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TESI DI LAUREA

MARKED TIP CATHETER FOR LESS INVASIVE SURFACTANT ADMINISTRATION (LISA): A CROSSOVER RANDOMIZED CONTROLLED MANIKIN TRIAL.

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1. RIASSUNTO.

Background: La tecnica LISA (somministrazione meno invasiva di surfattante) offre dei vantaggi rispetto alla tecnica INSURE per quanto riguarda l'utilizzo della ventilazione meccanica e la sopravvivenza dei neonati, ma posizionare il catetere LISA alla corretta profondità in trachea si è rivelato difficile. Questa difficoltà inficia la somministrazione del surfattante riducendo l'efficacia della procedura e prolunga la durata della laringoscopia rendendo la procedura maggiormente invasiva.

Obiettivi: L'obiettivo primario di questo studio è stato quello di confrontare il posizionamento del dispositivo alla profondità corretta nella trachea con un catetere LISA con punta marcata rispetto a un catetere LISA con punta non marcata in un manichino che simulava un neonato di peso estremamente basso. Ulteriori obiettivi erano confrontare il tempo totale e il numero di tentativi per raggiungere la profondità corretta nella trachea e l'opinione dei partecipanti sull'utilizzo del dispositivo.

Metodi: Abbiamo condotto uno studio crossover (AB/BA), non cieco, randomizzato e controllato in cui è stato confrontata la somministrazione di surfattante con tecnica LISA in un manichino simulante un neonato con peso estremamente basso usando un catetere LISA con punta marcata e uno con punta non marcata. I partecipanti arruolati nello studio erano medici neonatologi e specializzandi che lavorano in una terapia intensiva neonatale (TIN) di terzo livello.

Risultati: Lo studio ha incluso 50 partecipanti con esperienza mediana in TIN di 1 anno. La corretta profondità del catetere in trachea (outcome primario) è stata raggiunta da 38 partecipanti (76%) usando il catetere con la punta marcata e da 28 partecipanti (56%) usando il catetere con la punta non marcata (p=0,04). Il tempo mediano per il posizionamento del catetere LISA è stato di 19 secondi usando il catetere con la punta marcata e di 20 secondi usando il catetere con la punta non marcata (p=0,08). Il posizionamento del catetere in trachea al primo tentativo è stato raggiunto da tutti i partecipanti usando il catetere LISA con punta marcata e da 46 partecipanti su 50 (92%) usando il catetere con punta non marcata (p=0,13). I partecipanti hanno espresso di aver trovato maggiormente agevole

l'utilizzo del catetere LISA con la punta marcata (p=0,007), in modo particolare riferendosi all'inserimento in trachea (p=0,04) e alla facilità di posizionamento alla corretta profondità (p=0,004). Le opinioni riguardo al maneggiamento del catetere sono sulla soglia della significatività statistica (p=0,06).

Conclusioni: Questo studio su manichino dimostra che il catetere LISA con la punta marcata è superiore allo stesso con la punta non marcata per quanto riguarda la facilità di raggiungimento della corretta profondità in trachea. Per quanto riguarda il tempo totale e i tentativi necessari per posizionare correttamente il catetere, i dati sono sulla soglia della significatività statistica e non sono clinicamente rilevanti. Dall'opinione dei partecipanti è emersa maggiore facilità di utilizzo del catetere con la punta marcata rispetto a quello con la punta non marcata, in particolare per il posizionamento alla corretta profondità in trachea. Sono necessari ulteriori studi clinici per confermare questi risultati e verificarne la loro applicabilità nella pratica clinica.

Registrazione dello studio: Lo studio è stato registrato su clinicaltrial.gov NCT05399628.

2. ABSTRACT.

Background: Although LISA offers some advantages in ventilation procedure and neonatal outcomes, achieving the correct depth in the trachea using LISA catheter may be difficult. This may have some drawbacks such as impaired surfactant administration (reducing the efficacy of the procedure) or prolonged duration of the laryngoscopy (aggravating the invasiveness of the procedure).

Objectives: The primary objective of this trial was to compare the positioning of the device at the correct depth in the trachea with a LISA catheter with marked tip vs. a LISA catheter with unmarked tip in a manikin simulating an extremely low birth weight infant. Further objectives were to compare the total time and the number of attempts to achieve the correct depth in the trachea, and participant's opinion on using the device.

Methods: This was an unblinded, randomized, controlled, crossover (AB/BA) trial of surfactant treatment with LISA catheter with marked tip vs. LISA catheter with unmarked tip in a manikin simulating an extremely low birth weight infant. Participants were level III NICU consultants and residents. Randomization was performed using a computer-generated random assignment list. The primary outcome measure was the positioning of the device at the correct depth in the trachea. The secondary outcome measures were the time and number of attempts to achieve the correct depth, and participant's satisfaction.

Results: The analysis included 50 participants with a median experience in neonatal intensive care of 1 year. The correct depth of the device in the trachea (primary outcome measure) was achieved by 38 participants (76%) using the catheter with marked tip and 28 participants (56%) using the catheter with unmarked tip (p=0.04). Median time of device positioning was 19 seconds using the catheter with marked tip and 20 seconds using the catheter with unmarked tip (p=0.08). Inserting the device in the trachea at first attempt was achieved by all participants (100%) when using the catheter with marked tip and by 46/50 participants (92%) when using the catheter with unmarked tip (p=0.13). The participants found the catheter with the marked tip easier to use (p=0.007), especially concerning the insertion in the trachea (p=0.04) and the positioning at

the correct depth (p=0.004). The different opinion about handling the devices was only close to statistical significance (p=0.06).

Conclusion: LISA catheter with marked tip emerged as superior to the unmarked one in terms of easiness to achieve the correct depth of the device in the trachea. Differences between the two devices about total time of positioning and number of attempts needed were on the threshold of statistical significance and were not clinically relevant. Participants expressed that the marked tip catheter is easier to use than the unmarked one, especially regarding difficulty of reaching the correct depth in the trachea. Further trials are necessary to confirm our findings in clinical settings and real infants.

Trial registration: This trial has been registered at clinicaltrial.gov NCT05399628.

3. INTRODUCTION.

3.1 Definition of RDS and epidemiology.

Respiratory Distress Syndrome (RDS), previously known as Hyaline Membrane Disease (HMD), is a disease characteristic of prematurity. It occurs in premature babies who are born with immature lungs and therefore lack production of surfactant.

An infant born before 37 weeks of gestation is considered premature (full term is 37 to 42 weeks' gestational age). Standard definitions categorize the period of gestation of an infant's birth: babies born before 32 weeks' GA are "very preterm", while infants born before 28 weeks' GA are considered "extremely preterm".[1]

The risk of RDS decreases with the increase of gestational age: incidence of RDS is about 60% at 28 weeks' gestation, 30% in babies born between 28 and 34 weeks' gestation, and fewer than 5% in babies born after 34 weeks after conception.
With the advent of antenatal steroids and surfactant replacement therapy, mortality from RDS has plummeted in recent years, going from 100% to 10%.[2]

3.2 Pathophysiology and risk factors.

Surfactant deficiency is the primary cause of RDS, often complicated by an overly compliant chest wall. In the absence of pulmonary surfactant there is a significantly increased alveolar surface tension which leads to atelectasis, and the ability to attain an adequate functional residual capacity (FRC) is impaired.

Surfactant is produced by airway epithelial cells called type II pneumocytes and its synthesis begins at 24 to 28 weeks' gestation, but mature levels are present at around 35 weeks' GA. Surfactant is a mixture of phospholipids, of which the most abundant in mature lungs is phosphatidylcholine, and proteins, in particular surfactant proteins A, B, C, and D which all have different roles, from regulation of

surfactant secretion to aiding the phospholipids' spreading in the alveoli's surface. Surfactant is stored within type II pneumocytes in structures called lamellar bodies which give these cells a distinctive appearance.[2]
These surfactant-active agents are released into the alveoli, where they reduce surface tension and help maintain alveolar stability at end-expiration.

