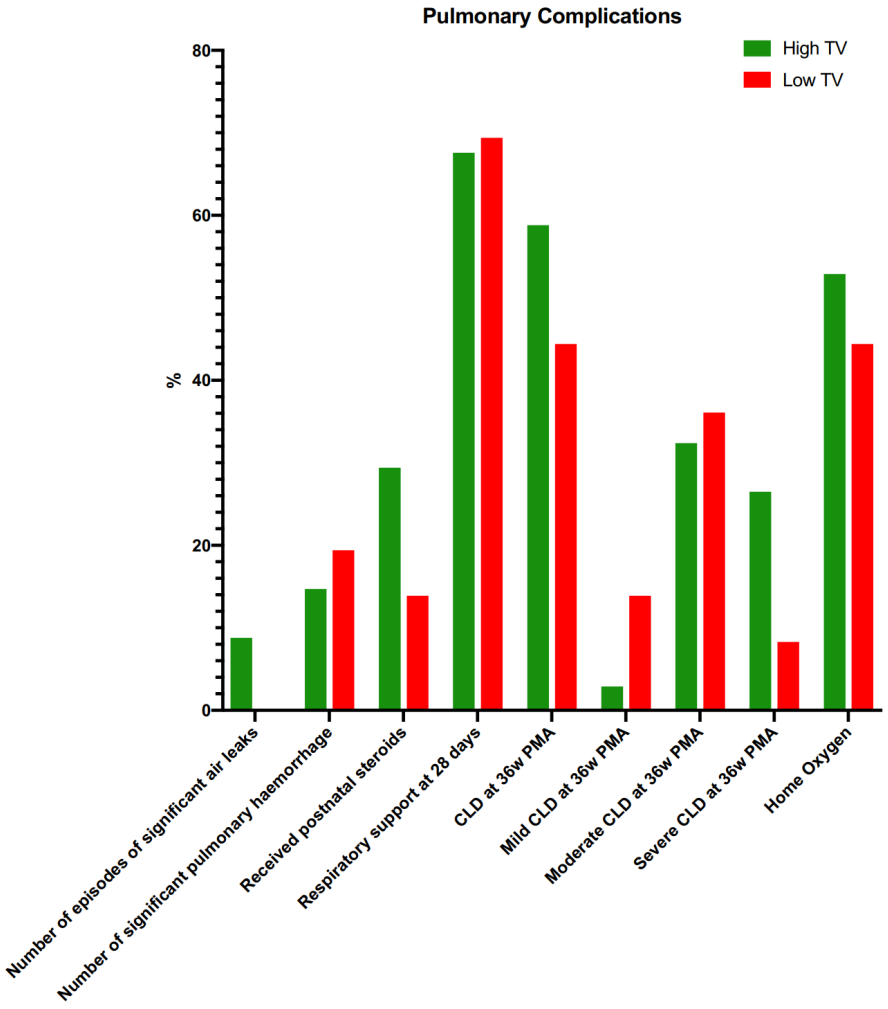


Figure 5.22: Pulmonary complications (% of total babies)

Table 5.30: Comparison of use of therapeutic agents between the two groups

Pharmacological treatment	**High TV** N= 34	**Low TV** N=36	**p-value**
Dose of surfactant in mg/kg, mean (SD)	245.3 (105.4)	230.5 (66.5)	0.486
Postnatal steroids for BPD, count (%)	10 (29.4%)	5 (13.9%)	0.201
Ibuprofen for PDA, count (%)	5 (14.7%)	6 (16.7%)	0.542

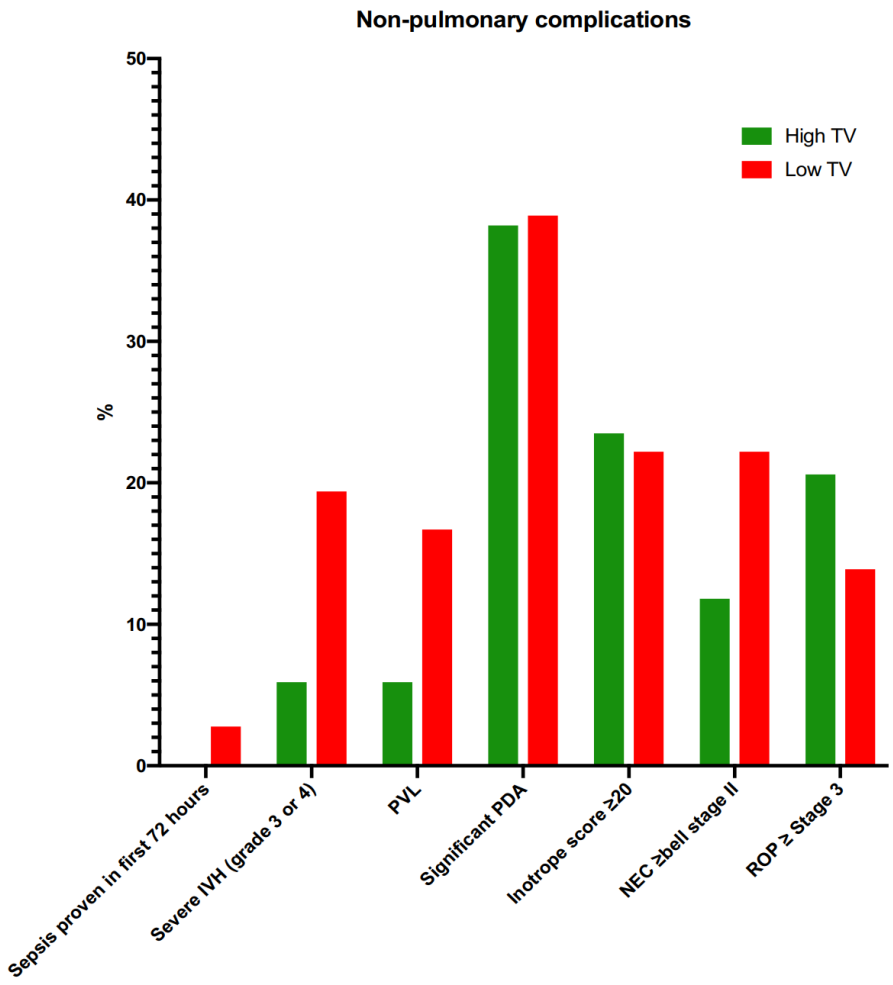


Figure 5.23: Non-pulmonary complications (% of total babies)

of days admitted and birth weight was – 19.7 and 19.6 gram/kg/day respectively. None of them showed any significant difference between the two groups. The data was also assessed using weight z-score (Fenton's) and when the two groups were compared, the mean change in z-score were -1.65 and -1.45 respectively in high and low tidal volume group. This was also not statistically significant (p=0.335, Table 5.31, Figure 5.24, Figure 5.25).

Table 5.31: Comparison of weight gain during hospitalisation in two groups

Weight gain	High TV	Low TV	p-value
Total weight gain, mean (SD)	1466.1 (613.4)	1566.17 (637.2)	0.541
daily weight gain, mean (SD) g/kg/day	19.7 (6.4)	19.6 (4.9)	0.966
Change in the z-score, mean (SD)	-1.65 (0.92)	-1.45 (0.57)	0.335

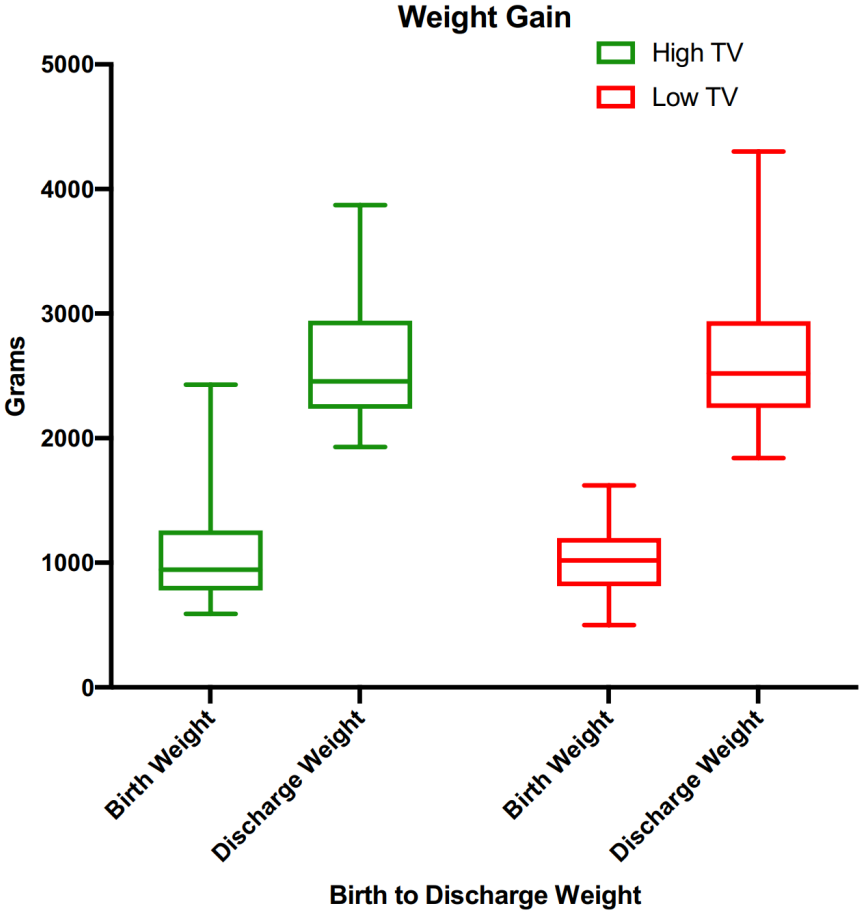


Figure 5.24: Weight gain from birth to discharges

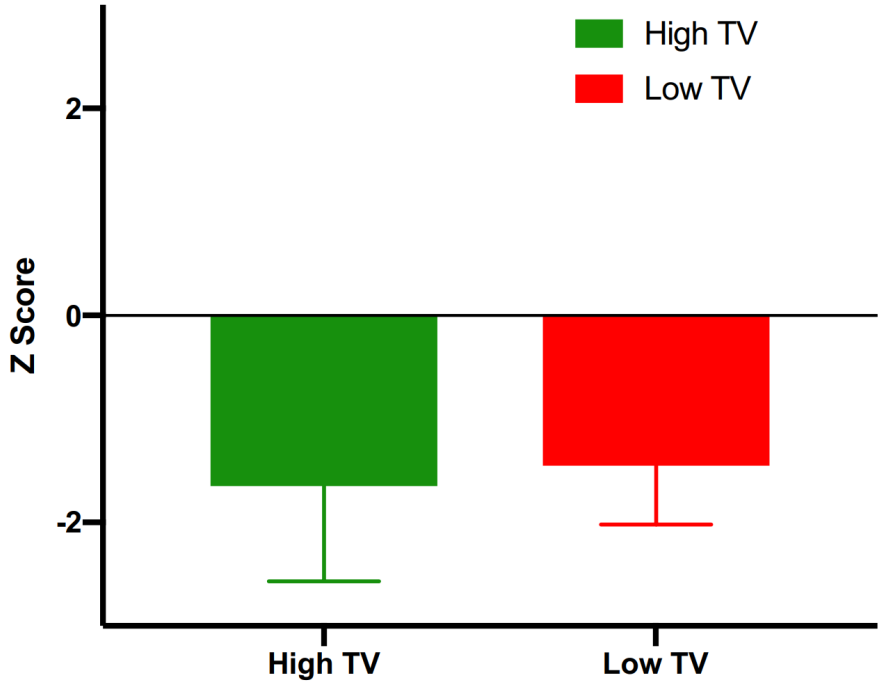


Figure 5.25: Change in z-score

5.12 Cytokines/Inflammatory markers

We obtained tracheal aspirate at pre-intubation and pre-extubation/48 hours.

The baseline characteristic of the inflammatory markers were not different between the two groups (Table 5.32). Many babies did not have a second sample because: it was early extubation, patient exited, patient transferred etc. Hence there were 28 pairs of sample of the babies. We then compared the difference between the two samples in the same baby (Table 5.33). The difference between the two groups were not statistically significant.

Table 5.32: Comparative analysis of pre-intubation tracheal aspirates between the two groups

Inflammatory marker	High TV	Low TV	p-value
IL-6, mean (SD)	2.74 (4.14)	2.65 (4.2)	0.933
IL-8, mean (SD)	53.94 (159.5)	7.31 (12.89)	0.158
TNF-α, mean (SD)	0.15 (0.32)	0.17 (0.37)	0.864

IL-6 – Internleukin 6, IL8-8 – Internleukin 8, TNF-α Tumour Necrosis Factor – Alpha

Table 5.33: Comparative analysis of change in tracheal aspirates between the two groups

Difference between pre and post intubation	High TV	Low TV	p-value
IL-6, mean (SD)	- 0.02 (4.02)	0.57 (3.2)	0.727
IL-8, mean (SD)	- 58.9 (189.89)	26.18 (32.27)	0.178
TNF-α, mean (SD)	-0.07 (0.19)	-0.06 (0.28)	0.924

IL-6 – Internleukin 6, IL-8 – Internleukin 8, TNF-α – Tumour Necrosis Factor – Alpha

5.13 Survival to discharge

Overall 70 babies were enrolled in the trial. Out of which 34 and 36 babies were randomised to high and low TV arm respectively. 5 and 10 babies exited due to meeting the exit criteria in high and low arm respectively before achieving the primary outcome.

On intention to treat analysis, out of total 70 enrolled babies, 30/34 and 29/36 babies survived to discharge home in high and low tidal volume respectively.

Out of these, 20/34 and 16/36 babies were still requiring oxygen at 36 weeks post menstrual age in in high and low arm respectively. Eventually 26 babies survived without chronic lung disease, out of which, 11 and 13 babies were in high and low arm respectively (p=0.80, Table 5.34, Figure 5.26).

Table 5.34: Comparison of Survival to discharge between two groups

	High TV N=34 Count (%)	**Low TV N=36 Count (%)**	**p-value**
Exited	5 (14.7%)	10 (27.8%)	0.398
Survived to discharge	30 (88.2%)	29 (80.6)	0.515
Survived to discharge without BPD at 36 weeks PMA	11(32.3%)	13 (36.1%)	0.804

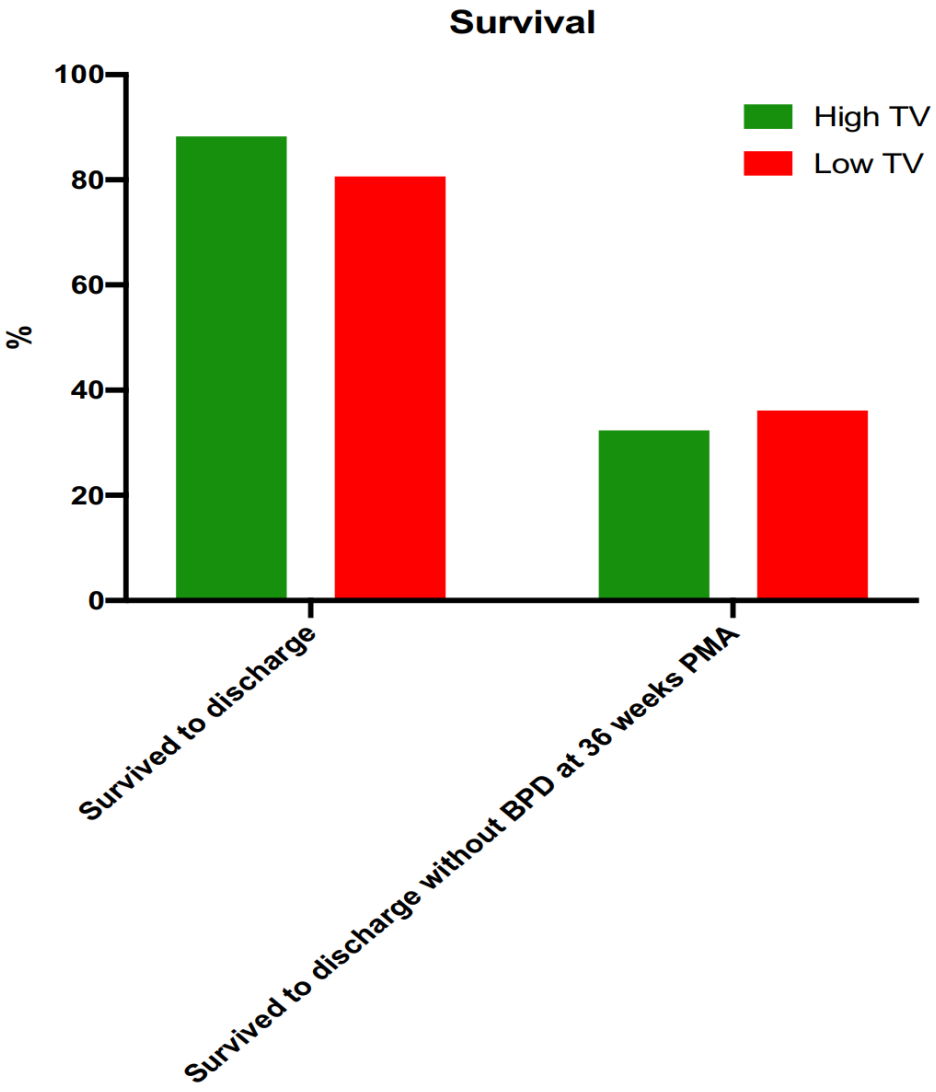


Figure 5.26: Comparison of survival between two groups

5.14 Profile of enrolled babies who did not survive

Out of the 11 babies died, 9 remained intubated until death. Four babies died in the high tidal volume group as opposed to 7 babies in the low tidal volume group. Of all these 7 babies had exited before death (4 in high TV and 3 in low TV). The common causes of death were – extreme prematurity, multiple organ failure, severe IVH, pulmonary haemorrhage, maternal chorioamnionitis, pneumothorax, sepsis, NEC, intestinal perforation, severe BPD, severe RDS, and pulmonary hypoplasia as shown in Table 5.35.