In preterm neonates the lack of surfactant causes the lungs to collapse because the babies aren't strong enough to generate the necessary inflation's pressure.
This condition leads to a decrease in FRC, and progressively injuries epithelial and endothelial cells, also decreasing level of oxygen in the child's blood. As a result, effusion of proteinaceous material and cellular debris into the alveolar spaces forms the so-called hyaline membranes and further impairs oxygenation. Atelectasis, hyaline membranes formation and interstitial edema make the lungs less compliant in RDS, so greater pressure is required to expand the alveoli and small airways. The edema is also influenced by the contraction of diuresis which is characteristic of the first days of life. Additionally, compared with the mature infant, the highly compliant chest wall of the preterm infant offers less resistance to the natural tendency of the lungs to collapse. Thus, at end-expiration, the volume of the thorax and lungs tend to approach residual volume. Atelectasis results in perfused but not ventilated alveoli, leading to hypoxia. All these conditions (asphyxia, hypoxemia, pulmonary ischemia), particularly in association with hypovolemia, hypotension and cold stress, further slow down surfactant production. Decreased lung compliance, small tidal volumes, increased physiologic dead space, and insufficient alveolar ventilation eventually result in hypercapnia.
The combination of hypercapnia, hypoxia, and acidosis produces pulmonary arterial vasoconstriction with increased right-to-left shunting through the foramen ovale and ductus arteriosus, and within the lung itself.[3]

The risk of RDS increases in preterm birth, particularly <28 weeks' GA, low birth weight and cesarean delivery, either elective or unplanned. Males have a higher risk of developing RDS, probably due to a different hormonal profile. It is not sure

whether parity of the mother impacts the risk of developing RDS.[4] In addition to prematurity, other factors that increase the risk of RDS include maternal gestational diabetes, perinatal asphyxia, hypothermia, and multiple gestations.[5]

There are some factors which reduce the risk of RDS, such as maternal heroin use, pregnancies with chronic or pregnancy-associated hypertension, prolonged rupture of membranes and antenatal corticosteroids prophylaxis.[3]

3.3 Lung development.

The development of the lungs starts during the embryonic period of the gestation. There is great debate as to when the development of the lungs ends but is well clear that premature birth interrupts their physiological development.[1]
The lungs' development is composed of five periods: the embryonic period going from week 0 to week 6 of the gestation, the pseudoglandular period (weeks 6 to 16), the canalicular period (weeks 16 to 24), the saccular period (weeks 24 to 36) and the alveolar period (36 weeks GA to postnatal life). During each period the lungs' circulation system develops parallel to the lungs.[1]

The embryonic period is the organogenesis' one: lungs growth begins in the third week of gestation with the outgrowth of a small diverticulum from the ventral wall of the foregut called the primitive respiratory diverticulum or lung bud. This extends in the ventral caudal direction into the surrounding mesoderm, growing anterior and parallel to the primitive esophagus. Within a few days, the grove between the diverticulum and the foregut closes with the only luminal attachment remaining at the site of the future hypopharynx and larynx. By gestational day 28, the respiratory diverticulum bifurcates into the right and left primary bronchial buds (main stem)(Figure 1).[6] At the end of this period the segmental portions of the airway tree are tubes of high columnar epithelium. The vascular system buds off the sixth pair of aortic arches and organize themselves into a vascular plexus which surrounds the pulmonary branches. The pulmonary vein is a small vessel starting from the left atrial portion of the embryonic heart.[1]

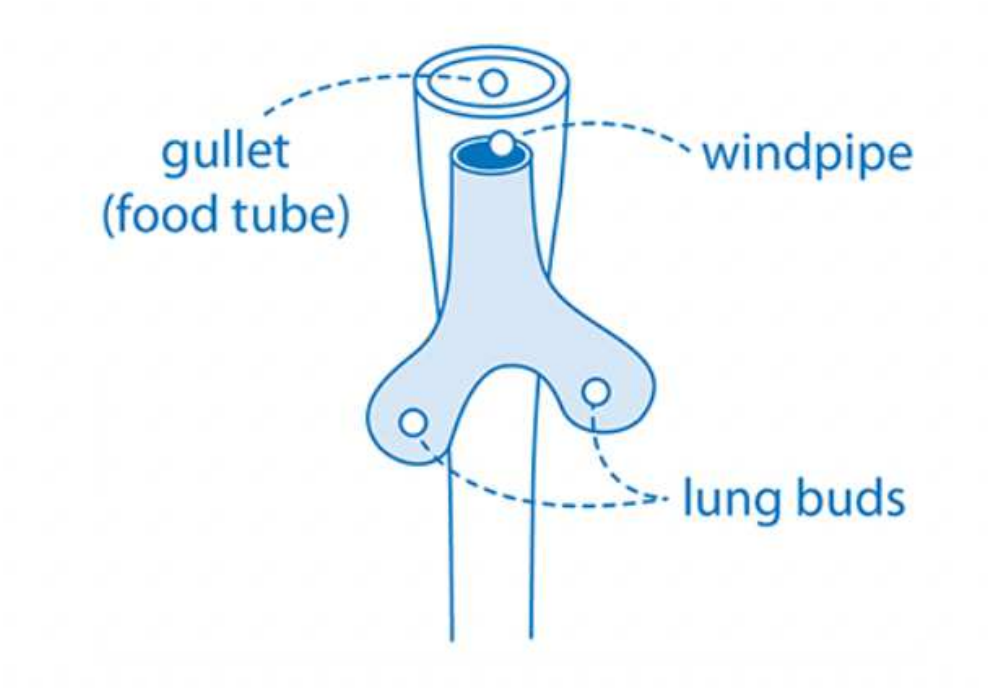

Figure 1. Buds forming the embrionic's stage lungs.

The pseudoglandular period starts when the lungs resemble a primitive gland around the sixth week of gestation. During this period the vascular development is completed, and capillaries resembles the adults' ones except for a thicker endothelium. The two lung buds divide themselves into the lobar bronchi (three for the right lung and two for the left lung). Cellular differentiation of the airways starts from the proximal end towards the distal end: the airway tubes are lined with high columnar epithelium and by 12 weeks' GA there are cartilage and smooth muscle cells in the trachea and segmental bronchi, along with mucus glands. In the distal regions differentiation into cuboidal epithelium also starts, with cells filled with glycogen which is fuel for cellular differentiation and a surfactant component.[1]

The lobar bronchi will dichotomously divide themselves and form the bronchial structure (Figure 2). At the end of the 17th week of gestation the acinus is formed, and it starts to widen until small airway spaces come into contact and the air-blood barrier appears. This period is called the canalicular one and sees the appearance

of type I and II pneumocytes with lamellar bodies, which will start the production of surfactant between the 6th and 7th month of gestation.[1]

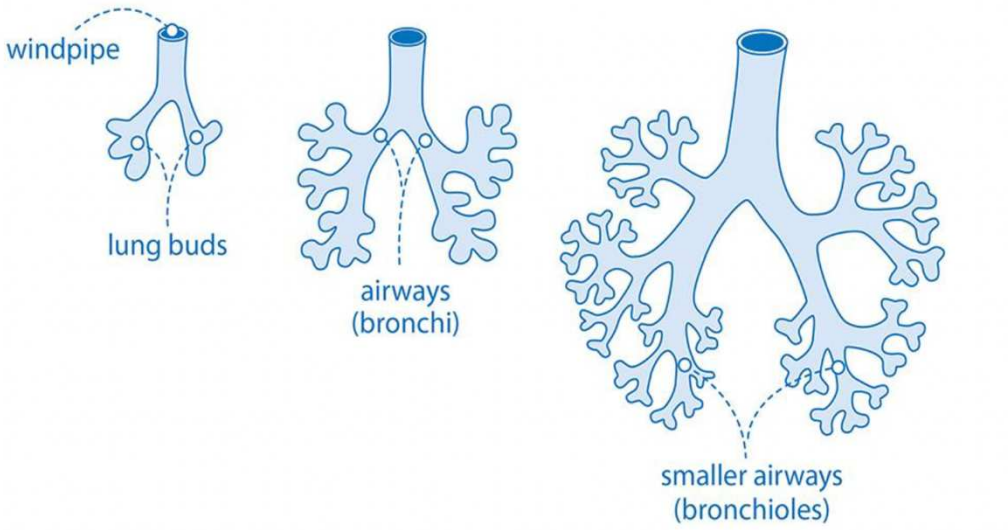

Figure 2. The respiratory tree is formed from the lung buds.

The saccular period is characterized by the presence of small buds called saccules which cluster at the end of the airways. The alveoli will be recognizable after the 32nd week of gestation. Although gas exchanges can be possible during this period, they are not effective due to the limited gas exchange area and the high distance compared to body weight and metabolic rate.[2]

The fifth stage of lung developments, the alveolar one, starts at the end of the physiological pregnancy and continues through the first 2 years of the baby's life (40 weeks GA to 2 years postnatal). During this period the alveoli continue to develop.[1]

3.4 Clinical manifestations.

Signs of RDS usually appear within minutes of birth, although they may not be recognized for several hours in larger premature infants, until rapid shallow respirations become more obvious.[3] Respiratory distress in the neonate most commonly presents as one or all the following physical signs: tachypnea, grunting,

nasal flaring, retractions, and cyanosis. In the newborn the normal respiratory rate is between 30 and 60 breaths per minute, tachypnea is defined by a respiratory rate higher than 60 breaths per minute. Tachypnea is a compensatory mechanism for hypercarbia, hypoxemia, or acidosis (both metabolic and respiratory), making it a common but nonspecific finding in a large variety of respiratory, cardiovascular, metabolic, or systemic diseases.[7] It is important to remember that some infants who have RDS exhibit all these symptoms, and others may show none.[2]

Grunting occurs when an infant attempts to maintain an adequate FRC in the face of poorly compliant lungs by partial glottic closure. As the infant prolongs the expiratory phase against this partially closed glottis, there is a prolonged and increased residual volume that maintains the airway opening and an audible expiratory sound. Infants with RDS have cyanosis and require supplemental oxygen. Retractions are visible in the subcostal, intercostal, and/or suprasternal areas.[7]

Breath sounds may be normal or diminished with a harsh tubular quality, and on deep inspiration, fine crackles may be heard. The natural course of untreated RDS is characterized by worsening cyanosis and dyspnea. If the condition is inadequately treated, blood pressure may fall; cyanosis and pallor increase, and grunting decreases or disappears, as the condition worsens. Apnea and irregular respiration are ominous signs requiring immediate intervention. Untreated patients may also have mixed respiratory-metabolic acidosis, edema, ileus, and oliguria. In most cases the signs peak at 3 days postnatal, after which improvement is gradual. Improvement can be heralded by spontaneous diuresis and improved blood gas values at lower inspired O_2 and/or lower ventilator support.[3]

Death can result from severe impairment of gas exchange, pulmonary interstitial emphysema, pneumothorax, pulmonary hemorrhage, or intraventricular hemorrhage (IVH).[3]

3.5 Diagnosis and differential diagnosis.

Along with the history and physical examination, a chest radiograph is needed for the diagnosis of RDS. The typical chest radiograph shows diffuse atelectasis and the classic "ground glass" appearance of the lung fields. Air bronchograms, which are air-filled bronchi superimposed on the relatively airless parenchyma of the lung tissue, are also commonly seen on chest radiographs. Importantly, the appearance of GBS pneumonia on chest radiographs can be identical to that of RDS. Empiric antibiotics to address GBS infection should be started until such disease is ruled out.[2] The "ground glass" appearance of the lungs at the chest x-ray is characteristic but not pathognomonic. The initial x-ray appearance is occasionally normal, with the typical pattern developing during the 1st day of life. Considerable variations in radiographic appearance may be seen, especially in infants who have already received treatment with surfactant replacement and/or positive pressure respiratory support. This variation often results in poor correlation between radiographic findings and the clinical course.[3]

ABG measurements demonstrate hypercarbia and hypoxia and eventually, in the unsupported infant, metabolic acidosis.[5]

Some rare, genetic disorders may contribute to respiratory distress. Abnormalities in surfactant protein B (Congenital Alveolar Proteinosis) and C genes as well as ABCA3, a gene responsible for transporting surfactant across membranes, are associated with severe and often lethal familial respiratory disease. In atypical cases of RDS, a lung profile (lecitin:sphyngomielin ratio and phosphatidylglycerol determination) performed on a tracheal aspirate can be helpful in establishing a diagnosis of surfactant deficiency.[3]

The differential diagnosis of respiratory distress in the newborn includes both pulmonary and non-pulmonary processes. Common pulmonary causes include RDS, meconium aspiration syndrome (MAS), and pneumonia. Non-pulmonary

causes include cardiac diseases, infection, metabolic disorders, central nervous system disorders, and several others miscellaneous disorders.[5]

Thus, after initial resuscitation and stabilization, it is important to use a detailed history, physical examination, and radiographic and laboratory findings to determine a more specific diagnosis and appropriately tailored management.

A thorough history may guide in identifying risk factors associated with common causes of neonatal respiratory distress. A detailed physical examination should focus beyond the lungs to identify non-pulmonary causes that may initially present as respiratory distress in a newborn. Radiographic findings can identify diaphragmatic paralysis, congenital pulmonary malformations, and intrathoracic space-occupying lesions, such as pneumothorax, a mediastinal mass, and congenital diaphragmatic hernia, that can compromise lung expansion. Significant tachypnea without increased work of breathing should prompt additional laboratory investigation to identify metabolic acidosis or sepsis.[7] Clinical factors such as maternal group B streptococcal colonization with inadequate intrapartum antibiotic prophylaxis, maternal fever (>38.6°C) or chorioamnionitis, or prolonged rupture of membranes (>12 hours) are associated with an increased risk of early-onset sepsis. Although complete blood counts are neither sensitive nor specific in the diagnosis of early-onset sepsis, the presence of marked neutropenia has been associated with increased risk.[3]

Hypoglycemia, hypomagnesemia, and hematologic abnormalities may result in a depressed ventilatory drive or impaired oxygen transport to the peripheral tissues. Cardiovascular disease may be difficult to distinguish from pulmonary causes of respiratory distress because most congenital heart defects present with symptoms that are similar to the ones of pulmonary causes of RD. Timing may be useful to differentiate between the two causes because congenital heart defects often present several hours to days after delivery, when the ductus arteriosus closes.

A condition that neonatologists must keep in mind when treating an infant with respiratory distress and cyanosis is pulmonary hypertension. Persistent pulmonary hypertension of the newborn (PPHN) may be primary or secondary to respiratory disease, particularly congenital diaphragmatic hernia, MAS, or RDS. When PPHN

occurs without concurrent pulmonary disease, differentiation from cyanotic heart disease is difficult. The response to ventilation with 100% oxygen (hyperoxia test) can help distinguish the two conditions. In some neonates with PPHN, the PaO_2 will increase to above 100 mmHg, whereas it will not increase above 45 mmHg in infants with cyanotic heart defects that have circulatory mixing.[7]

Transient tachypnea may be distinguished by its shorter and milder clinical course and is characterized by low or no need for O_2 supplementation.
Some familial conditions can cause respiratory distress (not RDS) in the newborn, and therefore are included in the differential diagnosis of RDS (e.g.: mucopolysaccharidosis, acinar dysplasia, pulmonary lymphangiectasia, and alveolocapillary dysplasia).[3]

3.6 MANAGEMENT.

There have been great extents of improvements in the last decades in the management of RDS, even reducing the risk of bronchopulmonary dysplasia associated to mechanical ventilation, but respiratory distress syndrome remains a frequent and impacting condition in preterm neonates. The most recent European Guidelines for treatment of RDS state that the aim of management of RDS is to provide interventions to maximize survival whilst minimizing potential adverse effects including BPD.[8] Stabilizing neonates with RDS on a non-invasive respiratory support (NRS) such as continuous positive airway pressure (CPAP) and then instituting surfactant therapy in selective neonates who have an increased oxygen requirement has become the standard practice.[9]

The management of RDS starts in the prenatal period with the prediction of risk of prenatal delivery, need of maternal transfer to a perinatal center and, when necessary, the administration of antenatal steroids. After delivery, when the first symptoms begin to be clinically evident, clinicians need to stabilize the infant and determine whether SRT is necessary and, if it is, if the administration must be

prophylactic or rescue therapy. There are different techniques neonatologists can use to perform SRT, the most recent ones being LISA and MIST.[8]
A work from 2007 indicates that prophylactic SRT in patients who are at risk for RDS, particularly those infants born at <30 weeks' gestation, improves neonatal survival and reduces morbidity.[10]