5.15 Comparison of study infants and eligible non enrolled infants

This section compares the study infants with the eligible but not enrolled infants for the study period. During the study period there were 27 infants who fulfilled the inclusion criteria but could not be enrolled. The reason for non-enrolment of these infants are depicted in Table 5.36. Out of these, 12 died before discharge.

5.15.1 Baseline characteristics

The unenrolled infants were compared with enrolled infants to identify any possible underlying reasons or patterns behind their unenrollment. (Table 5.37, Figure 5.27).

5.15.2 Ventilation in study infants versus not-enrolled infants

High frequency mode was the most common mode of ventilation for unenrolled infants. The rest of the modes are shown in table 5.38.

There was no difference in duration of mechanical ventilation between two groups. The median duration of mechanical ventilation in study infants cohort was 37.4

Table 5.35: Infant characteristics of the enrolled babies who did not survive

Study ID	Study Arm	Gestation	Birth weight	Gender	Achieved Primary outcome?	Duration of MV(hrs)	Alive for (hrs)	Cause of death
HNTH 001	Low	25+3	560	M	Yes	Never extubated	48	Extreme prematurity Severe pulmonary haemorrhage Donor of twin to twin transfusion
HNTH 005	High	25+0	820	F	No	Never extubated	59.42	Extreme prematurity Pneumothorax Chorioamnionitis
HNTH 009	Low	23+4	760	M	No	Never extubated	360.03	Extreme prematurity Acute kidney injury Severe bilateral IVH
HNTH 010	Low	27+2	1195	M	Yes	4.22	932.77	Multiple organ failure Bilateral grade 4 IVH Prematurity
HNTH 023	Low	23+4	550	M	Yes	Never extubated	199.12	Extreme prematurity Intestinal perforation IVH with hydrocephalus
HNTH 024	Low	23+4	500	M	No	Never extubated	160.38	Extreme prematurity Severe RDS
HNTH 028	High	26+3	870	M	No	1475.57	3254.97	Severe BPD Pulmonary hypoplasia PPROM
HNTH 031	Low	28+1	1050	F	Yes	Never extubated	63.93	Multiple organ failure Severe E Coli sepsis Maternal chorioamnionitis
HNTH 032	Low	25+1	800	F	No	Never extubated	71.75	Extreme prematurity Severe IVH Placental abruption
HNTH 033	High	23+1	660	M	No	Never extubated	40.67	Extreme prematurity Severe pulmonary haemorrhage Placental abruption
HNTH 035	High	26+6	910	F	No	Never extubated	49.63	Extreme prematurity Severe RDS

IVH – Intraventricular Haemorrhage, RDS – Respiratory Distress Syndrome, BPD – Bronchopulmonary Dysplasia, PPROM – Prolonged Premature Rupture of Membranes

Table 5.36: Reasons for non-enrolment

Reason	**No of babies (N=27) Count (%)**
Born in very poor condition	10 (37%)
Consultant decision	12 (44.4%)
Severe congenital anomaly	2 (7.4%)
Refused consent	3 (11.1%)

Table 5.37: Comparison of baseline characteristics of the enrolled and non-enrolled infants in the study

Variable	**Enrolled infants N=70**	**Unenrolled infants N=27**	**p-value**
Birth weight in grams, mean (SD)	1021.04 (389.6)	1044.23 (334.1)	0.771
Gestation in weeks, mean (SD)	27.31 (3.05)	27.40 (2.14)	0.888
Male sex, count (%)	44 (69.8%)	19 (70.4%)	0.636
Ethnic group- White British, count (%)	67 (95.7%)	23 (85.2%)	0.92
Singleton, count (%)	51 (72.9%)	18 (66.7%)	0.619
Normal Serology and Anomaly Scan, count (%)	63 (90%)	24 (88.9%)	0.836
Normal Vaginal Delivery, count (%)	41 (58.6%)	12 (44.4%)	0.258
2 doses of Antenatal steroids, count (%)	64 (91.4%)	16 (59.3%)	**0.001***
Admission temperature in ^{o}C, mean (SD)	36.4 (1.36)	36.5 (0.88)	0.632
CRIB 2 score, mean (SD)	10.04 (4.89)	9.14 (3.5)	0.332

VG – Volume Guarantee, HFO – High-Frequency Oscillation Ventilation, Vol AC – Volume Assist Control, Pr AC – Pressure Assist Control

*Baby born in extremely poor condition and died before could be commenced on formal ventilation

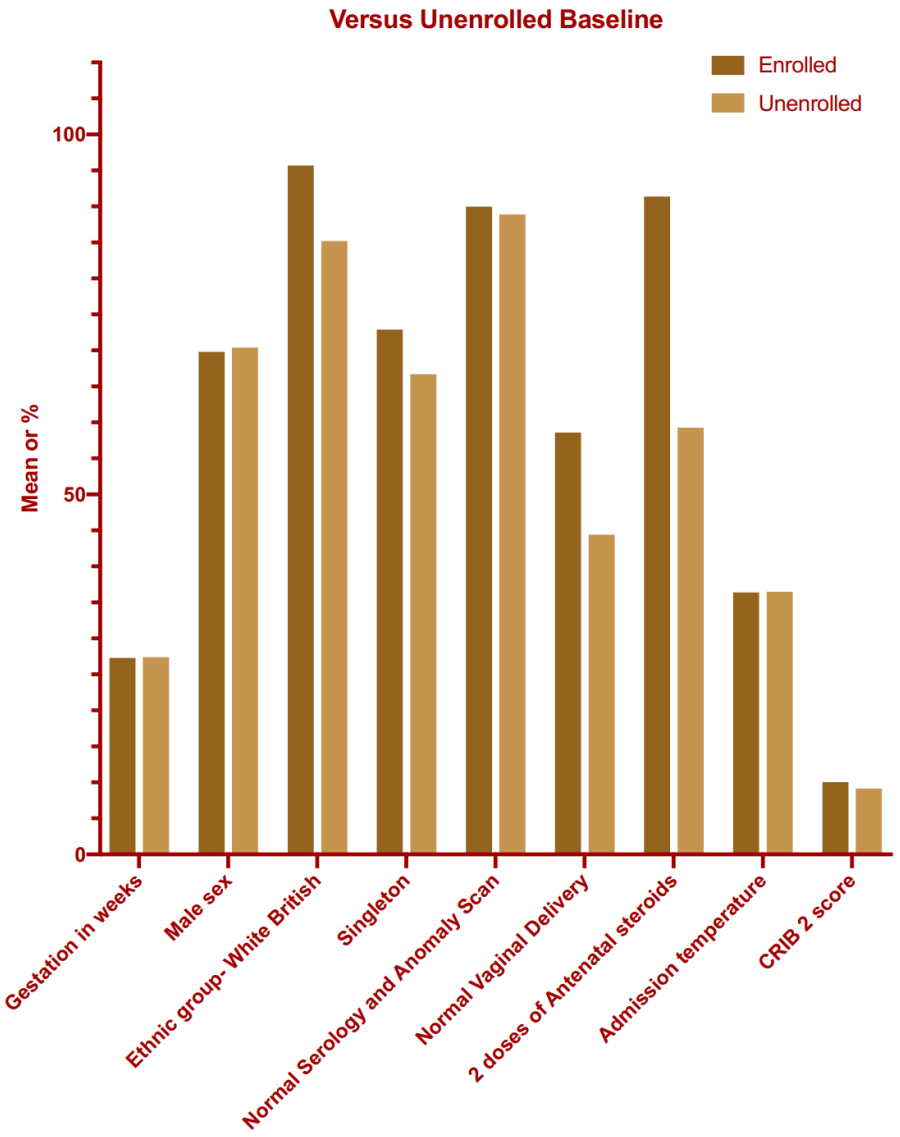


Figure 5.27: Comparison of baseline characteristics of the enrolled and non enrolled infants in the study

hours (95% CI, 18.96-55.95 hrs) and median duration of mechanical ventilation in those not enrolled was 96.57 hours (95% CI, 0 – 239.8 hrs) (p= 0.358, Table 5.39, Figure 5.28).

Table 5.38: Primary mode of ventilation for non-enrolled babies

Primary mode of ventilation	**No of babies (N=27) Count (%)**
VG	7 (25.9%)
HFO	8 (29.6%)
Vol AC	5 (18.5%)
Pr AC	6 (22.2%)
Never ventilated in NNU*	1 (3.7%)

*Baby perished before admission to NNU

Table 5.39: Comparison of duration of mechanical ventilation in enrolled versus not-enrolled infants

	Enrolled N=70 Median (95 % CI)	**Unenrolled N=27 Median (95 % CI)**	**p-value**
Duration of mechanical ventilation, hrs	37.4 (18.96-55.95)	96.57 (0 – 239.8)	0.358

5.15.3 Survival and complications related to prematurity

There were 11/70 (15.7%) and 12/27 (44.4%) deaths in enrolled and non-enrolled infants respectively which was statistically significant (p=0.<01).

There were also differences in incurring complications of prematurity between the two groups. The groups had significant differences in severe IVH (12.9% versus 25.%, p=<0.05), PVL (11.4% versus 3.7%, p=<0.05), significant ROP (17.1% versus 7.4%, p=<0.05), respiratory support at 28 days (68.6% versus 51.9%, p=<0.05), need for home oxygen (48.6% versus 14.8%, p=<.01) and survival (84.3% versus 55.6%, p =<.01). The difference in medium term complications can be explained on the basis that almost 45% of unenrolled babies did not survive

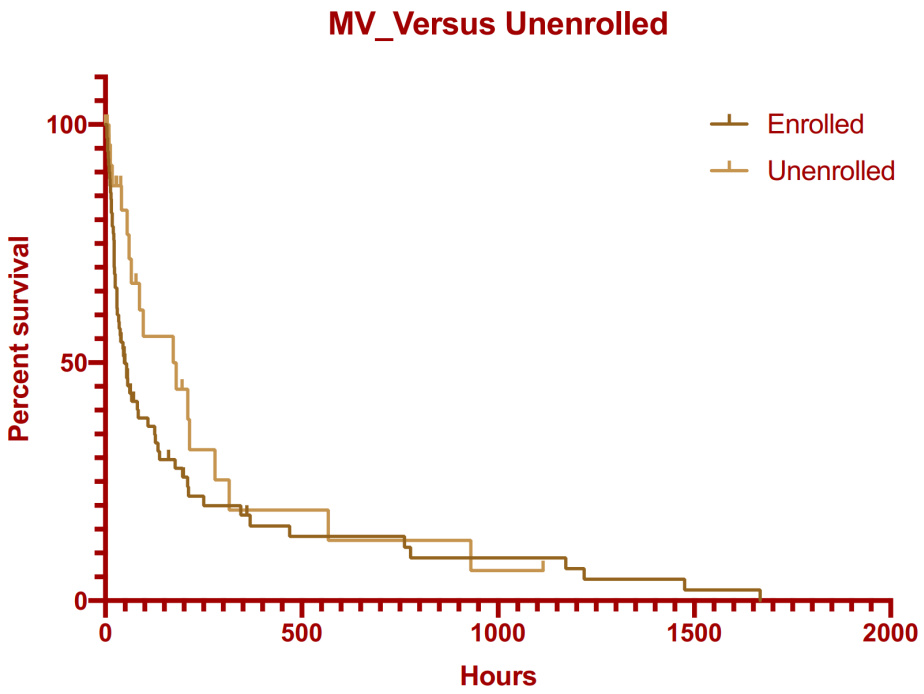


Figure 5.28: Kaplan Meier survival curves for the duration of mechanical ventilation in babies enrolled or not enrolled in the study

and hence the denominator was artificially higher in the group (Table 5.40, Figure 5.29).

5.16 Summary of important positive and negative findings

1. The trial enrolled babies from July 2013 to October 2016 and recruited 70 infants. (Table 5.1).

2. Comparison of maternal characteristics: there were no differences between the two groups. (Table 5.3).

3. Baseline infants' characteristics: No differences in demographics or clinical characteristics. (Table 5.8).

4. Primary Outcome measures

Table 5.40: Comparison of complications of prematurity of the enrolled and non-enrolled infants of the study

Parameter	Study-group (N=70) Count(%)	Non-enrolled infants (N=27) Count(%)	p-value
Significant Air Leak	3 (4.3%%)	4 (14.8%)	0.147
Significant Pulmonary haemorrhage	12 (17.1%)	4 (14.8%)	0.457
Severe IVH	9 (12.9%)	7 (25.9%)	**<0.05***
PVL	8 (11.4%)	1 (3.7%)	**<0.05***
NEC ≥2A	12 (17.1%)	1 (3.7%)	**<0.0005***
PDA requiring Medical/surgical treatment	9/34 (26.4%)	7/36 (19.4%)	0.574
BPD – 28 days	48 (68.6%)	14 (51.9%)	**<0.05***
BPD – 36 weeks PMA	36 (51.4%)	10 (37%)	0.18
Home oxygen	34 (48.6%)	4 (14.8%)	**<.01***
Survived	59 (84.3%)	15 (55.6%)	**<.01***
Survived without BPD at 36 weeks PMA	24 (34.3%)	6 (22.2%)	0.183

IVH– Intraventricular Haemorrhage, PVL – Periventricular leukomalacia, NEC – Necrotising Enterocolitis, ROP – Retinopathy of Prematurity, PDA – Patent Ductus Arteriosus, BPD – Bronchopulmonary Dysplasia

- The median duration of 25% drop in high and low TV group was 13.8 hours (95% CI, 6.4-21.3) and 18.1 hours (95% CI, 10.4-25.8) respectively. The log rank statistic for equality of survival distribution for randomisation arm was not significant (p=.931) (Table 5.12).

- On multiple linear regression analysis, gestation age less than 28 weeks, fractional inspired oxygen (FIO_2) and fluids in first week turned out to be significantly associated with primary outcome (Table 5.14).

- There were no differences between the two groups on subgroup analysis based on birth weight (≤1000 gram versus >1000 gram), gestation age (≤28 weeks versus >28 weeks) and severity of lung disease (as assessed by alveolar arterial oxygen gradient (A-aDO_2) and oxygenation index (OI) at initial ventilation) (Table 5.15, Table 5.16, Table 5.17, Table 5.18).