3.6.1 Prenatal care.

The European Guidelines indicate that interventions to improve outcome and prevent RDS begin before birth. There is often warning of impending preterm delivery, and in these cases, physicians need to consider interventions to prolong gestation or reduce risk of an adverse outcome by "preparing" the fetus. They suggest cervical length measurement, possibly in combination with a biomarker, to determine which women are at risk of delivery within 7 days and allow more judicious use of antenatal treatments. In case of a possible extremely preterm delivery the mother and child should be transported to tertiary centers where appropriate skills are available.[8]

The same guidelines indicate the use of antenatal corticosteroids to reduce the risk of RDS (antenatal corticosteroids reduce the risk of RDS by speeding up the fetus' lungs development). "A single course of prenatal corticosteroids given to mothers with anticipated preterm delivery improves survival, reduces RDS, NEC and intraventricular hemorrhage and does not appear to be associated with any significant maternal or short-term fetal adverse effects. Prenatal corticosteroids therapy is recommended in all pregnancies with threatened preterm birth before 34 weeks' gestation where active care of the newborn is anticipated. Observational studies suggest that antenatal steroids, together with other active management practices, reduce mortality at gestations as low as 22 weeks' GA. The optimal treatment to delivery interval is more than 24 hours and less than 7 days after the start of steroid treatment; beyond 14 days, benefits are diminished. WHO recommends that a single repeat course of steroids may be considered if preterm birth does not occur within 7 days after the initial course and there is a high risk of

preterm birth in the next 7 days. It is unlikely that repeat courses given after 32 weeks' GA improve outcome".[8]

3.6.2 Delivery room stabilization.

Although babies with RDS try to breathe on their own after delivery, they show difficulty breathing shortly thereafter because they're uncapable of maintaining adequate aeration. Therefore, proper supporting maneuvers are required to help these infants.

In the European Guidelines for treatment of RDS, timing of umbilical clamping is depicted as an important first step. Clamping the cord before initiation of respiration results in an acute transient reduction in left atrial filling, leading to an abrupt drop in left ventricular output. Delayed "physiological" clamping after lung aeration results in much smoother transition and less bradycardia in animal models.[8]

The 2021 edition of the European Guidelines for neonatal resuscitation highlight that an important point in the stabilization of the infant is maintaining the body temperature to prevent hypothermia. There are lots of maneuvers that can be implemented in this regard:

- Protect the infant from draughts. Ensure windows are closed and air-conditioning appropriately programmed.
- Keep the environment in which the infant is looked after (e.g., delivery room or theatre) warm at 23-25°C.
- For infants <28 weeks' gestation the delivery room or theatre temperature should be >25°C.
- Completely cover with polyethylene wrapping (apart from face) without drying and use a radiant warmer.
- If umbilical cord clamping is delayed and a radiant warmer is not accessible at this point, other measures (such as those listed below) will be needed to ensure thermal stability while still attached to the placenta.

- A combination of further interventions may be required in infants <32 weeks including increased room temperature, warm blanket, head cap and thermal mattress.
- Skin-to-skin care is feasible in less mature infants; however, caution is required in the more preterm or growth restricted infant in order to avoid hypothermia.
- For infants receiving respiratory support, use of warmed humidified respiratory gases should be considered.[11]

The European Guidelines for treatment of RDS go on to describe other important factors in delivery room stabilization: "stimulation of the infant during stabilization helps with establishing regular respirations. Spontaneously breathing babies should be started on CPAP rather than intubated in the delivery room to reduce risk of BPD. Provision of CPAP alone is ideal, and routine use of positive pressure breaths should be discouraged, although gentle positive pressure ventilation may be required for babies who remain apneic or bradycardic. Heart rate assessment is important in determining infant well-being during transition. Heart rate <100/min for >2 minutes in the first 5 minutes after birth is associated with 4.5 fold increase in mortality. When heart rate is satisfactory, the aim is to obtain normal saturations of 90% during the first minutes after birth. If the saturation is low, oxygen for resuscitation should be controlled using a blender. There is reduced mortality when using fraction of inspired oxygen (FiO_2) 0.21 rather than 1.0. Only a minority of babies should require intubation for stabilization. Intubation should be reserved for babies not responding to positive pressure ventilation via face mask or nasal prongs. Babies who require intubation for stabilization should be given surfactant."[8]

3.6.3 Surfactant therapy.

The administration of exogenous surfactant is called Surfactant Replacement Therapy (SRT). As highlighted in the UK consensus for treatment of RDS, surfactant administration plays an essential role in management of RDS, as it reduces requirement for positive pressure ventilation, mitigates risk of pulmonary air leak, and improves survival.[12]

Surfactant can be administered as a prophylactic or rescue therapy, and although literature shows its use is ideally reserved for babies showing clinical signs of RDS, the goal is to administer it as early as possible once its necessity is ascertained.

The aim is also to reduce damages deriving from mechanical ventilation (MV), which is why CPAP is preferred, especially in the more fragile babies.

Once stabilized with CPAP, many babies don't develop worsening clinical symptoms, but some will develop RDS. Several newborns with less severe disease may survive without surfactant administration. To determine if SRT is necessary, neonatologist can only combine their clinical evaluation of the infant's work of breathing, FiO_2 to maintain normal saturation, and degree of aeration of the lungs on chest X-ray, all of which can be influenced by the non-invasive respiratory support (e.g., Nasal-CPAP or NIPPV). Observational studies have confirmed that FiO_2 exceeding 0.30 in the first hours after birth in babies on CPAP is a reasonably good test for predicting subsequent CPAP failure. Therefore, it is recommended that the threshold of FiO_2>0.30 is used for all babies with a clinical diagnosis of RDS, especially in the early phase of worsening disease.[8] As demonstrated in a study by De Martino, LUS (lung ultrasound score) can be a valid tool to determine surfactant need, both in preterm and extremely preterm infants. A LUS can be used to accurately predict the need for the first surfactant dose and reveals fair accuracy when it comes to predicting surfactant re-treatment.[13]

Sardesai proved that different types of surfactants (animal-derived and synthetic) have been studied, and it emerged that animal-derived surfactants generally results in faster weaning of respiratory support, shorter duration of invasive

ventilation, and decreased mortality when compared to first- or second-generation synthetic surfactants, but some of the second-generation surfactants are at least not inferior to the animal-derived surfactants. Third-generation synthetic surfactants are currently being studied.[14] Animal-derived surfactants in clinical use are modified or purified from bovine or porcine lungs. All commercially available animal-derived surfactants are effective for prevention and treatment of respiratory distress syndrome. Adverse immunologic and infectious complications from exposure to proteins and other components of these animal products have not been identified.[15]

The European Guidelines for treatment of RDS show that "most of the head-to-head trials show that surfactants have similar efficacy when used in similar doses; however, there is a survival advantage when 200 mg/kg of poractant alfa is compared with 100 mm/kg of beractant or 100 mg/kg of poractant alfa to treat RDS."[8]

Effects of SRT on cerebral and systemic circulation and lung function have been studied by Schipper, with results showing a significant decrease in heart rate, mean arterial pressure, and mean cerebral blood flow velocity immediately after the administration of both the first and second dose of surfactant. The study concludes that within 30 minutes, normalization of the disturbed circulatory parameters was found despite persistent increase of FRC and significant decrease of the FiO_2.[16]

Initially, surfactant was administered using the INSURE technique, which uses tracheal intubation followed by administration of surfactant and a short period of mechanical ventilation. This technique reduces the time of MV and therefore the risk of BDP, and this is why it was endorsed. [8] A study by Calevo underlines that endotracheal intubation is an extremely distressing, painful procedure and has the potential for airway injury; in addition, it may be associated with significant hemodynamic instability including hypoxia, bradycardia, blood pressure fluctuations and increased intracranial pressure.[17] In the last decade there has been a surge in search for a less distressing procedure, and less invasive surfactant

administration (LISA) was born. As explained in the UK consensus for treatment of RDS, LISA is a technique for SRT via an endotracheally-placed catheter in infants spontaneously breathing on NIV (non-invasive ventilation). A soft-tipped, semi-rigid, fine bore surfactant administration catheter is placed under direct laryngoscopy, with or without the use of Magill forceps. Surfactant is then given slowly whilst the infant continues to breathe. Vital signs are continuously monitored, and patients comfort ensured throughout the procedure, during which NIV is uninterrupted. Moderate desaturation, with or without bradycardia, may occur. The catheter is removed once the surfactant delivery is complete.[12] Another alternate method for administering SRT uses a supraglottic airway device (SAD), as reported by Calevo. The reported advantages of SAD over endotracheal tubes comprise a reduced invasiveness, because the airway is not instrumented with a laryngoscope, and easier positioning.[17] However, the European Guidelines warn that the size of current available laryngeal masks limit use of the method to relatively mature preterm infants, and routine use for smaller infants at greatest risk of RDS is not recommended.[8] As stated in another study, "to avoid intubation, attempts have been made to give surfactant by aerosolization, but the results did not show beneficial effects."[18]