5. Duration of mechanical ventilation

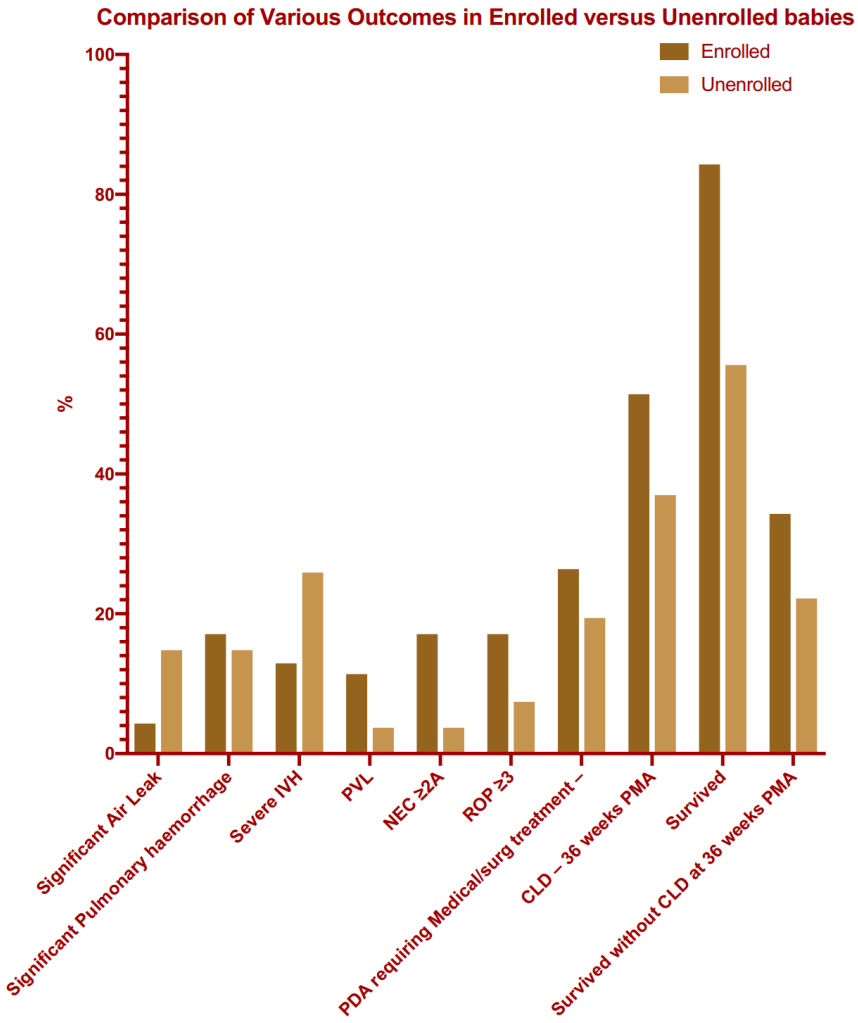


Figure 5.29: Comparison of Outcomes between enrolled and unenrolled babies

- The median duration of mechanical ventilation in the high TV group was 35 hours (95% CI (9.8-60.2 hrs)) as compared to 61.8 hours (95% CI 14.4 – 109.2 hrs) in low TV group. The log rank statistic for equality of survival distribution for randomisation arm was not significant (p=0.928) (Table 5.19).

- There were no differences between the two groups on subgroup analysis based on birth weight ($\leq$1000 gram versus >1000 gram), gestation age ($\leq$28 weeks versus >28 weeks) and severity of lung disease (as assessed by alveolar arterial oxygen gradient (A-aDO_2) and oxygenation index (OI) at initial ventilation)

(Table 5.20, Table 5.21).

6. Duration of admission

- The duration of admission was compared between the two study groups. The median duration of admission in high tidal volume group was 1932.7 hrs (95% CI 1416.1 – 2449.2 hrs) as compared to 1762.3 hrs (95% CI 1576.7 – 1957). The log rank statistic for equality of survival distribution for randomisation arm was p= 0.304 (Table 5.22).

7. Efficacy of the ventilation

- Mean (SD) tidal volume was different between the two groups – 6 (1.6) and 4.4 (1.4) in high and low tidal volume group respectively (p=<0.0001). There was no difference in minute volumes of the two groups. Mean (SD) set RR for high and low TV group was 33.7 (6.7) and 38.5 (10.2). This was statistically significant (p=<0.05). There was also difference in two groups for mean PIP which was proportional to the difference in the tidal volume in both statistical and clinical significance. While mean $paCO_2$ was not different between the two groups, the number of low $paCO_2$ values were higher in high tidal volume group (61.3% versus 32.6%, p=<.005) (Table 5.23).
- The mean maximum mean airway pressure in high tidal volume group was 12.3 .as compared to 10.1 cm H_2O in low tidal volume group. This was statistically significant (<.0001) probably because of increased peak inspiratory component in achieving the tidal volume (Table 5.24).

8. Duration of respiratory support in two groups: No differences between the two groups in duration of non-invasive (CPAP or HHFNC) or combined respiratory support (Table 5.25).

9. Other outcome measures

- Pulmonary complications: No differences in short term pulmonary complications (air leak, pulmonary haemorrhage) or medium term pulmonary complications (BPD at 28 days' life and 36 weeks PMA, severity of BPD, requirement and amount of home oxygen) (Table 5.26, Table 5.27).

- Non-pulmonary complications: There were no differences between the two groups when compared for sepsis, IVH, NEC, PDA and ROP (Table 5.28).

10. Use of Therapeutic agents: no differences in use of surfactant, postnatal steroids or ibuprofen (Table 5.30).

11. Weight gain/postnatal growth: there were no differences between the two groups while comparing for total weight gain, weight gain per day and change in z scores (Table 5.31).

12. Cytokine/inflammatory markers: no differences between the baseline or change in cytokines (IL6, IL8 and TNF alpha) (Table 5.32, Table 5.33).

13. Survival to discharge: no differences in number of babies that died or survived without BPD at 36 weeks (Table 5.34).

14. Profile of babies who did not survive: on analysing the profile of 11 babies who did not survive the admission, the common causes of death were found to be - extreme prematurity, multiple organ failure, Severe IVH, pulmonary haemorrhage, maternal chorioamnionitis, pneumothorax, sepsis, NEC, intestinal perforation, severe BPD, severe RDS, and pulmonary hypoplasia (Table 5.35).

15. Comparison of enrolled versus eligible non-enrolled infants: There were no differences in baseline demographic and clinical characteristics (Table 5.37).There was no difference in duration of mechanical ventilation (Table 5.39). However there were differences in incurring complications of prematurity between the two groups. The groups had significant differences in severe IVH (12.9% versus 25.9%, p=<0.05), PVL (11.4% versus 3.7%, p=<0.05), significant ROP (17.1% versus 7.4%, p=<0.05), respiratory support at 28 days (68.6% versus 51.9%, p=<0.05),

need for home oxygen (48.6% versus 14.8%, p=<0.01) and survival (84.3% versus 55.6%, p =<0.01) (Table 5.40).

Chapter 6

Discussion and Conclusions

6.1 Summary of Main results

6.1.1 Introduction

Preterm infants are born with a varying degree of respiratory distress syndrome known to be proportionate to the degree of prematurity. A moderate to severe spectrum of the disease requires support with mechanical ventilation. Traditionally this support was provided by targeting pressure ventilation. The physiological studies by Dreyfuss and Hernandez et al. (Dreyfuss et al. (1988), Hernandez et al. (1989), Dreyfuss and Saumon (1998)) proposed the superiority of volume targeting. A meta analysis (Klingenberg et al. (2017))vindicated the concept in providing a safer way of providing respiratory support to preterm infants. With developments in technology, hybrid modes like volume guarantee not only provide the best of both the worlds but are sophisticated enough to deliver the tiny volumes. These small volumes can be as low as 2 millilitres, a testament to the use of nanotechnology in modern clinical medicine. While the precision of the technology has helped us to achieve which was considered impossible not long ago it has created a dilemma regarding better tidal volume within the broad range of 4-8 ml/kg. This question has been addressed in adult and paediatric clinical care. In neonatal medicine, there have been a few studies addressing this but they are mostly experimental or

cross-over studies making it difficult for the clinician to base decision making on current evidence. Our study is the first ever clinical study to address this vital question.

6.1.2 Summary of the main results

Babies in both groups were similar at baseline with regards to maternal, demographic and clinical characteristics. There was no difference in the primary outcome of time difference to reach a 25% reduction in baseline peak pressure. There was also no difference in the duration of mechanical ventilation or hospitalisation. There was no difference between the baseline or any change in cytokines (IL-6, IL-8 and TNF-α). Whilst the tidal volume, peak inspiratory pressure and mean airway pressures were different and higher in the assigned high tidal volume group, the minute volume, $paCO_2$ or FIO_2 requirements were not significantly different. There was no difference in short term secondary outcomes (air leak, pulmonary haemorrhage, sepsis, IVH, NEC, PDA and ROP) or medium-term complications (CLD at 28 days' life and 36 weeks PMA, severity of CLD, amount of home oxygen, survival to discharge and survival without BPD at 36 weeks PMA).

The study highlights that it is possible that both tidal volumes ranges selected for this study are at the functional residual capacity and that more considerable differences might be necessary to show a change (Figure 6.1).

6.2 Relationship with other studies

Our study is the first ever randomised controlled trial to compare low versus high tidal volume in a real life clinical situation. Without any precedence for such a study, this warranted an innovation to make it feasible. We chose a 25% reduction in baseline peak inspiratory pressure, as our pilot demonstrated that it was generally the average drop in peak inspiratory pressure when the baby was deemed to be

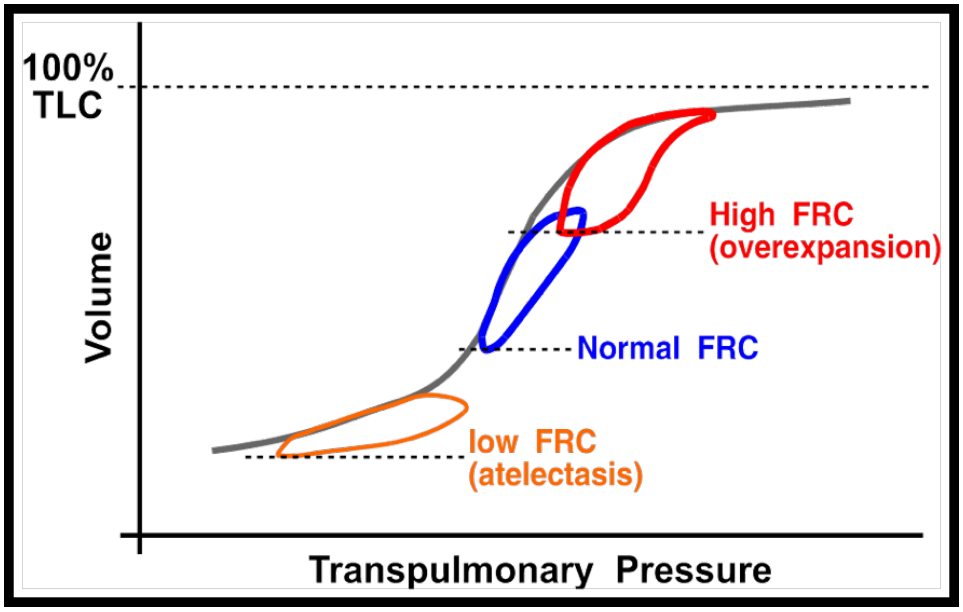


Figure 6.1: Functional Residual capacity

ready for extubation. However, the uniqueness of the parameter also meant that we could not compare this with any previous study. We hope that this will lead to further studies using the same parameters, providing us adequate data to help us interpret this in the future.

The trial did include other parameters as significant secondary outcomes. One of the most studied secondary outcomes is the impact on the duration of mechanical ventilation by the intervention. Sousse et al. (Sousse et al. (2015)), in their prospective cross over study of mechanical ventilation of children with inhalation injury, showed that use of high tidal volume reduced the duration of ventilation. In our study, though the median duration was less in high TV group, the statistical analysis failed to show any significant difference.

Pneumothorax has been correlated with the magnitude of lung injury. Rouby et al. (Rouby and Brochard (2007)) found that the incidence of pneumothorax was significantly higher (8 versus 2) in the patient group ventilated with higher pressures and tidal volumes. Sousse et al. (Sousse et al. (2015)) in their study on children with inhalation injury also demonstrated that children with high tidal

volume had a higher incidence of pneumothorax: 52 (28%) versus 75 (19%) ($p < 0.03$). Our study had 3 (8.8%) infants in high tidal volume group had significant air leak as opposed to 0 (0%) in the low tidal volume group. It was not statistically significant (p=0.109).

In our study, when compared for survival to discharge, there was no difference between the two groups (High TV – 88.2%, Low TV – 80.5%, p= 0.51). Sousse LE et al. (Sousse et al. (2015)) reported that mortality was approximately 22% for those ventilated with high TV, as compared with 15% for those ventilated with low TV which was statistically insignificant ($p = 0.10$). A meta-analysis performed by de Jager et al. (de Jager et al. (2014)) studying the impact of tidal volume on critically ill mechanically ventilated children did not find any difference in mortality of two groups. Hence it can be safely concluded that tidal volumes do not appear to be having an impact on survival to discharge.

Our study showed that while the respiratory rate (RR) in the low TV group was higher as suggested in various other studies, differences between both groups were not statistically significant. As an interesting observation, we also looked at the set RR (back up RR for pressure assist control and SIMV mode) between the two groups and found that this was not only higher in low TV group, but the difference was statistically significant. This could have been because of using SIMV as weaning mode which meant that weaning of support in low TV groups was slower, keeping them intubated longer (median duration of mechanical ventilation, statistically insignificant).

Our study showed that while PIP and TV were altered significantly due to intervention. It showed a higher PIP among infants ventilated with high tidal volume. While this has theoretically plausible explanation, this has also been replicated in the study by Sousse et al. (Sousse et al. (2015)) who showed a significantly increased maximum peak inspiratory pressure (43 ± 18 versus 38 ± 16, $p=< 0.02$) compared with those in patients with LTV. There were no differences in MV and pCO_2. This is consistent with the findings of a prospective interventional study

by Mishra et al(Mishra et al. (2003)) where authors showed that the preterm infants showed fantastic capability to maintain pCO_2 despite the change in PIP by modulating their respiratory rate.

Patel et al. (Patel et al. (2009)) in their study of infants' work of breathing also found that babies had increased work of breathing at lower tidal volume. The authors speculated that this increased work of breathing could be due to the need for increased respiratory rate in order to maintain the minute volume and normocapnoea. While we did not measure the work of breathing as part of our study, our study corroborates a similar observation of increased respiratory rate at lower tidal volumes. In our study, this difference, however, was not statistically significant.

Caffeine

We emphasised having caffeine at the beginning of the mechanical ventilation as it is known to affect ventilator parameters. In fact, Kraaijenga et al. (Kraaijenga et al. (2015)) studied the impact of caffeine on tidal volume and found that it increased tidal volume by a median of 30% (IQR, 7%-48%).

Growth

Deficient postnatal growth is known to be associated with poor neurological outcome for AGA and SGA preterm in EPIPAGE study.(Guellec et al. (2016))Babies in this study were assessed using Fenton z scores. The mean change in z-scores were -1.65 and -1.45 respectively in the high and low tidal volume group. This was also not statistically significant.

$paCO_2$

Hypocapnia is a significant problem in the early stages of life of premature babies. Luyt et al. (Luyt et al. (2001)) in their study of premature infants reported an incidence of inadvertent hyperventilation to the partial pressure of arterial carbon dioxide $PaCO_2$ <25 torrs (<3.3 kPa) was reported in 30% of ventilated newborn infants during the first day of life.

Dawson et al. (Dawson and Davies (2005)) set out to review the arterial carbon dioxide tensions ($paCO_2$) in newborn infants ventilated using synchronised intermittent mandatory ventilation (SIMV) in volume guarantee mode with a TV of 4 mL/kg in preterm infants less than 33 weeks in their prospective observational study. They reported that Severe hypo- or hypercapnoea occurred in 8% of infants at the time of their first blood gas measurement and in <4% of blood gas measurements in the first 48 h. In our study, 43.5% of the values in the low tidal volume group were within range. This was not statistically different from a corresponding value of 27.6% in the high tidal volume group. The difficulty in maintaining normal $paCO_2$ in extremely preterm infants was demonstrated by Cheema et al. (Cheema et al. (2007)) when researchers used volume guarantee with the TV of 4 ml/kg and reported that 100% babies less than 25 weeks' gestation had out-of-range $paCO_2$ values with 2/4 had hypercarbia and 2/4 hypocarbia.

Cytokines

Cytokines are an increasingly studied class of compounds because of their relationship with short term inflammation and long term outcomes. There have been, however, conflicting reports about their relationship to tidal volumes. In a non-blinded prospective randomised controlled trial by Determann et al., (Determann et al. (2010)) the authors reported that in critically ill adult patients, that Plasma interleukin 6 (IL-6) levels decreased significantly more strongly in the lower tidal volume group. The authors concluded that mechanical ventilation with conventional tidal volumes is associated with sustained cytokine production and contributes to the development of lung injury in patients without ALI at the onset of mechanical ventilation. A similar study assessing cytokines levels in preterm infants by Lista et al. (Lista et al. (2006)), found that cytokine (IL-8 and TNF-α) levels collected on day 7 were significantly higher in the low tidal volume group. The authors concluded that when compared with 3mL/kg, ventilation with tidal volume of 5 mL/kg reduced the inflammatory response. In our study, though we did not compare the two (3 mL/kg versus 5 mL/kg), we did not find any differences

between our two groups of low and high tidal volumes.

We speculate that using SIMV as weaning mode did not lead to a drop in PIP as anticipated. Scopesi et al. (Scopesi et al. (2007)) in their cross over study of comparing SIMV and SIPPV with VG in the weaning phase found that PIP was lower in SIPPV as compared to SIMV.

6.3 Justification of methods

We chose SIMV as weaning mode as it is a widely recognised mode for weaning and study. We did not reduce the tidal volume as a means of weaning. This was because, firstly, VG is considered an auto weaning modality as demonstrated by a drop in peak pressures as demonstrated by Lista et al. (Lista et al. (2004)) in their study of using VG against PSV. Secondly, there are concerns about the increasing work of breathing at low tidal volumes as found by Patel et al. (Patel et al. (2009)) who reported an increase in the work of breathing at lower tidal volumes and thus advised against the strategy. Of note, they did not show any difference in work of breathing between pressure assist control (PAC) and synchronised intermittent mandatory ventilation (SIMV) modes while used with volume-targeted ventilation. Chowdhury et al. (Chowdhury et al. (2012)) and Lista et al. (Lista et al. (2006)) also demonstrated that lower tidal volume did not translate into lower work of breathing or less inflammation. Hence while it is a conventional practice of reducing tidal volume as part of weaning, we stuck to maintaining the purity of tidal volume.

Statistical analysis

We used survival statistics using Kaplan Meier and log-rank test to compare the durations rather than a chi-square test. Sousse et al. (Sousse et al. (2015)) in their study have demonstrated the difference practically. The authors found the difference between the two groups which while considered censoring as used in survival statistics, it vanished.

6.4 Strengths of the study

Our study is the first ever effort to compare the two spectra of the tidal volumes in the normal range in the clinical setting. The study dealt with real life clinical dilemmas like the suitable primary outcome and appropriate secondary outcomes. The study is extension of previous smaller studies. As the previous cross over studies demonstrated that a lower tidal volume could be associated with increase in work of breathing, we desisted from using strategy of reducing tidal volume during weaning. We also used cytokines assessment to ascertain the difference between the two groups. The study was randomised controlled trial where randomisation was carried out by blocked envelope to avoid bias. The study groups represented the clinical population and did not have any differences between them which compromised the methodology of efficient randomisation. Finally, during the study we encountered some technical difficulties and we hope that identification of such issues will help and facilitate future studies in this direction.

6.5 Limitations of the study

The study did not have enough participants to satisfy the initial power calculations. In the absence of precedence, we decided to go for a pilot study so that lessons learnt from this study could be used for designing future studies. The study obtained permission for a "waiver of consent" due to the narrow time window required for the randomisation process. However, the narrow window also led to us missing some babies for the study. The study was accompanied by the introduction of volume guarantee in the baby unit and we discovered errors in the software and problems with the hardware, leading to glitches. These glitches appeared in the form of delivery to auto cycling, too high or too low pressures being delivered than expected. Some of these were picked up during the pilot phase and addressed by an update in software. The hardware related issues mainly involved the flow

sensor. The error-prone flow sensors needed replacing. In our experience, reusable flow sensors were found to be more reliable. We learnt that the VG mode is more sensitive to ventilator malfunction than conventional modes and hence instituted a close observation of equipment and a programme of continuing education among staff to deal with such a situation. With more experience in the modality, the staff grew in confidence, and incidences of mishaps came down drastically to allow us to conduct the study with scientific vigour. We are not alone in encountering difficulties in introducing new modes. Keszler et al. (Keszler and Abubakar (2004)) carried out an RCT to compare VG combined with SIPPV to SIPPV alone with regards to maintaining normal tidal volume and normocarbia. The authors highlighted the difficulties associated with the use of new and unfamiliar technologies in which human errors also contributed to the alteration of results. They suggested similar caution in dealing with new and unfamiliar technologies

PIP appeared to be higher than traditional PIP in pressure targeted modes. Polimeni et al. (Polimeni et al. (2006)) in their study of comparing VG with and without SIMV also reported that PIP was higher in babies with VG. We also acknowledge that the infants who received CPR were included in the study and so introducing an element of error related to lung injury.

The absence of precedence meant that sample size analysis was difficult to carry out. We chose to recruit 70 infants as per the feasibility of recruitment. While this is a significant number for a clinical study, it remains a small number for a study to provide conclusive answers. It is possible that sample size may have been too small to detect a real difference in the outcome. Whilst efforts were taken to equipoise the two arms, it was difficult to blind the study from caregivers or the research team.

6.6 Clinical relevance

The study addressed the vital clinical question of differences in low versus high tidal volume in providing mechanical ventilation in preterm infants. While there are several studies to address this in adult and paediatric clinical care it presents a genuine dilemma in neonatal units across the world. Preterm infants are born with respiratory distress syndrome and require mechanical ventilation for life saving support. The modality of mechanical ventilation is known to have short, medium and long term impacts and it remains vital to improve upon the technique to maximise effectiveness while minimising its side effects. Our study is the first ever such effort in the real life clinical setting managing preterm infants with respiratory distress syndrome. Whilst bearing in mind the important limitations of sample size, the study did, however, helped to dispel the myths regarding the perceived risks associated with higher tidal volumes. We understand that it is a negative study with limited final conclusions. The negative results, however, add to confidence about ventilating at higher tidal volumes as demanded by clinical situations. The study, therefore, provides a template for future work to address this important question.

6.7 Summary and Conclusions

The results of the study can be summarised as follows.

The study recruited 70 babies from July 2013 to September 2016. There were no differences in baseline maternal and infants' characteristics between the two groups.

When assessed for the primary outcome measure of time difference to drop in peak pressure by 25%, the median duration of 25% drop in PIP in high and low TV group was 13.8 hours (95% CI, 6.4-21.3 hrs) and 18.1 hours (95% CI, 10.4-25.8 hrs) respectively. The log rank statistic for equality of survival distribution for randomisation arm was not significant (p=.931) (Table 5.12). On multiple linear

regression analysis, gestation age less than 28 weeks, fractional inspired oxygen (FIO_2) and fluids in the first week turned out to be significantly associated with the primary outcome (Table 5.14).

When durations of mechanical ventilation were compared between the two groups, median duration in the high TV group was 35 hours (95% CI 9.8-60.2 hrs)) as compared to 61.8 hours (95% CI 14.4 – 109.2 hrs) in low TV group. The log rank statistic for equality of survival distribution for randomisation arm was not significant (p=0.928) (Table 5.19). Similarly, there were no differences between the two groups in the duration of hospitalisation, non-invasive (CPAP or HHFNC) or combined respiratory support (Table 5.22, Table 5.25).

With regards to the tidal volumes, these were different between the two groups: 6 (1.6) and 4.4 (1.4) in the high and low tidal volume group respectively (p=<.0001). There was also a difference in two groups for mean PIP and MAP which was proportional to the difference in the tidal volume in both statistical and clinical significance. However, there was no difference in minute volumes of the two groups. We also noted that while mean $paCO_2$ was not different between the two groups, the number of low $paCO_2$ values were higher in high tidal volume group (Table 5.23).

We did not find any differences with regards to pulmonary complications (air leak, pulmonary haemorrhage, CLD at 28 days' life and 36 weeks PMA, severity of CLD, requirement and amount of home oxygen) and non-pulmonary complications (sepsis, IVH, NEC, PDA and ROP). Also, there was no difference in number of babies who died or survived without BPD at 36 weeks. We also found that the cytokines (IL-6, IL-8 and TNF-α) levels were comparable in both groups.

In summary, the study was the first ever to compare low versus high tidal volume in preterm infants in clinical circumstances. The study did not show any difference in respiratory or non-respiratory complications of prematurity.

The study also highlighted the difficulties in carrying out a research project with

a narrow eligibility window in critically sick babies. The study speculates that it is possible that both tidal volumes ranges selected for studies are at the functional residual capacity and that more substantial differences might be necessary to show a change.

6.8 Suggestions for further research

From this experience, we would recommend a multicentre randomised controlled trial to address the relevant clinical questions. This, we hope, would provide the necessary numbers to address the essential secondary outcomes of chronic lung disease and survival without chronic lung disease.

We would also recommend devising algorithms to address the technical difficulties in dealing with volume guarantee both for patient care as well as research purpose.

6.9 Reflections

When I took to this project, I was full of enthusiasm. I had participated in other people's trials and looked forward to conducting my own and to make a contribution to improving the lives of babies I am passionate about.

While I was prepared for the hard work, I was not ready for the bureaucracy in research work which takes much time to get things running. I also encountered difficulty in getting everyone on the same page to help recruit babies. While the unit had run trials before, it remained primarily a clinical unit which meant that there was an extra burden on the existing workload. I tried to put in extra hours in acknowledgement of their extra effort to help recruit the patients. It was challenging to convince the ethics committee of the need to permit a "waiver of consent" to randomise babies while awaiting formal consent from parents at a less stressful circumstances. This was to ensure the consistency and vigour of the methodology in maintaining a constant tidal volume. As the majority of the preterm infants

required mechanical support soon after birth, a waiver of consent also meant that we had a very narrow time-window to randomise the baby. This led to missing babies in the early period of the research but this was something we could address, and eventually, we managed to recruit the vast majority of eligible infants.

This was clinical research within the pragmatic working environment and I am grateful to have been funded for the time and for the opportunity to carry this out. The trade off was to return the favour as a clinician, which I later realised was quite demanding. I had to continue the recruitment even after leaving the job to ensure the required babies for the trial. This, and the write, up was to be done while fulfilling other commitments was demanding. I am sure I am not the only one to have had difficulties in the pragmatic clinical research world, but it did appear to be a mammoth task and sometimes nearly impossible. I am grateful to everyone around me, especially my mentors to help me complete this project.

Appendix A

Ethics Approval

NRES Committee North East - Sunderland
Room 002
TEDCO Business Centre
Viking Business Park
Jarrow
Tyne & Wear
NE32 3DT

Telephone: 0191 4283563

22 May 2013

Professor Samir Gupta
Clinical Professor of Neonatology & Consultant Neonatologist
North Tees & Hartlepool NHS Foundation Trust
Department of Neonatal Medicine,
University of Durham & North Tees University Hospital
Hardwick Road, Stockton-on-Tees
TS19 8PE

Dear Professor Gupta

Study title:	**High vs. Low Tidal Volume for Mechanical Ventilation in Preterm Babies with respiratory distress syndrome - A randomised controlled trial**
REC reference:	**13/NE/0110**
IRAS project ID:	**126072**

Thank you for your letter of 15 May 2013, responding to the Committee's request for further information on the above research [and submitting revised documentation].

The further information has been considered on behalf of the Committee by myself as Chair.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Mrs Helen M Wilson, nrescommittee.northeast-sunderland@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a **favourable ethical opinion** for the above research on the basis described in the application form, protocol and supporting documentation [as revised], subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering Letter		
Covering Letter		15 May 2013
GP/Consultant Information Sheets	Version 1	12 March 2013
Investigator CV	Dr Anupam Gupta	
Other: CV	Professor Samir Gupta	05 November 2012
Other: CV	Professor A Pali S Hungin	
Participant Consent Form	Version 3	21 March 2013
Participant Information Sheet	Version 3	21 March 2013
Participant Information Sheet	Version 5	15 May 2013

Protocol	Version 3	21 March 2013
REC application	Version 3.4	22 March 2013
Response to Request for Further Information		15 May 2013

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

13/NE/0110	**Please quote this number on all correspondence**

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely

Mr Paddy Stevenson
Chair

Email: nrescommittee.northeast-sunderland@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: Ms Jane Greenaway, R&D Manager

Dr Anup Gupta

Appendix B

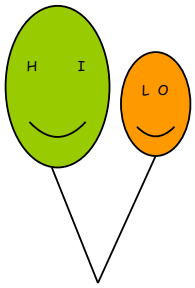
Patient Information Leaflet

Contact Details:

Prof S Gupta, Consultant Neonatologist
Dr C Harikumar, Consultant Neonatologist
Dr B Reichert, Consultant Neonatologist
Dr J Sundaram, Consultant Neonatologist
Dr A Gupta, Neonatal Research Fellow
Ms W Cheadle, Neonatal Research Nurse

North Tees and Hartlepool NHS
NHS Foundation Trust

Information leaflet for Parents



HILO Trial

(High vs. Low Tidal Volume for Mechanical Ventilation in Preterm Babies)

What is the purpose of the study?
Babies born prematurely can have breathing problems due to immaturity of the lungs. This is due to lack of a chemical called surfactant (normally present in the lungs of babies born at full term). Surfactant keeps airways open by preventing collapse of the lungs. A proportion of these premature babies can require help for breathing. This is provided by a plastic tube placed in the wind pipe (endotracheal tube) and attached to a breathing machine called a ventilator.

The ventilator works by pushing a volume of gas (oxygen/air) called the tidal volume into baby's lungs with every breath. The normal reference range for this tidal volume is between 4 and 8 ml per kg. This is a wide range and it is not known whether there are any advantages of targeting tidal volumes at narrow upper or lower end of normal range.

Why am I being asked to participate in the study?
You are being asked to take part in this study as your baby is born very early (more than 8 weeks before the due date) and/or weighs less than 1500g and requires breathing support on a ventilator.

Does my baby have to take part?
Taking part in the study is voluntary and your decision would not affect the care provided to your baby.

What will happen to my baby if I agree to take part in the trial?
Your baby will be provided breathing support through a ventilator delivering tidal volumes within normal range. This could be low normal (4-5 ml/Kg) or high normal (7-8 ml/kg) tidal volume from birth. The allocation to these two groups is done using a computer generated random sequence to allow comparison of any differences between the two study groups scientifically.

We would also request for your permission to test the fluids from your baby's windpipe for inflammatory markers. This fluid is routinely suctioned from the windpipe to clear airways and would be discarded after testing. No extra blood tests or injections are

necessary over and above routine care. Your baby would continue to receive the same care on the neonatal unit independent of your decision regarding taking part in the study or not.

What will happen to my baby if I do not agree to take part in the trial?
Your decision whether or not to take part will not affect the care provided to your baby. If you are not able to decide or decline to take part, your baby will receive the same standard care provided to all babies born prematurely and would receive breathing support utilising tidal volume in the normal wide range of 4-8 ml/kg as per current practice.