Sedation and analgesia are controversial issues in RDS management. The European Guidelines highlight that the comfort of the baby must be taken into account, but there are doubts about the effects of sedation which can cause harm to the spontaneous respiratory drive of the infant. More importantly, LISA has a better chance of success without sedation when compared to INSURE. The Guidelines recommend not to use long-lasting muscle relaxants because they can increase the need for ventilation. They also non recommend the use of opiates.[8] A recent study has confronted ketamine and propofol for premedication for LISA technique. Neither of these drugs is ideal, as propofol has no analgesic effect and ketamine may carry a risk of neurological toxicity at high (10-40 mg/kg) single doses. The dose of ketamine used in the study was two times higher than expected, whereas propofol doses where more in line with the study's expectations. In conclusion, this study's findings suggest that ketamine or propofol

can be used for premedication before LISA, as they show comparable efficacy and tolerance.[19] A study published in 2019 by Kort showed that sedative premedication used before performing SRT with LISA technique should help obtain better success rate of the first attempt of surfactant administration, better adequacy of technical quality and reduce oxygen desaturations.[20]

A publication by Masmonteil posit that, since qualitative or quantitative deficit of surfactant often is involved in the pathogenesis of various respiratory disorders in late preterm or term babies (meconium aspiration syndrome (MAS), pneumonia/sepsis, congenital diaphragmatic hernia (CDH)), and a number of preterm infants who have or do not have initial RDS also eventually may exhibit a secondary surfactant deficiency during the course of a chronic lung disease or after an acute episode of lung injury (such as pulmonary hemorrhage (PH) or pneumonia/sepsis), all these disorders may represent potential targets for surfactant therapy.[21]

3.6.4 Respiratory support.

Owen explains that treatments for infants with respiratory distress (not RDS) include oxygen therapy, exogenous surfactant, various modes of respiratory support, and postnatal corticosteroids.[22]
Per the European Guidelines, in preterm babies targeting lower saturations (85-89% vs. 91-95%) reduces risk of severe retinopathy of prematurity (ROP) but at expense of increasing mortality and necrotizing enterocolitis (NEC). Recommended target saturations are between 90 and 94%, setting alarm limits between 89 and 95%.[8]

Since invasive ventilation has led to deleterious consequences for babies, non-invasive ventilation (NIV) strategies have been studied. In his publication, Permall explains that NIV has improved mortality rates of preterm infants with RDS. [23] The European Guidelines recommend CPAP as the optimal first mode of respiratory support, seen that it improves oxygenation, regulates breathing and is effective at

reducing reintubation following extubation. They also state that despite best intentions to maximize NIV, many small infants still require MV. The aim of MV is to provide acceptable ABG whilst avoiding lung injury and its functioning is based on the inflation of the collapsed lung. The risks of MV are over-distention of the lungs, which can cause air leaks, and atelectasis, which generates inflammation and can lead to BPD (to limit the risk of developing BPD, postnatal corticosteroids can be considered). Hence, once the infant is stabilized and breaths spontaneously, weaning should be immediately started. While weaning the infant, hypocarbia and severe hypercarbia are to be avoided, although it is reasonable to tolerate a modest degree of hypercarbia provided the pH remains above 7.22. Caffeine can be used to facilitate weaning from MV.[8] A meta-analysis from 2014 indicates that avoiding MV reduces the incidence of death or CPD in premature infants <30 weeks' GA without increasing the risk of intra ventricular hemorrhage (IVH).[24]

3.6.5 Monitoring and supportive care.

Recommendations from the European Guidelines to achieve best outcomes for preterm babies with RDS state that optimal supportive care with monitoring physiological variables is important. These recommendations state:

- Core temperature should be maintained between 36.5 and 37.5 ° C at all times.
- Most babies should be started on intravenous fluids of 70–80 mL/kg/day in a humidified incubator, although some very immature babies may need more. Fluids must be tailored individually according to serum sodium levels, urine output and weight loss.
- Parenteral nutrition should be started from birth. Amino acids 1–2 g/kg/day should be started from day one and quickly built up to 2.5–3.5 g/kg/day. Lipids should be started from day one and built up to a maximum of 4.0 g/kg/day if tolerated.
- Enteral feeding with mother's milk should be started from the first day if the baby is hemodynamically stable.

- Treatment of hypotension is recommended when it is confirmed by evidence of poor tissue perfusion such as oliguria, acidosis and poor capillary return rather than purely on numerical values.[8]

3.7 LISA TECHNIQUE.

In recent years a great number of studies have demonstrated at least the non-inferiority of LISA compared with INSURE.

A Germany study published in 2015 observed that rates of pneumothorax and severe IVH were lower in LISA-treated infants, and LISA showed increased rate of survival without major complications.[25]

In 2019, an Indian study compared SURE (SRT without intubation) and INSURE techniques in premature babies. The need for MV in the first 72 hours of life was significantly lower in the SURE group compared to the INSURE group. Similarly, duration of oxygen therapy and hospital stay were significantly shorter in the SURE group. BPD rate was significantly lower among the infants who were treated with the SURE technique. In preterm neonates with RDS who are stabilized on CPAP, the SURE technique for surfactant delivery resulted in the reduced need for MV and may also decrease the rate of BPD in some vulnerable subpopulations.[26]

A 2016 survey assessed the rate of utilization, premedication, as well as technique and equipment used for LISA in Europe. The results indicated that the use of LISA is widespread throughout Europe but there are variations concerning all aspects of LISA technique. Most of the neonatologists who participated in the survey considered LISA even in extremely preterm babies, but since a failure of this approach was likely to be insufficient and would be followed by invasive methods of surfactant administration, LISA was regarded as a safe first-line attempt in less severe RDS, while babies with severe RDS were treated with other approaches. Although it is recommended to ensure the comfort of the infant during LISA procedures, 52% of the participating neonatologists would not use any premedication to perform it. Possible explanations for such a limited use of premedication drugs include the believe that LISA is perceived to be less traumatic

than other methods, or spontaneous breathing is regarded being superior to analgesia or sedation. Different devices have been used to perform LISA: some neonatologists prefer using a catheter and holding it in place with a Magill forceps, while others prefer more rigid catheters that can be inserted without forceps.[27]

A study used surfactant-deficient newborn piglets to evaluate the impact of the neonatal brain of LISA and INSURE. Both procedures have been associated with side effects such as transient hypoxemia, bradycardia, and desaturation. Such alterations may produce changes in cerebral hemodynamics, oxygenation, and electrical brain activity, which could negatively influence long-term neurodevelopment. In spontaneously breathing surfactant-deficient newborn piglets, short lasting decreases in cerebral oxygenation are associated with SRT by the INSURE or LISA method using a nasogastric tube (NT), while no cerebral oxygenation changes occurred with LISA using a catheter created for this technique. Notably, none of the treatments studied seems to have a negative impact on the neonatal brain.[28]