What are the possible side effects or complications of the treatment?
As, your baby would be ventilated utilising standard practice, there are no perceived side effects of participating in this study. Also, as the study follows standard practice, complications and risks associated with premature birth are expected to be comparable between study groups. The study conforms to the manufacturers' recommendations for the ventilators and utilises the recommended tidal volume range for use in newborn infants.

Irrespective of allotted group, your baby would be monitored very closely by the medical and nursing staff in the neonatal intensive care unit and in accordance with standard practices on the neonatal unit.

What are the possible advantages and disadvantages of taking part in this trial?
While ventilators are life saving machines, they have the potential to cause injury to the lungs. This injury can be in the form of risk of chest infections in the short term and risk of damage to the lungs (chronic lung disease). The results of the study could improve our practice of ventilation and ventilation associated injury in very preterm babies in the future. There are no perceived disadvantages of participating in this trial.

What if new information becomes available?
The clinicians caring for your baby would inform you of any new information that becomes available during the course of the study.

What if something goes wrong?
As this study follows current standard practice, your baby is unlikely to have any additional adverse effects while participating in this trial. During the study period data in all expected or unexpected adverse events will be collected and monitored. You will be covered by the trust indemnity scheme and participating in the trial does not affect your legal rights.

Will taking part in this study be kept confidential?
We assure you that strict policies and procedures will be followed as per best practice guidance issued by the Department of Health in relation to confidentiality. Your GP would be informed about participation of in this study unless you decide not to inform. Your baby's details will be kept securely and will only be seen by the study team and people from the regulatory authorities. Information about your baby will not be used for any purpose other than to answer the research question.

What will happen to the results of the research study?

At the end of the study, the results will be analysed and published anonymously in peer reviewed medical journals and will be presented in medical seminars and conferences. We will send you a summary of the final results of the study if you would like. A copy of the full journal article can be requested from the Chief Investigator. You and your baby will not be identified in any report or publication of the study.

Who is organising and funding this study?
The study is sponsored by University Hospital of North Tees. This study is a topic for a higher degree with the Durham University pursued by the co-investigator.

Who has reviewed the study?
The study has been assessed and approved by Sunderland NHS Research Ethics Committee and Trust Research and Development Committee. It has also been reviewed by parents of babies and by an external expert in the field of newborn ventilation.

Approval means the Committees are satisfied that the study is safe, participants are protected and there are no additional risks. It also assures that you have been given sufficient information to make an informed decision regarding taking part in this study.

What if I have concerns?
If you have any concerns or questions about this study or the way it has been carried out, you could contact the Chief Investigator or member of the team. If you have any questions about this study you may also choose to get information from the Trust Research and Development department (Floor 2, South Wing, University Hospital of North Tees, Hardwick Road Stockton-on-Tees TS19 8PE). You could also alternatively contact **INVOLVE (http://www.invo.org.uk/resourcecentre/ or Tel 02380 651088)** for more advice and support.

If you have any complaint about the conduct of the study, you could also contact PALS (Patient Advice and Liaison Service) Tel 01642 624719/ mobile 07796 958658/ freephone 0800 092 0084 or email at PALS.NT@nth.nhs.uk.

Thank you for reading this information leaflet. Should you desire to discuss the study in more detail a member of the team would be happy to provide further information. Alternatively, the contact details of the study team are provided on this leaflet.

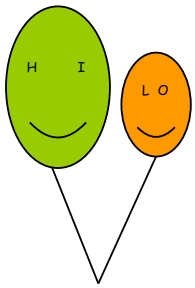
Local contact for your Hospital is

Co-investigator:
Dr Anup Gupta
Neonatal Research Registrar
01642 624247

Chief Investigator:
Professor Samir Gupta
Consultant Neonatologist
01642 624250

Appendix C

Consent Form



North Tees and Hartlepool NHS
NHS Foundation Trust

HILO Trial

Consent from Parents

Neonatal unit
University Hospital of North Tees
Stockton TS19 8PE
Tel: 01642 624247/48

Study No: CH-087 **Hospital Name: University Hospital of North Tees**

Formal Title: High vs. Low Tidal Volume for Mechanical Ventilation in Preterm Babies with respiratory distress syndrome - A randomised controlled trial
Name of Researcher: Dr Anupam Gupta **Please initial each box**

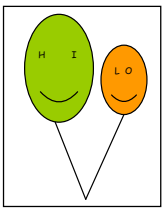
I, ……mother/father of …… Do hereby give my consent for my baby to be included in this study.	
I also give consent for testing of fluid from the windpipe (tracheal aspirate).	
I confirm that I have been given the information leaflet (version 5 May 15 2013) which I have read and understood. I have been given an opportunity to ask my questions which have been answered.	
I can confirm that my baby's participation in this trial is entirely voluntary. I understand what will happen to my baby during this trial. I am aware of my rights to withdraw my baby from the trial at any time. In such an event if I chose to do so, his/her future care or legal rights will not be affected in any way.	
I also give my consent to publish the trial results in scientific journals and to present the data in national and international meetings and conferences provided no personal details will be made available at any stage.	
I understand that relevant sections of any of my baby's medical notes and data collected during the study, may be looked at by responsible individuals from regulatory authorities or from the NHS trust, where it is relevant to my baby taking part in this research. I give permission for these individuals to have access to my baby's records.	
I agree for the Neonatal unit to inform my GP about my baby's participation in this trial	

Name of Parent	Name of health professional taking consent
Signature	Signature
Date	Date

Copy to – Parents
Case notes

Appendix D

Flow Chart



North Tees and Hartlepool NHS
NHS Foundation Trust

HILO PROTOCOL

Birth weight ≤1500 g OR Gestation ≤ 32+0 weeks
Evidence of RDS requiring intubation and ventilation

Exclusion criteria:
1. Serious underlying congenital anomaly
   a. Congenital diaphragmatic hernia
   b. Cyanotic congenital heart disease
   c. Airway anomalies
   d. Abdominal wall defects
2. **Multiple pregnancies – only the first-born will be randomized; the others will receive the same strategy AND WOULD BE ENROLLED**
3. Babies initiated on ventilation after 12 hours of life or transferred from other centres ventilated

Tracheal aspirate @ Birth
and then Surfactant 200 mg/Kg

Randomisation on basis of Birth Weight

- <1000 g
  - High Normal Vt 7 ml/Kg
  - Low Normal Vt 4 ml/Kg
- ≥1000g
  - High Normal Vt 8 ml/Kg
  - Low Normal Vt 5 ml/Kg

Pr A/C+VG
Back up rate 40/min
PEEP 5 cm
PIP – 10 cm above baseline
I time – < 1 Kg - 0.26 and for ≥ 1 Kg - 0.30 sec

Monitor oxygenation and ventilation as standard care
spO2 91-95% and pCO2 4.5-8 cm H2O

FIO2

- <0.3 → Decrease PEEP to 4 cm H2O
- 0.3-0.5 → Continue same
- > 0.5 → Increase PEEP to 6 cm H2O

pCO2 (kPa)

- <4.5 & pH>7.4 → Switch to SIMV mode with back up rate 40/min and No pressure support if MAP≥10 PS 5-6cm H20 if MAP≤10
- 4.5-6.5 → Continue same
- 6.5-8 → Increase rate
- >8 & pH<7.25 → Start HFOV

Collection of tracheal aspirate @ 48 hours/ pre-extubation whichever is earlier

Exit Criteria:
Babies may be discontinued on volume guarantee ventilation and switched to high frequency oscillatory ventilation (HFOV) if any of the following occurs
1. Inadequate ventilation (pH <7.25 and pCO2 >8 kPa);
2. Inadequate oxygenation (FIO2 >0.6 to maintain SpO_2 >91%)
3. Massive pulmonary haemorrhage or pulmonary hypertension

Wean onto SIMV mode with back up rate 40/min and same Vt with pressure support 5-6 cm H2O if
1. >50% breaths are spontaneous
2. FIO2 <0.4
3. MAP <10 cm H2O

→ If switched onto SIMV - Reduce rate by 10 every 6-12 hrs as applicable

Minute ventilation test if
MAP <8
FIO2 <0.3

- Not passed → Continue ventilation and retry after 6-12 hours
- Passed → Extubate

Sequence of events

Enrol if - Intubated at birth OR less than 12 hours of age + GA≤32+0 OR B. wt ≤1500g

Tracheal aspirate before surfactant – sample stays in local fridge/freezer with baby's label and time of collection

Randomised to arm based on randomisation envelope (different for <1Kg and ≥1Kg)

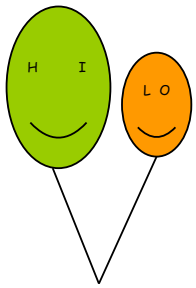
Manage the baby as per standard practice keeping the tidal volume same and weaning by switching over to SIMV and coming down on rate

Second tracheal aspirate at 48 hours or before extubation whichever is earlier

Appendix E

Minute Volume Test Protocol

HILO TRIAL Minute Ventilation TEST



Name

Hospital Number:

Date: **Time:**

Whilst on Mechanical ventilation (SIMV):

Time (mins)	Heart Rate	Respiratory Rate	FIO2	Saturations	Tidal volume	Minute ventilation (Ml/kg/min)	
End of 1st minute						MV1	
End of 2nd Minute						MV2	
End of 3rd minute						MV3	
End of 4th minute						MV4	
End of 5th minute						MV5	
Average Minute ventilation on mechanical ventilation (MV_{simv}): $\frac{MV1+MV2+MV3+MV4+MV5}{5}$ =							

Whilst on ETCPAP:

Time (mins)	Heart Rate	Respiratory Rate	FIO2	Saturations	Tidal volume	Minute ventilation (Ml/kg/min)	
End of 1st minute						CPAP1	
End of 2nd Minute						CPAP2	
End of 3rd minute						CPAP3	
End of 4th minute						CPAP4	
End of 5th minute						CPAP5	
Average Minute ventilation on ETCPAP (MV_{etcpap}): $\frac{CPAP1+CPAP2+CPAP3+CPAP4+CPAP5}{5}$ =							

MV_{etcpap}/MV_{simv} =

Baby is ready for extubation if

- **MV_{etcpap}/MV_{simv} is more than 0.5**
- **No bradycardia needing IPPV**
- **Rise of FIO2 no more than 10%**

Date and time of extubation:

Appendix F

Proforma

HILO Proforma

Patient Number:	**HNTH**
Gestation Age:	
Gender:	**M / F**
Multiple Pregnancy:	Singleton / Twin / Triplet / Quad
Birth weight:	**grams** **centiles**
Weight ≤10th centile:	**Yes / No**
Birth weight z score:	
OFC	**cm** **centiles**
Inborn	**Yes / No**
Randomised to:	**High (7 / 8 ml/kg) OR Low (4 / 5 ml/kg)**

MATERNAL DETAILS:

Booked at	UHNT / UHH /
Parity:	G P
Steroids:	**Complete / Incomplete / None**
PROM (>18 hrs):	Yes / No
Pre-labour ROM:	Yes / No
Chorioamnionitis (↑CRP/WCC/Histology)	Yes / No
Maternal Temperature	Yes / No
Antibiotics in labour	Yes / No
Antepartum H'ge (needing intervention)	Yes / No
Diabetes	Yes / No
PIH	Yes / No
Medical Problems	
Obstetric Problems	

Rubella	Immune / Non-Immune / Not performed
Syphilis	Negative / Positive / Not performed
Hep B	Negative / Positive / Not performed
HIV	Negative / Positive / Not performed
Anomaly scan	Normal / Not performed /

PERINATAL EVENTS:

Reason for delivery:	Spontaneous onset /		
Delivered by :	NVD / EmLSCS / ElLSCS / Ventouse / Forceps		
SP1 –Birth	**Date:**	**Time:**	
Resuscitation:			
Time first Gasp	min		
Time regular respiration	min		
Intubated at Birth	Yes / No		
Intubated at	mins		
Apgars:	@1 min	@5 mins	@10 min
External cardiac massage	Yes / No		
Drugs during resuscitation	No /		
Tracheal aspirate collected?	Yes / No		

NEONATAL COURSE:

SP2 – Admission to NNU	**Date:**	**Time:**
Age admitted to NNU		
Initial Temperature	ºC	
Worst base excess in first 12 hours	kPa	
CRIB 2 score		

SP3 – 1st intubation	**Date:**	**Time:**
Age at ventilation		

CPAP before ventilation Yes / No

Received surfactant Yes / No

Number of surfactant doses

OI admission

A-DO2 on admission

Initial FIO2 Max FIO2

Initial PIP Max PIP

Initial MAP Max MAP

X-ray report of Chest x-ray on admission:

Chest ribs seen

Gases until 1st cycle of MV

Age of Life	Date	Time	C/V/A	pH	PCO2	BE	Comments

SP4 –25% reduction in PIP: **Date:** **Time:**

Age at 25% reduction in PIP

Time to 25% extubation in PIP (SP4-SP3)

SP5 –MVT **Date:** **Time:**

MVT done before ext Yes / No

MVT passed ? Yes / No

MVT number when MVT passed

Ve (M) – MVT passed

Ve (S) – MVT passed

Ve (M) / Ve (S) –

SP6 – Extubation **Date:** **Time:**

Age at extubation

Duration of intubaton (SP6-SP3)

Cydn – Ext

FIO2 – Ext

Total RR

Did baby receive caffeine before extubation Yes / No

Extubated to: CPAP / HHFO2 / LFO2 / SVIA

From -	Changed to:	When: Date	When: Time	Why?	Total Time spent

Met Exit Criteria **Yes / No**

If yes, when Date: Time:

If yes, which ventilation

Total fluid intake in first week of life

SP7 –came off CPAP and remained off for 7 days **Date:** **Time:**

SP8 –came off low flow and stayed off for 7 days **Date:** **Time:**

On CPAP for

Max CPAP pressures

Max CPAP FIO2

On HHFNC for

Max HF Flow

Max HF FIO2

On low flow oxygen for **(SP8-SP6)**

Total duration of invasive respiratory support

Total duration of Invasive and Non-invasive Respiratory support (MV+CPAP+HF)

Tracheal aspirate – 1st / pre-intubation

Tracheal aspirate – 2nd / at 48 hr / pre-extubation

Received steroids? Yes / No

O2 or respiratory support at 28 days Yes / No

O2 or respiratory support at 36 CGA Yes / No

Survival without CLD at 36 weeks Yes / No

Discharged on home oxygen: Yes / No If yes, -

Oxygen trace performed Yes / No

If yes, on how much oxygen

Trace report

Median

Time spent <92%

Time spent <94%

OUTCOME:	**Discharge / Death / Transfer**	
SP9 - Date and time of discharge/death	**Date:**	**Time:**
Age at discharge	days	CGA
If transferred, Base hospital	NA /	
Discharge weight	**grams**	**centiles**
If died, Censor Primary		
If died, Censor death		

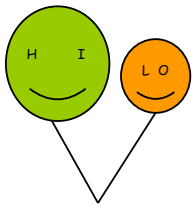
Complications of prematurity:

Sepsis :	Yes / No	
Blood culture positive	Yes / No	
Air Leak	No / Pneumothorax / Pneumomediastinum / drained	
IVH at 1st week	Rt Nil / Gr 1 / 2 / 3 / 4	Lt Nil / Gr 1 / 2 / 3 / 4
IVH at 6th week	Rt Nil / Gr 1 / 2 / 3 / 4	Lt Nil / Gr 1 / 2 / 3 / 4
PVL	Yes / No	
NEC	Nil / Gr 1/ Gr 2 / Gr 3 / Surgically treated	
ROP	Rt- Nil / Stage / Zone ;Lt - Nil/ Stage / Zone ;Lasered	
PDA	Nil Significant / Significant but not treated / Medically treated / Surgically ligated	

Protocol violations (and Other Comments):

Appendix G

Letter to GP



North Tees and Hartlepool NHS
NHS Foundation Trust

To,

Date: / / 201
Neonatal Unit
University Hospital of North Tees
Hardwick Road TS19 8PE
01642 624247/4248

Dear Dr

Re: Baby ……..; DoB……..
Gestation at birth……
Infant of ……; DoB……..
Address……..

Subject: HILO Trial (REC Ref No: ……..)

This is to inform you that above baby was admitted to the neonatal unit in view of prematurity and needed ventilatory support. After obtaining parental consent the infant was randomised to ……..group of the trial. Full results of the trial are not available yet.

A summary of the trial will be made available to the parent when the trial is completed. If the parent wishes a complete published report can be made available by requesting to the chief investigator. The participation into the trial should not change any approach to the healthcare needs in the future.

If you need any further information, please do not hesitate to contact us.

Many thanks.

Yours sincerely

Dr Anupam Gupta
Neonatal research Fellow to
Chief Investigator: Professor Samir Gupta
Consultant Neonatologist
University Hospital of North Tees

Bibliography

Who: recommended definitions, terminology and format for statistical tables related to the perinatal period and use of a new certificate for cause of perinatal deaths. modifications recommended by figo as amended october 14, 1976. *Acta Obstet Gynecol Scand*, 56(3):247–53, 1977.

E. S. Abd El-Moneim, H. O. Fuerste, M. Krueger, A. A. Elmagd, M. Brandis, J. Schulte-Moenting, and R. Hentschel. Pressure support ventilation combined with volume guarantee versus synchronized intermittent mandatory ventilation: a pilot crossover trial in premature infants in their weaning phase. *Pediatr Crit Care Med*, 6(3):286–92, 2005.

K. M. Abubakar and M. Keszler. Patient-ventilator interactions in new modes of patient-triggered ventilation. *Pediatr Pulmonol*, 32(1):71–5, 2001.

J. C. Aldana-Aguirre, M. Pinto, R. M. Featherstone, and M. Kumar. Less invasive surfactant administration versus intubation for surfactant delivery in preterm infants with respiratory distress syndrome: a systematic review and meta-analysis. *Arch Dis Child Fetal Neonatal Ed*, 102(1):F17–F23, 2017.

M. B. Amato, C. S. Barbas, D. M. Medeiros, R. B. Magaldi, G. P. Schettino, G. Lorenzi-Filho, R. A. Kairalla, D. Deheinzelin, C. Munoz, R. Oliveira, T. Y. Takagaki, and C. R. Carvalho. Effect of a protective-ventilation strategy on

mortality in the acute respiratory distress syndrome. *N Engl J Med*, 338(6): 347–54, 1998.

E. P. Argiras, C. R. Blakeley, M. S. Dunnill, S. Otremski, and M. K. Sykes. High peep decreases hyaline membrane formation in surfactant deficient lungs. *Br J Anaesth*, 59(10):1278–85, 1987.

K. P. Bach, C. A. Kuschel, M. H. Oliver, and F. H. Bloomfield. Ventilator gas flow rates affect inspiratory time and ventilator efficiency index in term lambs. *Neonatology*, 96(4):259–64, 2009.

K. P. Bach, C. A. Kuschel, S. B. Hooper, J. Bertram, S. McKnight, S. E. Peachey, V. A. Zahra, S. J. Flecknoe, M. H. Oliver, M. J. Wallace, and F. H. Bloomfield. High bias gas flows increase lung injury in the ventilated preterm lamb. *PLoS One*, 7(10):e47044, 2012.

L. J. Bjorklund, J. Ingimarsson, T. Curstedt, J. John, B. Robertson, O. Werner, and C. T. Vilstrup. Manual ventilation with a few large breaths at birth compromises the therapeutic effect of subsequent surfactant replacement in immature lambs. *Pediatr Res*, 42(3):348–55, 1997.

H. Blencowe, S. Cousens, M. Z. Oestergaard, D. Chou, A. B. Moller, R. Narwal, A. Adler, C. Vera Garcia, S. Rohde, L. Say, and J. E. Lawn. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. *Lancet*, 379(9832):2162–72, 2012.

C. L. Bose, C. E. Dammann, and M. M. Laughon. Bronchopulmonary dysplasia and inflammatory biomarkers in the premature neonate. *Arch Dis Child Fetal Neonatal Ed*, 93(6):F455–61, 2008.

N. Brew, S. B. Hooper, B. J. Allison, M. J. Wallace, and R. Harding. Injury and repair in the very immature lung following brief mechanical ventilation. *Am J Physiol Lung Cell Mol Physiol*, 301(6):L917–26, 2011.

R. G. Brower, C. B. Shanholtz, H. E. Fessler, D. M. Shade, J. White, P., C. M. Wiener, J. G. Teeter, J. M. Dodd-o, Y. Almog, and S. Piantadosi. Prospective, randomized, controlled clinical trial comparing traditional versus reduced tidal volume ventilation in acute respiratory distress syndrome patients. *Crit Care Med*, 27(8):1492–8, 1999.

R. G. Brower, M. A. Matthay, A. Morris, D. Schoenfeld, B. T. Thompson, and A. Wheeler. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med*, 342(18):1301–8, 2000.

A. L. Byrne, M. Bennett, R. Chatterji, R. Symons, N. L. Pace, and P. S. Thomas. Peripheral venous and arterial blood gas analysis in adults: are they comparable? a systematic review and meta-analysis. *Respirology*, 19(2):168–175, 2014.

W. M. Callaghan, M. F. MacDorman, S. A. Rasmussen, C. Qin, and E. M. Lackritz. The contribution of preterm birth to infant mortality rates in the united states. *Pediatrics*, 118(4):1566–73, 2006.

D. P. Carlton, K. H. Albertine, S. C. Cho, M. Lont, and R. D. Bland. Role of neutrophils in lung vascular injury and edema after premature birth in lambs. *J Appl Physiol (1985)*, 83(4):1307–17, 1997.

I. U. Cheema and J. S. Ahluwalia. Feasibility of tidal volume-guided ventilation in newborn infants: a randomized, crossover trial using the volume guarantee modality. *Pediatrics*, 107(6):1323–8, 2001.

I. U. Cheema, A. K. Sinha, S. T. Kempley, and J. S. Ahluwalia. Impact of volume guarantee ventilation on arterial carbon dioxide tension in newborn infants: a randomised controlled trial. *Early Hum Dev*, 83(3):183–9, 2007.

O. Chowdhury, G. F. Rafferty, S. Lee, S. Hannam, A. D. Milner, and A. Greenough. Volume-targeted ventilation in infants born at or near term. *Arch Dis Child Fetal Neonatal Ed*, 97(4):F264–6, 2012.

R. H. Clark, D. R. Gerstmann, A. H. Jobe, S. T. Moffitt, A. S. Slutsky, and B. A. Yoder. Lung injury in neonates: causes, strategies for prevention, and long-term consequences. *J Pediatr*, 139(4):478–86, 2001.

C. J. Cote, A. J. Petkau, J. F. Ryan, and J. P. Welch. Wasted ventilation measured in vitro with eight anesthetic circuits with and without inline humidification. *Anesthesiology*, 59(5):442–6, 1983.

S. E. Courtney, D. J. Durand, J. M. Asselin, M. L. Hudak, J. L. Aschner, C. T. Shoemaker, and G. Neonatal Ventilation Study. High-frequency oscillatory ventilation versus conventional mechanical ventilation for very-low-birth-weight infants. *N Engl J Med*, 347(9):643–52, 2002.

C. A. Crowther, C. J. McKinlay, P. Middleton, and J. E. Harding. Repeat doses of prenatal corticosteroids for women at risk of preterm birth for improving neonatal health outcomes. *Cochrane Database Syst Rev*, (6):CD003935, 2011.

P. G. Davis and C. J. Morley. Volume control: a logical solution to volutrauma? *J Pediatr*, 149(3):290–1, 2006.

C. Dawson and M. W. Davies. Volume-targeted ventilation and arterial carbon dioxide in neonates. *J Paediatr Child Health*, 41(9-10):518–21, 2005.

P. de Jager, J. G. Burgerhof, M. van Heerde, M. J. Albers, D. G. Markhorst, and M. C. Kneyber. Tidal volume and mortality in mechanically ventilated children: a systematic review and meta-analysis of observational studies*. *Crit Care Med*, 42(12):2461–72, 2014.

R. M. Determann, A. Royakkers, E. K. Wolthuis, A. P. Vlaar, G. Choi, F. Paulus, J. J. Hofstra, M. J. de Graaff, J. C. Korevaar, and M. J. Schultz. Ventilation with lower tidal volumes as compared with conventional tidal volumes for patients without acute lung injury: a preventive randomized controlled trial. *Crit Care*, 14(1):R1, 2010.

I. Donald and J. Lord. Augmented respiration; studies in atelectasis neonatorum. *Lancet*, 1(6749):9–17, 1953.

L. W. Doyle, P. G. Davis, C. J. Morley, A. McPhee, J. B. Carlin, and D. S. Investigators. Low-dose dexamethasone facilitates extubation among chronically ventilator-dependent infants: a multicenter, international, randomized, controlled trial. *Pediatrics*, 117(1):75–83, 2006.

D. Dreyfuss and G. Saumon. Ventilator-induced lung injury: lessons from experimental studies. *Am J Respir Crit Care Med*, 157(1):294–323, 1998.

D. Dreyfuss, P. Soler, G. Basset, and G. Saumon. High inflation pressure pulmonary edema. respective effects of high airway pressure, high tidal volume, and positive end-expiratory pressure. *Am Rev Respir Dis*, 137(5):1159–64, 1988.

D. Dreyfuss, P. Soler, and G. Saumon. Mechanical ventilation-induced pulmonary edema. interaction with previous lung alterations. *Am J Respir Crit Care Med*, 151(5):1568–75, 1995.

M. R. El Tahan, L. Pasin, N. Marczin, and G. Landoni. Impact of low tidal volumes during one-lung ventilation. a meta-analysis of randomized controlled trials. *J Cardiothorac Vasc Anesth*, 31(5):1767–1773, 2017.

A. Erdemir, Z. Kahramaner, E. Turkoglu, H. Cosar, S. Sutcuoglu, and E. A. Ozer. Effects of synchronized intermittent mandatory ventilation versus pressure support plus volume guarantee ventilation in the weaning phase of preterm infants*. *Pediatr Crit Care Med*, 15(3):236–41, 2014.

EuroNeoStat. Annual report for very low gestational age infants 2010. the ens project. 2010.

A. A. Fanaroff, B. J. Stoll, L. L. Wright, W. A. Carlo, R. A. Ehrenkranz, A. R. Stark, C. R. Bauer, E. F. Donovan, S. B. Korones, A. R. Laptook, J. A. Lemons, W. Oh, L. A. Papile, S. Shankaran, D. K. Stevenson, J. E. Tyson, W. K. Poole,

and N. N. R. Network. Trends in neonatal morbidity and mortality for very low birthweight infants. *Am J Obstet Gynecol*, 196(2):147 e1–8, 2007.

T. R. Fenton and R. S. Sauve. Using the lms method to calculate z-scores for the fenton preterm infant growth chart. *Eur J Clin Nutr*, 61(12):1380–5, 2007.

H. S. Fischer and C. Buhrer. Avoiding endotracheal ventilation to prevent bronchopulmonary dysplasia: a meta-analysis. *Pediatrics*, 132(5):e1351–60, 2013.

L. Frank and I. R. Sosenko. Development of lung antioxidant enzyme system in late gestation: possible implications for the prematurely born infant. *J Pediatr*, 110(1):9–14, 1987.

U. Frey, J. Stocks, A. Coates, P. Sly, and J. Bates. Specifications for equipment used for infant pulmonary function testing. ers/ats task force on standards for infant respiratory function testing. european respiratory society/ american thoracic society. *Eur Respir J*, 16(4):731–40, 2000.

J. G. Fryer and J. R. Ashford. Trends in perinatal and neonatal mortality in england and wales 1960-69. *Br J Prev Soc Med*, 26(1):1–9, 1972.

E. Futier, J. M. Constantin, C. Paugam-Burtz, J. Pascal, M. Eurin, A. Neuschwander, E. Marret, M. Beaussier, C. Gutton, J. Y. Lefrant, B. Allaouchiche, D. Verzilli, M. Leone, A. De Jong, J. E. Bazin, B. Pereira, S. Jaber, and I. S. Group. A trial of intraoperative low-tidal-volume ventilation in abdominal surgery. *N Engl J Med*, 369(5):428–37, 2013.

O. Gajic, S. I. Dara, J. L. Mendez, A. O. Adesanya, E. Festic, S. M. Caples, R. Rana, J. L. St Sauver, J. F. Lymp, B. Afessa, and R. D. Hubmayr. Ventilator-associated lung injury in patients without acute lung injury at the onset of mechanical ventilation. *Crit Care Med*, 32(9):1817–24, 2004.

L. J. Greenfield, P. A. Ebert, and D. W. Benson. Effect of positive pressure ventilation on surface tension properties of lung extracts. *Anesthesiology*, 25:312–6, 1964.

G. A. Gregory, J. A. Kitterman, R. H. Phibbs, W. H. Tooley, and W. K. Hamilton. Treatment of the idiopathic respiratory-distress syndrome with continuous positive airway pressure. *N Engl J Med*, 284(24):1333–40, 1971.

P. Groneck, B. Gotze-Speer, M. Oppermann, H. Eiffert, and C. P. Speer. Association of pulmonary inflammation and increased microvascular permeability during the development of bronchopulmonary dysplasia: a sequential analysis of inflammatory mediators in respiratory fluids of high-risk preterm neonates. *Pediatrics*, 93(5):712–8, 1994.

A. Grover and D. Field. Volume-targeted ventilation in the neonate: time to change? *Arch Dis Child Fetal Neonatal Ed*, 93(1):F7–13, 2008.

I. Guellec, A. Lapillonne, S. Marret, J. C. Picaud, D. Mitanchez, M. L. Charkaluk, J. Fresson, C. Arnaud, C. Flamand, G. Cambonie, M. Kaminski, J. C. Roze, P. Y. Ancel, and G. Etude Epidemiologique sur les Petits Ages Gestationnels Study. Effect of intra- and extrauterine growth on long-term neurologic outcomes of very preterm infants. *J Pediatr*, 175:93–99 e1, 2016.

S. Guven, S. Bozdag, H. Saner, M. Cetinkaya, A. S. Yazar, and M. Erguven. Early neonatal outcomes of volume guaranteed ventilation in preterm infants with respiratory distress syndrome. *The Journal Of Maternal-Fetal & Neonatal Medicine: The Official Journal Of The European Association Of Perinatal Medicine, The Federation Of Asia And Oceania Perinatal Societies, The International Society Of Perinatal Obstetricians*, 26(4):396–401, 2013.

F. J. Halbertsma, M. Vaneker, G. J. Scheffer, and J. G. van der Hoeven. Cytokines and biotrauma in ventilator-induced lung injury: a critical review of the literature. *Neth J Med*, 63(10):382–92, 2005.

M. Hallman, M. Kulovich, E. Kirkpatrick, R. G. Sugarman, and L. Gluck. Phosphatidylinositol and phosphatidylglycerol in amniotic fluid: indices of lung maturity. *Am J Obstet Gynecol*, 125(5):613–7, 1976.

A. Harada, N. Sekido, T. Akahoshi, T. Wada, N. Mukaida, and K. Matsushima. Essential involvement of interleukin-8 (il-8) in acute inflammation. *J Leukoc Biol*, 56(5):559–64, 1994.

M. Hashizume, N. Hayakawa, M. Suzuki, and M. Mihara. Il-6/sil-6r trans-signalling, but not tnf-alpha induced angiogenesis in a huvec and synovial cell co-culture system. *Rheumatol Int*, 29(12):1449–54, 2009.

P. C. Heinrich, J. V. Castell, and T. Andus. Interleukin-6 and the acute phase response. *Biochem J*, 265(3):621–36, 1990.

L. A. Hernandez, K. J. Peevy, A. A. Moise, and J. C. Parker. Chest wall restriction limits high airway pressure-induced lung injury in young rabbits. *J Appl Physiol (1985)*, 66(5):2364–8, 1989.