The developmental outcome of extremely preterm infants treated with LISA was evaluated in a study published in 2020. There is a growing body of evidence that LISA-treated infants are at a decreased risk of developing BPD compared to intubation and MV and other NIV. In contrast, data on long-term outcome after LISA compared to intubation are scarce, therefore there are safety concerns regarding long-term outcome especially outside of Europe. Lisa reduces duration of ventilation and risk of BPD, both well-defined risk factors of adverse development. There are concerns that the LISA procedure, which is predominantly performed without sedation or analgesia to prevent apnea, may cause discomfort and pain, thus increasing the risk of cerebral complications by impairing cerebral venous return. The study evaluated neurocognitive development at 24 months of corrected age after LISA in preterm infants born at 23-26 weeks of gestational age. Infants born at 25 and 26 weeks showed improved neurodevelopment after less invasive surfactant administration. Infants born at 23 and 24 weeks showed improved psychomotor development after LISA. The result shows that this

technique is safe and may be superior regarding developmental outcome of extremely preterm infants.[29] A 2021 study conducted in surfactant-depleted adult rabbits compared administration of surfactant via LISA and INSURE, and those with the outcome without SRT. No signs of recovery were found in the untreated animals. In an acute setting, three hours post-treatment, LISA method seems to be effective as INSURE and showed similar surfactant lung delivery. This study should reassure some of the concerns raised by the clinical community on LISA adoption in neonatal units.[30] Another study observed no differences in outcome at 2 years in babies treated with LISA vs babies who received standard treatment.[31]

Although evidence form randomized trials and meta-analyses suggest that LISA is superior in terms of reducing need for MV and the combined outcome of death or BPD,[32] [33] [34] [35] health care providers may experience some difficulties in achieving the correct positioning of the LISA catheter in the trachea. Previous unpublished observations from this study group suggested that achieving the correct depth in the trachea may be difficult, as also reported by health care providers. This may have some drawbacks such as impaired surfactant administration (if the device is not positioned at the correct depth) or prolonged duration of the laryngoscopy (to achieve the correct depth). This is likely to reduce the efficacy of the procedure or aggravate the invasiveness of the procedure (resulting in stressful consequences such as bradycardia, hypoxia and hemodynamic changes), respectively.

The “PICOT” question of this study was:
P: in extremely low birth weight infants with RDS
I: does LISA catheter with marked tip
C: compared to LISA catheter with unmarked tip,
O: change the positioning of the device at the correct depth in the trachea?

4. OBJECTIVES.

The primary objective of this trial was to compare the positioning of the device at the correct depth in the trachea with a LISA catheter with marked tip vs. a LISA catheter with unmarked tip in a manikin simulating an extremely low birth weight infant.

Further objectives were to compare the total time and the number of attempts to achieve the correct depth in the trachea, and participant's opinion on using the device.

5. METHODS.

5.1 Study design.

This was a randomized, controlled, crossover (AB/BA) trial of surfactant administration with LISA catheter with marked tip vs. LISA catheter with unmarked tip in a manikin simulating an extremely low birth weight infant (clinicaltrial.gov NCT05399628). Written informed consent was obtained from participants.

5.2 Setting.

This simulation study was conducted at the University Hospital of Padua (Italy) and the Fondazione Poliambulanza of Brescia (Italy) between 6th and 11th June 2022. The scenario consisted of an extremely low birth weight infant needing surfactant administration (neonatal simulator manikin: Premature Anne, Laerdal Medical Corporation, Stavanger, Norway) (figure 3).

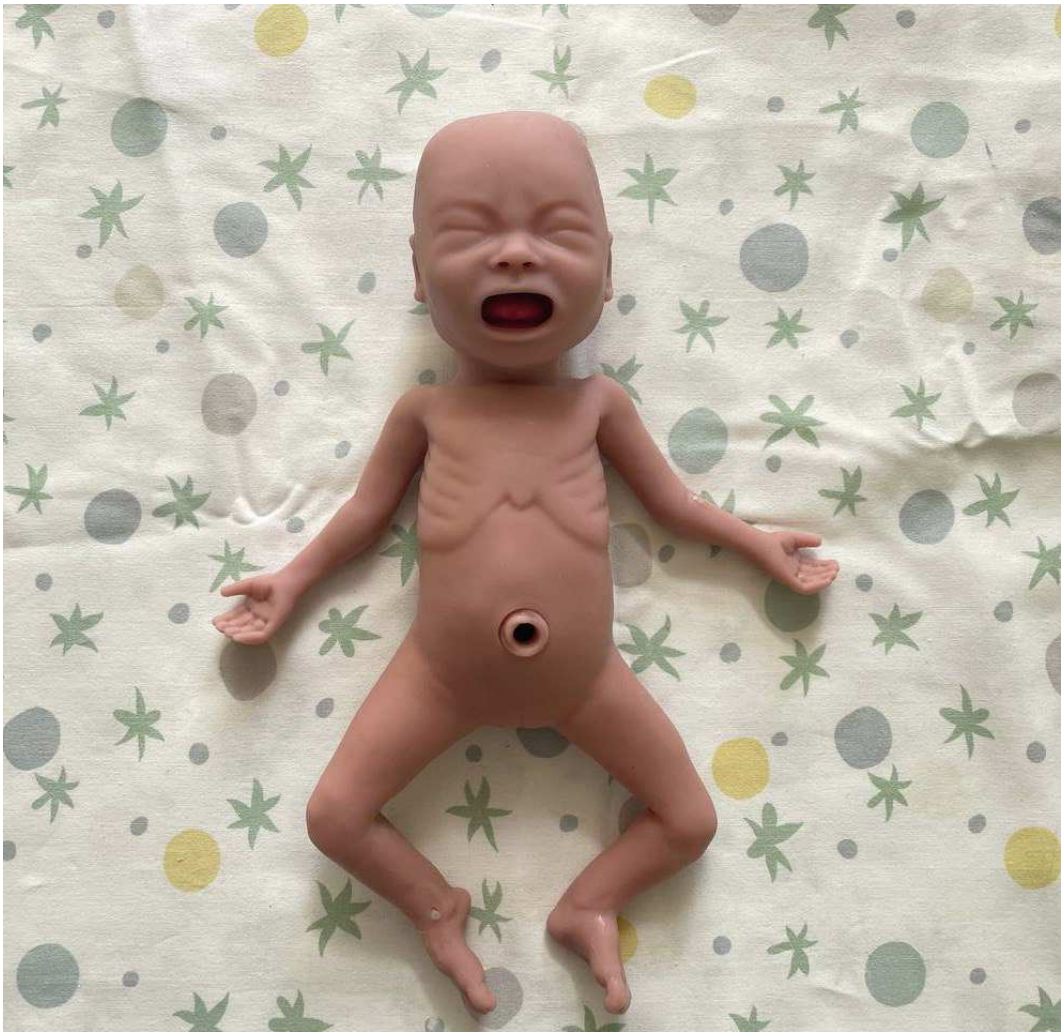

Figure 3. Neonatal simulator manikin used in this trial: Premature Anne, Laerdal Medical Corporation, Stavanger, Norway.

5.3 Participants.

Level III neonatal intensive care unit (NICU) consultants and pediatric residents who had previous experience with LISA and INSURE were eligible to participate in the study. Refusal to participate was the only exclusion criteria.

5.4 Randomization.

Participants were randomly allocated to AB or BA arms (1:1 ratio) using a computer-generated random assignment list. Arm assignments were put in sequentially numbered, sealed, opaque envelopes.

5.5 Procedures.

Participants in AB arm were designated to perform the procedure with LISA (LISAcath®, Chiesi Farmaceutici, Parma Italy) catheter with unmarked tip, followed by the procedure with LISA catheter with marked tip (Figures 4 and 5). Participants in BA arm were designated to the reverse sequence. A washout period of 6 hours was included to reduce any carryover effect. During each simulation, an external observer recorded the study outcomes.

After each attempt, the positioning of the device was evaluated by the external observer using a laryngoscope, and the procedure was repeated if the device was not in the trachea. The maximum time allowed for each attempt was 30 seconds. If the procedure was not completed within 30 seconds, the participant paused for 30 seconds, then he/she performed another attempt. The procedure was repeated until the device was positioned in the trachea. If the device reached the trachea during the attempt, the procedure was not repeated in case of incorrect depth in the trachea. The total time of device positioning was calculated as the sum of the times of all attempts needed to achieve the device positioning in the trachea.

At the end of each simulation, participants were asked to grade the difficulty in using the device (not difficult; mildly difficult; moderately difficult; very difficult; extremely difficult) overall and regarding three specific aspects (handling the device, inserting the device in the trachea, achieving the correct depth). All procedures were video recorded.