C. M. Herrera, T. Gerhardt, R. Everett, N. Claure, G. Musante, and E. Bancalari. Randomized, crossover study of volume guarantee (vg) versus synchronized intermittent mandatory ventilation (simv) in very low birth weight (vlbw) infants recovering from respiratory failure. *Pediatr Res*, 45(4, Part 2 of 2):304A–304A, 1999.

C. M. Herrera, T. Gerhardt, N. Claure, R. Everett, G. Musante, C. Thomas, and E. Bancalari. Effects of volume-guaranteed synchronized intermittent mandatory ventilation in preterm infants recovering from respiratory failure. *Pediatrics*, 110 (3):529–33, 2002.

N. H. Hillman, T. J. Moss, I. Nitsos, and A. H. Jobe. Moderate tidal volumes and oxygen exposure during initiation of ventilation in preterm fetal sheep. *Pediatr Res*, 72(6):593–9, 2012.

J. Hitti, M. A. Krohn, D. L. Patton, P. Tarczy-Hornoch, S. L. Hillier, E. M. Cassen, and D. A. Eschenbach. Amniotic fluid tumor necrosis factor-alpha and the risk of respiratory distress syndrome among preterm infants. *Am J Obstet Gynecol*, 177(1):50–6, 1997.

A. R. Huber, S. L. Kunkel, r. Todd, R. F., and S. J. Weiss. Regulation of transendothelial neutrophil migration by endogenous interleukin-8. *Science*, 254(5028): 99–102, 1991.

H. T. Idriss and J. H. Naismith. Tnf alpha and the tnf receptor superfamily: structure-function relationship(s). *Microsc Res Tech*, 50(3):184–95, 2000.

Y. Imai, T. Kawano, K. Miyasaka, M. Takata, T. Imai, and K. Okuyama. Inflammatory chemical mediators during conventional ventilation and during high frequency oscillatory ventilation. *Am J Respir Crit Care Med*, 150(6 Pt 1):1550–4, 1994.

P. International Committee for the Classification of Retinopathy of. The international classification of retinopathy of prematurity revisited. *Arch Ophthalmol*, 123(7):991–9, 2005.

T. Ishibashi, H. Kimura, Y. Shikama, T. Uchida, S. Kariyone, T. Hirano, T. Kishimoto, F. Takatsuki, and Y. Akiyama. Interleukin-6 is a potent thrombopoietic factor in vivo in mice. *Blood*, 74(4):1241–4, 1989.

B. Jonsson, K. Tullus, A. Brauner, Y. Lu, and G. Noack. Early increase of tnf alpha and il-6 in tracheobronchial aspirate fluid indicator of subsequent chronic lung disease in preterm infants. *Arch Dis Child Fetal Neonatal Ed*, 77(3):F198–201, 1997.

T. Kawano, S. Mori, M. Cybulsky, R. Burger, A. Ballin, E. Cutz, and A. C. Bryan. Effect of granulocyte depletion in a ventilated surfactant-depleted lung. *J Appl Physiol (1985)*, 62(1):27–33, 1987.

M. Keszler. Volume guarantee and ventilator-induced lung injury: Goldilock's rules apply. *Pediatr Pulmonol*, 41(4):364–6, 2006.

M. Keszler. State of the art in conventional mechanical ventilation. *J Perinatol*, 29(4):262–75, 2009.

M. Keszler and K. Abubakar. Volume guarantee: stability of tidal volume and incidence of hypocarbia. *Pediatr Pulmonol*, 38(3):240–5, 2004.

M. Keszler, S. Nassabeh-Montazami, and K. Abubakar. Evolution of tidal volume requirement during the first 3 weeks of life in infants <800 g ventilated with volume guarantee. *Arch Dis Child Fetal Neonatal Ed*, 94(4):F279–82, 2009.

R. Kirby, E. Robison, J. Schulz, and R. A. DeLemos. Continuous-flow ventilation as an alternative to assisted or controlled ventilation in infants. *Anesth Analg*, 51(6):871–5, 1972.

H. Kirpalani, D. Millar, B. Lemyre, B. A. Yoder, A. Chiu, R. S. Roberts, and N. S. Group. A trial comparing noninvasive ventilation strategies in preterm infants. *N Engl J Med*, 369(7):611–20, 2013.

C. Klingenberg, K. I. Wheeler, L. S. Owen, P. I. Kaaresen, and P. G. Davis. An international survey of volume-targeted neonatal ventilation. *Arch Dis Child Fetal Neonatal Ed*, 96(2):F146–8, 2011.

C. Klingenberg, K. I. Wheeler, N. McCallion, C. J. Morley, and P. G. Davis. Volume-targeted versus pressure-limited ventilation in neonates. *Cochrane Database Syst Rev*, 10:CD003666, 2017.

M. Kluckow and N. Evans. Early echocardiographic prediction of symptomatic patent ductus arteriosus in preterm infants undergoing mechanical ventilation. *J Pediatr*, 127(5):774–9, 1995.

T. Kobayashi, K. Nitta, M. Ganzuka, S. Inui, G. Grossmann, and B. Robertson. Inactivation of exogenous surfactant by pulmonary edema fluid. *Pediatr Res*, 29 (4 Pt 1):353–6, 1991.

A. Kozian, T. Schilling, H. Schutze, M. Senturk, T. Hachenberg, and G. Hedenstierna. Ventilatory protective strategies during thoracic surgery: effects of alveolar recruitment maneuver and low-tidal volume ventilation on lung density distribution. *Anesthesiology*, 114(5):1025–35, 2011.

J. V. Kraaijenga, G. J. Hutten, F. H. de Jongh, and A. H. van Kaam. The effect of caffeine on diaphragmatic activity and tidal volume in preterm infants. *J Pediatr*, 167(1):70–5, 2015.

H. Kumar, T. Kawai, and S. Akira. Pathogen recognition by the innate immune system. *Int Rev Immunol*, 30(1):16–34, 2011.

B. Larroque, H. Samain, and E. Groupe. [epipage study: mortality of very premature infants and state of progress at follow up]. *J Gynecol Obstet Biol Reprod (Paris)*, 30(6 Suppl):S33–41, 2001.

B. Larroque, P. Y. Ancel, S. Marret, L. Marchand, M. Andre, C. Arnaud, V. Pierrat, J. C. Roze, J. Messer, G. Thiriez, A. Burguet, J. C. Picaud, G. Breart, M. Kaminski, and E. S. group. Neurodevelopmental disabilities and special care of 5-year-old children born before 33 weeks of gestation (the epipage study): a longitudinal cohort study. *Lancet*, 371(9615):813–20, 2008.

J. A. Lemons, C. R. Bauer, W. Oh, S. B. Korones, L. A. Papile, B. J. Stoll, J. Verter, M. Temprosa, L. L. Wright, R. A. Ehrenkranz, A. A. Fanaroff, A. Stark, W. Carlo, J. E. Tyson, E. F. Donovan, S. Shankaran, and D. K. Stevenson. Very low birth weight outcomes of the national institute of child health and human development neonatal research network, january 1995 through december 1996. nichd neonatal research network. *Pediatrics*, 107(1):E1, 2001.

M. Licker, J. Diaper, Y. Villiger, A. Spiliopoulos, V. Licker, J. Robert, and J. M. Tschopp. Impact of intraoperative lung-protective interventions in patients undergoing lung cancer surgery. *Crit Care*, 13(2):R41, 2009.

G. Lista, M. Colnaghi, F. Castoldi, V. Condo, R. Reali, G. Compagnoni, and F. Mosca. Impact of targeted-volume ventilation on lung inflammatory response in preterm infants with respiratory distress syndrome (rds). *Pediatr Pulmonol*, 37(6):510–4, 2004.

G. Lista, F. Castoldi, P. Fontana, R. Reali, A. Reggiani, S. Bianchi, and G. Compagnoni. Lung inflammation in preterm infants with respiratory distress syndrome: effects of ventilation with different tidal volumes. *Pediatr Pulmonol*, 41 (4):357–63, 2006.

G. Lista, F. Castoldi, S. Bianchi, M. Battaglioli, F. Cavigioli, and M. A. Bosoni. Volume guarantee versus high-frequency ventilation: lung inflammation in preterm infants. *Arch Dis Child Fetal Neonatal Ed*, 93(4):F252–6, 2008.

J. P. Liuzzi, L. A. Lichten, S. Rivera, R. K. Blanchard, T. B. Aydemir, M. D. Knutson, T. Ganz, and R. J. Cousins. Interleukin-6 regulates the zinc transporter zip14 in liver and contributes to the hypozincemia of the acute-phase response. *Proc Natl Acad Sci U S A*, 102(19):6843–8, 2005.

S. M. Lozano and K. M. Newnam. Modalities of mechanical ventilation: Volume-targeted versus pressure-limited. *Adv Neonatal Care*, 16(2):99–107; quiz E1–2, 2016.

K. Luyt, D. Wright, and J. H. Baumer. Randomised study comparing extent of hypocarbia in preterm infants during conventional and patient triggered ventilation. *Arch Dis Child Fetal Neonatal Ed*, 84(1):F14–7, 2001.

C. S. Ma, E. K. Deenick, M. Batten, and S. G. Tangye. The origins, function, and regulation of t follicular helper cells. *J Exp Med*, 209(7):1241–53, 2012.

B. J. Manley, L. S. Owen, L. W. Doyle, C. C. Andersen, D. W. Cartwright, M. A. Pritchard, S. M. Donath, and P. G. Davis. High-flow nasal cannulae in very preterm infants after extubation. *N Engl J Med*, 369(15):1425–33, 2013.

T. R. Martin, B. P. Pistorese, E. Y. Chi, R. B. Goodman, and M. A. Matthay. Effects of leukotriene b4 in the human lung. recruitment of neutrophils into the alveolar spaces without a change in protein permeability. *J Clin Invest*, 84(5): 1609–19, 1989.

S. P. Martin J.A., Osterman M.J. Are preterm births on the decline in the united states? recent data from the national vital statistics system. 39, 2010.

A. D. Maslow, T. S. Stafford, K. R. Davignon, and T. Ng. A randomized comparison of different ventilator strategies during thoracotomy for pulmonary resection. *J Thorac Cardiovasc Surg*, 146(1):38–44, 2013.

K. Matsushima, K. Morishita, T. Yoshimura, S. Lavu, Y. Kobayashi, W. Lew, E. Appella, H. F. Kung, E. J. Leonard, and J. J. Oppenheim. Molecular cloning of a human monocyte-derived neutrophil chemotactic factor (mdncf) and the induction of mdncf mrna by interleukin 1 and tumor necrosis factor. *J Exp Med*, 167(6):1883–93, 1988.

N. McCallion, P. G. Davis, and C. J. Morley. Volume-targeted versus pressure-limited ventilation in the neonate. *Cochrane Database Syst Rev*, (3):CD003666, 2005.

N. McCallion, R. Lau, C. J. Morley, and P. A. Dargaville. Neonatal volume guarantee ventilation: effects of spontaneous breathing, triggered and untriggered inflations. *Arch Dis Child Fetal Neonatal Ed*, 93(1):F36–9, 2008.

M. C. McCormick. The contribution of low birth weight to infant mortality and childhood morbidity. *N Engl J Med*, 312(2):82–90, 1985.

J. Meneses, V. Bhandari, and J. G. Alves. Nasal intermittent positive-pressure ventilation vs nasal continuous positive airway pressure for preterm infants with respiratory distress syndrome: a systematic review and meta-analysis. *Arch Pediatr Adolesc Med*, 166(4):372–6, 2012.

Q. Mian, P. Y. Cheung, M. O'Reilly, G. Pichler, S. van Os, K. Kushniruk, K. Aziz, and G. M. Schmolzer. Spontaneously breathing preterm infants change in tidal volume to improve lung aeration immediately after birth. *J Pediatr*, 167(2):274–8 e1, 2015.

P. Michelet, X. B. D'Journo, A. Roch, C. Doddoli, V. Marin, L. Papazian, I. Decamps, F. Bregeon, P. Thomas, and J. P. Auffray. Protective ventilation influences systemic inflammation after esophagectomy: a randomized controlled study. *Anesthesiology*, 105(5):911–9, 2006.

R. Mishra, S. G. Golombek, S. R. Ramirez-Tolentino, S. Das, and E. F. La Gamma. Low-birth-weight neonates exhibit a physiological set-point to regulate co2: an untapped potential to minimize volutrauma-associated lung injury. *Am J Perinatol*, 20(8):453–63, 2003.

U. K. Munshi, J. O. Niu, M. M. Siddiq, and L. A. Parton. Elevation of interleukin-8 and interleukin-6 precedes the influx of neutrophils in tracheal aspirates from preterm infants who develop bronchopulmonary dysplasia. *Pediatr Pulmonol*, 24 (5):331–6, 1997.

J. G. Muscedere, J. B. Mullen, K. Gan, and A. S. Slutsky. Tidal ventilation at low airway pressures can augment lung injury. *Am J Respir Crit Care Med*, 149(5): 1327–34, 1994.

S. M. Nafday, R. S. Green, J. Lin, L. P. Brion, I. Ochshorn, and I. R. Holzman. Is there an advantage of using pressure support ventilation with volume guarantee in the initial management of premature infants with respiratory distress syndrome? a pilot study. *J Perinatol*, 25(3):193–7, 2005.

A. S. Naik, S. G. Kallapur, C. J. Bachurski, A. H. Jobe, J. Michna, B. W. Kramer, and M. Ikegami. Effects of ventilation with different positive end-expiratory pressures on cytokine expression in the preterm lamb lung. *Am J Respir Crit Care Med*, 164(3):494–8, 2001.

E. Nemeth, S. Rivera, V. Gabayan, C. Keller, S. Taudorf, B. K. Pedersen, and T. Ganz. Il-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J Clin Invest*, 113(9):1271–6, 2004.

A. S. Neto, F. D. Simonis, C. S. Barbas, M. Biehl, R. M. Determann, J. Elmer, G. Friedman, O. Gajic, J. N. Goldstein, R. Linko, R. Pinheiro de Oliveira, S. Sundar, D. Talmor, E. K. Wolthuis, M. Gama de Abreu, P. Pelosi, M. J. Schultz, and P. R. V. N. Investigators. Lung-protective ventilation with low tidal volumes and the occurrence of pulmonary complications in patients without acute respiratory distress syndrome: A systematic review and individual patient data analysis. *Crit Care Med*, 43(10):2155–63, 2015.

K. Nitta and T. Kobayashi. Impairment of surfactant activity and ventilation by proteins in lung edema fluid. *Respir Physiol*, 95(1):43–51, 1994.

J. Northway, W. H., R. C. Rosan, and D. Y. Porter. Pulmonary disease following respirator therapy of hyaline-membrane disease. bronchopulmonary dysplasia. *N Engl J Med*, 276(7):357–68, 1967.

M. Okada, M. Kitahara, S. Kishimoto, T. Matsuda, T. Hirano, and T. Kishimoto. Il-6/bsf-2 functions as a killer helper factor in the in vitro induction of cytotoxic t cells. *J Immunol*, 141(5):1543–9, 1988.

L. A. Papile, J. Burstein, R. Burstein, and H. Koffler. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. *J Pediatr*, 92(4):529–34, 1978.

P. E. Parsons, M. D. Eisner, B. T. Thompson, M. A. Matthay, M. Ancukiewicz, G. R. Bernard, A. P. Wheeler, and N. A. R. D. S. C. T. Network. Lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury. *Crit Care Med*, 33(1):1–6; discussion 230–2, 2005.

D. S. Patel, A. Sharma, M. Prendergast, G. F. Rafferty, and A. Greenough. Work of breathing and different levels of volume-targeted ventilation. *Pediatrics*, 123 (4):e679–84, 2009.

N. Petrucci and W. Iacovelli. Ventilation with smaller tidal volumes: a quantitative systematic review of randomized controlled trials. *Anesth Analg*, 99(1):193–200, 2004.