Figure 4. Devices for surfactant administration used in the trial: LISA (LISAcath®, Chiesi Farmaceutici, Parma Italy) catheter with marked and unmarked tip.

Figure 5. Qr-code redirecting to a video on how to perform Surfactant Replacement Therapy with LISA technique using LISA catheter in a manikin simulating an extremely low birth weight infant.

5.6 Outcome measures.

The primary outcome measure was the positioning of the device at the correct depth in the trachea (as assessed by the external observer using a laryngoscope). The secondary outcome measures included the total time and the number of attempts for positioning the device in the trachea, and participant's opinion on using the device (evaluated using a Likert scale). The time of device positioning was defined as the time elapsed from the positioning of the laryngoscope in the manikin mouth to the connection of the syringe to the catheter. As the procedure was repeated in case of device not in the trachea, the total time of device positioning will be calculated as the sum of the time of device positioning in all attempts.

5.6 Data collection.

Participant characteristics (age, sex, experience) and trial data (randomization sequence and outcome measures) were collected by an observer who was not involved in the simulation. Data were recorded on a dedicated data sheet and stored in a password-protected computer to ensure confidentiality before, during, and after the trial.

5.7 Masking.

The characteristics of the intervention did not allow the masking of participants and outcome assessors. The statistician performing data analysis was masked to treatment allocation.

5.8 Sample size.

A minimum sample size of 32 participants was required to have an 80% chance of detecting, as significant at the 5% level, an increase of 20% in the primary outcome measure in a crossover design. The calculation was based on previous

observations[36] and was performed using R 4.1 (R Foundation for Statistical Computing, Vienna, Austria).[37]

6. STATISTICAL ANALYSIS.

This crossover study used an AB/BA scheme, which is uniform within sequences and periods (thus removing any period and sequence effects), and included a washout period that was chosen to reasonably prevent any carryover effects. Numerical data were summarized as median and interquartile range (IQR), and categorical data as absolute frequency and percentage. Numerical outcome measures were not Normally distributed (according to the q-q plots), hence were compared between the two arms using the Wilcoxon signed-rank test and effect sizes were reported as median difference with bootstrap 95% confidence interval. Binary outcome measures were compared between the two arms using the McNemar test and effect sizes were reported as difference in proportion for paired data with 95% confidence interval. Participants' opinions about difficulty in using the device were evaluated using a Likert scale and compared between the two arms using the Wilcoxon signed-rank test. All tests were 2-sided and a p-value less than 0.05 was considered statistically significant. Statistical analysis was performed using R 4.1 (R Foundation for Statistical Computing, Vienna, Austria).[37]

7. RESULTS.

All eligible participants were enrolled in the trial (Figure 5). The analysis included 50 participants (21 males and 29 females, median age 32 years, IQR 30-38) with a median experience in neonatal intensive care of 1 year (IQR 1-6). Experience in surfactant treatment with INSURE was >20 cases in 15 participants, 10-20 cases in six participants, 5-10 cases in four participants and <5 cases in 25 participants. Experience in surfactant treatment with LISA catheter was >20 cases in four participants, 10-20 cases in five participants, 5-10 cases in two participants and <5 cases in 39 participants.

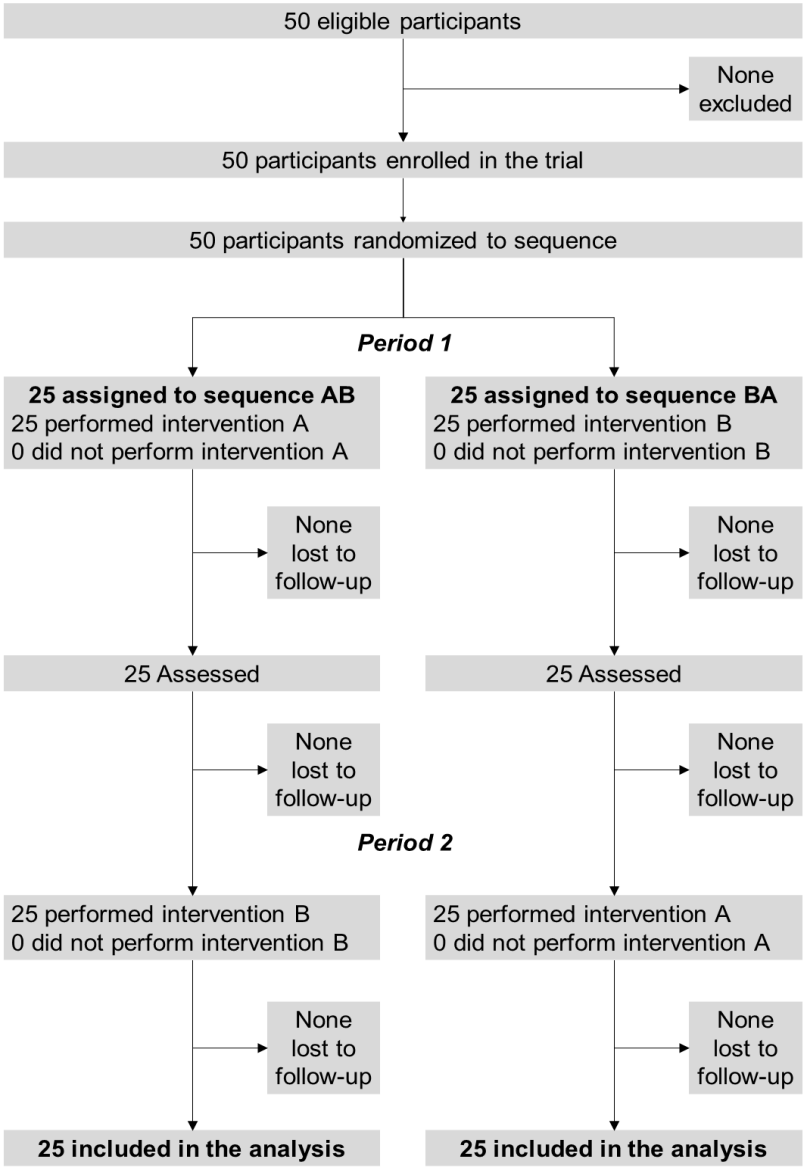

Figure 5. CONSORT flow diagram.

The correct depth of the device in the trachea (primary outcome measure) was achieved by 38 participants (76%) using the catheter with marked tip and 28 participants (56%) using the catheter with unmarked tip (difference in proportion 20%, 95% confidence interval 1 to 37%; p=0.04; Table 1).

Median time of device positioning was 19 seconds (IQR 14-22) using the catheter with marked tip and 20 seconds (IQR 15-22) using the catheter with unmarked tip (p=0.08; Table 1). Inserting the device in the trachea at first attempt was achieved by all participants (100%) when using the catheter with marked tip and 46/50 participants (92%) when using the catheter with unmarked tip (p=0.13; Table 1).

	Outcome measure	Procedure with LISA catheter with marked tip	Procedure with LISA catheter with unmarked tip	Comparison of LISA catheters with marked vs. unmarked tip	
Primary outcome		n (%)	n (%)	p-value (McNemar test)	Difference in proportion for paired data (95% confidence interval)
	Correct depth of the device in the trachea	38 (76%)	28 (56%)	0.04	20% (1% to 37%)
Secondary outcomes		median (IQR)	median (IQR)	p-value (Wilcoxon signed-rank test)	Median difference (bootstrap 95% confidence interval)
	Total time of device positioning, seconds	19 (14-22)	20 (15-22)	0.08	-1 (-4 to 0)
		n (%)	n (%)	p-value (McNemar test)	Difference in proportion for paired data (95% confidence interval)
	Number of attempts to insert the device in the trachea: 1 attempt 2 attempts	 50 (100%) 0 (0%)	 46 (92%) 4 (8%)	0.13	8% (-2% to 19%)

Table I. Outcome measures.

Participants' opinions about difficulty in using the catheters are displayed in Figure 6 (numerical results in Supplementary Table 1). Overall, the participants found the catheter with the marked tip easier to use (Figure 6D, p=0.007), especially concerning the insertion in the trachea (p=0.04, Figure 6B) and the positioning at the correct depth (p=0.004, Figure 6C). The different opinion about handling the devices was only close to statistical significance (p=0.06, Figure 6A).