V. Pierrat, L. Marchand-Martin, C. Arnaud, M. Kaminski, M. Resche-Rigon, C. Lebeaux, F. Bodeau-Livinec, A. S. Morgan, F. Goffinet, S. Marret, P. Y. Ancel, and E.-w. group. Neurodevelopmental outcome at 2 years for preterm children born at 22 to 34 weeks' gestation in france in 2011: Epipage-2 cohort study. *BMJ*, 358:j3448, 2017.

A. Piotrowski, W. Sobala, and P. Kawczynski. Patient-initiated, pressure-regulated, volume-controlled ventilation compared with intermittent mandatory ventilation in neonates: a prospective, randomised study. *Intensive Care Med*, 23(9):975–81, 1997.

V. Poli, R. Balena, E. Fattori, A. Markatos, M. Yamamoto, H. Tanaka, G. Ciliberto, G. A. Rodan, and F. Costantini. Interleukin-6 deficient mice are protected from bone loss caused by estrogen depletion. *EMBO J*, 13(5):1189–96, 1994.

V. Polimeni, N. Claure, C. D'Ugard, and E. Bancalari. Effects of volume-targeted synchronized intermittent mandatory ventilation on spontaneous episodes of hypoxemia in preterm infants. *Biol Neonate*, 89(1):50–5, 2006.

J. Pugin, I. Dunn, P. Jolliet, D. Tassaux, J. L. Magnenat, L. P. Nicod, and J. C. Chevrolet. Activation of human macrophages by mechanical ventilation in vitro. *Am J Physiol*, 275(6 Pt 1):L1040–50, 1998.

V. M. Ranieri, P. M. Suter, C. Tortorella, R. De Tullio, J. M. Dayer, A. Brienza, F. Bruno, and A. S. Slutsky. Effect of mechanical ventilation on inflammatory mediators in patients with acute respiratory distress syndrome: a randomized controlled trial. *JAMA*, 282(1):54–61, 1999.

V. Ratner, S. A. Sosunov, Z. V. Niatsetskaya, I. V. Utkina-Sosunova, and V. S. Ten. Mechanical ventilation causes pulmonary mitochondrial dysfunction and delayed alveolarization in neonatal mice. *Am J Respir Cell Mol Biol*, 49(6): 943–50, 2013.

E. O. Reynolds and A. Taghizadeh. Improved prognosis of infants mechanically ventilated for hyaline membrane disease. *Arch Dis Child*, 49(7):505–15, 1974.

P. B. Rich, C. A. Reickert, S. Sawada, S. S. Awad, W. R. Lynch, K. J. Johnson, and R. B. Hirschl. Effect of rate and inspiratory flow on ventilator-induced lung injury. *J Trauma*, 49(5):903–11, 2000.

P. C. Rimensberger. Neonatal respiratory failure. *Curr Opin Pediatr*, 14(3):315–21, 2002.

C. T. Roberts, L. S. Owen, B. J. Manley, D. H. Froisland, S. M. Donath, K. M. Dalziel, M. A. Pritchard, D. W. Cartwright, C. L. Collins, A. Malhotra, P. G. Davis, and H. T. Investigators. Nasal high-flow therapy for primary respiratory support in preterm infants. *N Engl J Med*, 375(12):1142–51, 2016.

D. Roberts, J. Brown, N. Medley, and S. R. Dalziel. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. *Cochrane Database Syst Rev*, 3:CD004454, 2017.

M. X. Rojas-Reyes, C. J. Morley, and R. Soll. Prophylactic versus selective use of surfactant in preventing morbidity and mortality in preterm infants. *Cochrane Database Syst Rev*, (3):CD000510, 2012.

J. J. Rouby and L. Brochard. Tidal recruitment and overinflation in acute respiratory distress syndrome: yin and yang. *Am J Respir Crit Care Med*, 175(2): 104–6, 2007.

J. J. Rouby, T. Lherm, E. Martin de Lassale, P. Poete, L. Bodin, J. F. Finet, P. Callard, and P. Viars. Histologic aspects of pulmonary barotrauma in critically ill patients with acute respiratory failure. *Intensive Care Med*, 19(7):383–9, 1993.

R. M. Ryan. A new look at bronchopulmonary dysplasia classification. *J Perinatol*, 26(4):207–9, 2006.

B. K. Sandhar, D. J. Niblett, E. P. Argiras, M. S. Dunnill, and M. K. Sykes. Effects of positive end-expiratory pressure on hyaline membrane formation in a rabbit model of the neonatal respiratory distress syndrome. *Intensive Care Med*, 14(5): 538–46, 1988.

F. Scopesi, M. G. Calevo, P. Rolfe, C. Arioni, C. Traggiai, F. M. Risso, and G. Serra. Volume targeted ventilation (volume guarantee) in the weaning phase of premature newborn infants. *Pediatr Pulmonol*, 42(10):864–70, 2007.

N. Seger and R. Soll. Animal derived surfactant extract for treatment of respiratory distress syndrome. *Cochrane Database Syst Rev*, (2):CD007836, 2009.

A. Serpa Neto, S. O. Cardoso, J. A. Manetta, V. G. Pereira, D. C. Esposito, O. Pasqualucci Mde, M. C. Damasceno, and M. J. Schultz. Association between use of lung-protective ventilation with lower tidal volumes and clinical outcomes among patients without acute respiratory distress syndrome: a meta-analysis. *JAMA*, 308(16):1651–9, 2012.

A. Sharma, A. D. Milner, and A. Greenough. Performance of neonatal ventilators in volume targeted ventilation mode. *Acta Paediatr*, 96(2):176–80, 2007.

S. Sharma, K. M. Abubakar, and M. Keszler. Tidal volume in infants with congenital diaphragmatic hernia supported with conventional mechanical ventilation. *Am J Perinatol*, 32(6):577–82, 2015.

S. K. Sinha, S. M. Donn, J. Gavey, and M. McCarty. Randomised trial of volume controlled versus time cycled, pressure limited ventilation in preterm infants with respiratory distress syndrome. *Arch Dis Child Fetal Neonatal Ed*, 77(3):F202–5, 1997.

A. S. Slutsky and L. N. Tremblay. Multiple system organ failure. is mechanical ventilation a contributing factor? *Am J Respir Crit Care Med*, 157(6 Pt 1): 1721–5, 1998.

P. D. Sly, P. K. Nicholls, L. J. Berry, Z. Hantos, and V. Cannizzaro. High tidal volume ventilation does not exacerbate acid-induced lung injury in infant rats. *Respir Physiol Neurobiol*, 189(1):129–35, 2013.

R. Soll and E. Ozek. Prophylactic protein free synthetic surfactant for preventing morbidity and mortality in preterm infants. *Cochrane Database Syst Rev*, (1): CD001079, 2010.

R. F. Soll. Synthetic surfactant for respiratory distress syndrome in preterm infants. *Cochrane Database Syst Rev*, (2):CD001149, 2000.

L. E. Sousse, D. N. Herndon, C. R. Andersen, A. Ali, N. C. Benjamin, T. Granchi, O. E. Suman, and R. P. Mlcak. High tidal volume decreases adult respiratory distress syndrome, atelectasis, and ventilator days compared with low tidal volume in pediatric burned patients with inhalation injury. *J Am Coll Surg*, 220 (4):570–8, 2015.

L. Stern, A. D. Ramos, E. W. Outerbridge, and P. H. Beaudry. Negative pressure artificial respiration: use in treatment of respiratory failure of the newborn. *Can Med Assoc J*, 102(6):595–601, 1970.

A. Stewart, E. O. R. Reynolds, and A. P. Lipscomb. Outcome for infants of very low birthweight: survey of world literature. *The Lancet*, 317(8228):1038–1041, 1981.

G. M. Stoelhorst, M. Rijken, S. E. Martens, R. Brand, A. L. den Ouden, J. M. Wit, S. Veen, and P. Leiden Follow-Up Project on. Changes in neonatology: comparison of two cohorts of very preterm infants (gestational age <32 weeks): the project on preterm and small for gestational age infants 1983 and the leiden follow-up project on prematurity 1996-1997. *Pediatrics*, 115(2):396–405, 2005.

B. J. Stoll, N. I. Hansen, E. F. Bell, S. Shankaran, A. R. Laptook, M. C. Walsh, E. C. Hale, N. S. Newman, K. Schibler, W. A. Carlo, K. A. Kennedy, B. B. Poindexter, N. N. Finer, R. A. Ehrenkranz, S. Duara, P. J. Sanchez, T. M. O'Shea, R. N. Goldberg, K. P. Van Meurs, R. G. Faix, D. L. Phelps, r. Frantz, I. D., K. L. Watterberg, S. Saha, A. Das, R. D. Higgins, H. Eunice Kennedy Shriver National Institute of Child, and N. Human Development Neonatal Research. Neonatal outcomes of extremely preterm infants from the nichd neonatal research network. *Pediatrics*, 126(3):443–56, 2010.

R. M. Strieter, S. L. Kunkel, H. J. Showell, D. G. Remick, S. H. Phan, P. A. Ward, and R. M. Marks. Endothelial cell gene expression of a neutrophil chemotactic factor by tnf-alpha, lps, and il-1 beta. *Science*, 243(4897):1467–9, 1989.

R. M. Strieter, J. A. Belperio, and M. P. Keane. Cytokines in innate host defense in the lung. *J Clin Invest*, 109(6):699–705, 2002.

F. Stuber, H. Wrigge, S. Schroeder, S. Wetegrove, J. Zinserling, A. Hoeft, and C. Putensen. Kinetic and reversibility of mechanical ventilation-associated pulmonary and systemic inflammatory response in patients with acute lung injury. *Intensive Care Med*, 28(7):834–41, 2002.

M. Sugiura, P. R. McCulloch, S. Wren, R. H. Dawson, and A. B. Froese. Ventilator pattern influences neutrophil influx and activation in atelectasis-prone rabbit lung. *J Appl Physiol (1985)*, 77(3):1355–65, 1994.

T. Tanaka, M. Narazaki, and T. Kishimoto. Il-6 in inflammation, immunity, and disease. *Cold Spring Harb Perspect Biol*, 6(10):a016295, 2014.

A. J. Thornton, R. M. Strieter, I. Lindley, M. Baggiolini, and S. L. Kunkel. Cytokine-induced gene expression of a neutrophil chemotactic factor/il-8 in human hepatocytes. *J Immunol*, 144(7):2609–13, 1990.

L. Tremblay, F. Valenza, S. P. Ribeiro, J. Li, and A. S. Slutsky. Injurious ventilatory strategies increase cytokines and c-fos m-rna expression in an isolated rat lung model. *J Clin Invest*, 99(5):944–52, 1997.

L. N. Tremblay and A. S. Slutsky. Ventilator-induced lung injury: from the bench to the bedside. *Intensive Care Med*, 32(1):24–33, 2006.

L. N. Tremblay, D. Miatto, Q. Hamid, A. Govindarajan, and A. S. Slutsky. Injurious ventilation induces widespread pulmonary epithelial expression of tumor necrosis factor-alpha and interleukin-6 messenger rna. *Crit Care Med*, 30(8):1693–700, 2002.

A. van Kaam. Lung-protective ventilation in neonatology. *Neonatology*, 99(4): 338–41, 2011.

A. H. van Kaam, P. C. Rimensberger, D. Borensztajn, A. P. De Jaegere, and G. Neovent Study. Ventilation practices in the neonatal intensive care unit: a cross-sectional study. *J Pediatr*, 157(5):767–71 e1–3, 2010.

Y. E. Vaucher, M. Peralta-Carcelen, N. N. Finer, W. A. Carlo, M. G. Gantz, M. C. Walsh, A. R. Laptook, B. A. Yoder, R. G. Faix, A. Das, K. Schibler, W. Rich, N. S. Newman, B. R. Vohr, K. Yolton, R. J. Heyne, D. E. Wilson-Costello, P. W. Evans, R. F. Goldstein, M. J. Acarregui, I. Adams-Chapman, A. Pappas, S. R. Hintz, B. Poindexter, A. M. Dusick, E. C. McGowan, R. A. Ehrenkranz, A. Bodnar, C. R. Bauer, J. Fuller, T. M. O'Shea, G. J. Myers, R. D. Higgins, and S. S. G. o. t. E. K. S. N. N. R. Network. Neurodevelopmental outcomes in the early cpap and pulse oximetry trial. *N Engl J Med*, 367(26):2495–504, 2012.

J. Villar, R. M. Kacmarek, L. Perez-Mendez, and A. Aguirre-Jaime. A high positive end-expiratory pressure, low tidal volume ventilatory strategy improves outcome in persistent acute respiratory distress syndrome: a randomized, controlled trial. *Crit Care Med*, 34(5):1311–8, 2006.

M. C. Walsh and R. M. Kliegman. Necrotizing enterocolitis: treatment based on staging criteria. *Pediatr Clin North Am*, 33(1):179–201, 1986.

A. Walz, P. Peveri, H. Aschauer, and M. Baggiolini. Purification and amino acid sequencing of naf, a novel neutrophil-activating factor produced by monocytes. *Biochem Biophys Res Commun*, 149(2):755–61, 1987.

H. H. Webb and D. F. Tierney. Experimental pulmonary edema due to intermittent positive pressure ventilation with high inflation pressures. protection by positive end-expiratory pressure. *Am Rev Respir Dis*, 110(5):556–65, 1974.

A. P. Wheeler, G. R. Bernard, B. T. Thompson, D. Schoenfeld, H. P. Wiedemann, B. deBoisblanc, J. Connors, A. F., R. D. Hite, and A. L. Harabin. Pulmonary-artery versus central venous catheter to guide treatment of acute lung injury. *N Engl J Med*, 354(21):2213–24, 2006.

K. I. Wheeler, C. Klingenberg, C. J. Morley, and P. G. Davis. Volume-targeted versus pressure-limited ventilation for preterm infants: a systematic review and meta-analysis. *Neonatology*, 100(3):219–27, 2011.

E. M. Williams, N. Pickerd, M. Eriksen, K. Oygarden, and S. Kotecha. Estimation of tidal ventilation in preterm and term newborn infants using electromagnetic inductance plethysmography. *Physiol Meas*, 32(11):1833–45, 2011.

H. Wrigge, U. Uhlig, J. Zinserling, E. Behrends-Callsen, G. Ottersbach, M. Fischer, S. Uhlig, and C. Putensen. The effects of different ventilatory settings on pulmonary and systemic inflammatory responses during major surgery. *Anesth Analg*, 98(3):775–81, table of contents, 2004.

M. Yang, H. J. Ahn, K. Kim, J. A. Kim, C. A. Yi, M. J. Kim, and H. J. Kim. Does a protective ventilation strategy reduce the risk of pulmonary complications after lung cancer surgery?: a randomized controlled trial. *Chest*, 139(3):530–537, 2011.

B. A. Yoder, R. A. Stoddard, M. Li, J. King, D. R. Dirnberger, and S. Abbasi. Heated, humidified high-flow nasal cannula versus nasal cpap for respiratory support in neonates. *Pediatrics*, 131(5):e1482–90, 2013.

T. Yoshida, A. Uchiyama, N. Matsuura, T. Mashimo, and Y. Fujino. The comparison of spontaneous breathing and muscle paralysis in two different severities of experimental lung injury. *Crit Care Med*, 41(2):536–45, 2013.

G. S. Zavorsky, J. Cao, N. E. Mayo, R. Gabbay, and J. M. Murias. Arterial versus capillary blood gases: a meta-analysis. *Respir Physiol Neurobiol*, 155(3):268–79, 2007.

J. Zeitlin, E. S. Draper, L. Kollee, D. Milligan, K. Boerch, R. Agostino, L. Gortner, P. Van Reempts, J. L. Chabernaud, J. Gadzinowski, G. Breart, E. Papiernik, and M. r. group. Differences in rates and short-term outcome of live births before 32 weeks of gestation in europe in 2003: results from the mosaic cohort. *Pediatrics*, 121(4):e936–44, 2008.

G. Zick, G. Elke, T. Becher, D. Schadler, S. Pulletz, S. Freitag-Wolf, N. Weiler, and I. Frerichs. Effect of peep and tidal volume on ventilation distribution and end-expiratory lung volume: a prospective experimental animal and pilot clinical study. *PLoS One*, 8(8):e72675, 2013.