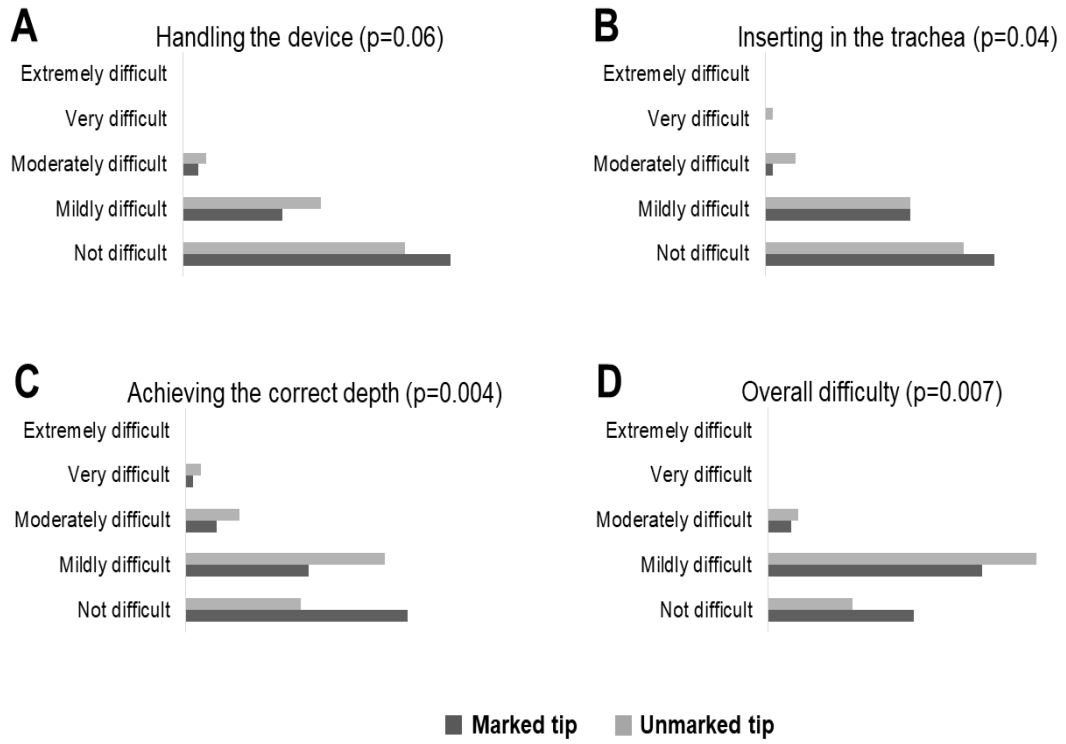

Figure 6. Participants' opinions about difficulty in using the catheters with marked and unmarked tips (evaluated using a Likert scale).

7.1 Supplementary material.

Aspect	Procedure with LISA catheter with marked tip: n (%)	Procedure with LISA catheter with unmarked tip: n (%)
Difficulty in handling the device:		
1 not difficult	35 (70)	29 (58)
2 mildly difficult	13 (26)	18 (36)
3 moderately difficult	2 (4)	3 (6)
4 very difficult	0 (0)	0 (0)
5 extremely difficult	0 (0)	0 (0)
Difficulty in inserting the device in the trachea:		
1 not difficult	30 (60)	26 (52)
2 mildly difficult	19 (38)	19 (38)
3 moderately difficult	1 (2)	4 (8)
4 very difficult	0 (0)	1 (2)
5 extremely difficult	0 (0)	0 (0)
Difficulty in achieving the correct depth:		
1 not difficult	29 (58)	15 (30)
2 mildly difficult	16 (32)	26 (52)
3 moderately difficult	4 (8)	7 (14)
4 very difficult	1 (2)	2 (4)
5 extremely difficult	0 (0)	0 (0)
Overall difficulty:		
1 not difficult	19 (38)	11 (22)
2 mildly difficult	28 (56)	35 (70)
3 moderately difficult	3 (6)	4 (8)
4 very difficult	0 (0)	0 (0)
5 extremely difficult	0 (0)	0 (0)

Supplementary Table I. Participant ratings (Likert scale) of the difficulty in using the devices.

8. DISCUSSION.

In our trial, the use of a marked tip LISAcath to perform SRT with LISA technique led to a 76% correct positioning of the device, evaluated as the proper depth of the device in the trachea, whilst using a non-marked tip LISAcath for the same procedure enabled only 56% of the participants to reach the right depth. This is coherent with our initial hypothesis which was based on a trial which is now under evaluation for publication. [36]

Catheter placement time differs only by 1 second between using marked vs. unmarked tip catheter, which slightly favors the marked tip device over the unmarked one, even if clinically it's not that much of a difference. However, it cannot be excluded that with a larger group of participants the difference in time of positioning may be more statistically and/or clinically significant. Even if the results would be the same as this trial, it would be understandable since the device is essentially the same, which resulted in this study's participants opinion about ease of handling being not that much different between the two devices.

The number of attempts to correctly positioning the device was 1 attempt in 100% of cases using the marked catheter, while 92% of the participants took 1 attempt to correctly position the unmarked catheter (4 out of 50 participants took 2 attempts, corresponding to 8% of the total participants).

Participants found placing the marked tip catheter at the proper depth easier than the unmarked one ($p=0.004$), a little easier positioning the marked device in the trachea compared to the unmarked one ($p=0.04$), whilst the difference between handling the two devices is minimal and on the threshold of statistical significance ($p=0.06$).

This result is also understandable since the two devices are only slightly different, and in line with the previous results on the little statistically significant difference in total positioning time and number of attempts to position them correctly. Overall, the opinion of the participants points towards a better ease of use of the marked catheter ($p=0.007$).

The minimal sample size required to have a statistically significant difference in proper depth positioning of the device was widely achieved.

It has been demonstrated that LISA technique is superior (or at least non-inferior) to the INSURE one in terms of lower use of MV and lower risk of developing BDP[26], improving survival without major complications[25], and developmental outcome of the babies treated with it[29]. These findings helped the diffusion of this technique, which is now used throughout Europe even in the smaller infants[27]. This wide use of the LISA method fueled the need to produce a device for this specific technique, but health care providers may experience some difficulties in achieving the correct positioning of the device in the trachea. As stated in previous unpublished observations from this study group, health care providers reported having some difficulties achieving the correct depth in the trachea, and this can affect the administration of the surfactant, rendering it inadequate, or prolonging the duration of the laryngoscopy to achieve it (resulting in stressful consequences for the infants treated with it such as bradycardia, hypoxia and hemodynamic changes)[36]. As emerged from the results of this study, marking the tip of the LISA catheter could be useful to more easily reach the correct depth of the device in the trachea, while making its use easier. These results lead to recommend the use of a marked tip LISA catheter in the clinical practice. This information suggests a necessary technical improvement to the exiting LISA catheter and should be useful for the companies producing the catheters used for surfactant administration.

The strengths of this trial are the design (this is a randomized, controlled crossover (AB/BA) trial), the use of materials normally used in the clinical practice, and the use of a manikin which is very close to reality. A further strength regards the wide experience of participants who were enrolled in the study; indeed, there were not only experienced consultants but also pediatric residents with less experience, which makes it a heterogeneous group and allows the results to be generalized also to non-level III NICUs.

8.1 Limitations.

This study has also some limitations. Although the manikin used was close to real babies, the results of the trial cannot be automatically transported in real children. Furthermore, the setting of the study lacked the stress, pressing and emotional involvement of clinical practice, which may influence the performance of the participants.

9. CONCLUSION.

The results of this trial demonstrate that it is easier to position a LISA catheter to the right depth in the trachea when the device has the tip marked, while the time for positioning the device and the number of attempts were similar between the marked tip catheter and the unmarked one. Health care professionals expressed an easier use of the marked tip device, especially when referring to the difficulty of achieving the correct depth in the trachea. However, the trial lacked the clinical practice's setting necessary to automatically transfer the results in real neonates and real clinical settings. Further trials are necessary to confirm our findings in the clinical practice.

10. TRIAL REGISTRATION.

This trial has been registered at clinicaltrial.gov NCT05399628.

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12. APPENDIX 1 – PROTOCOL OF THE STUDY.

Study design: this is an unblinded, randomized, controlled, crossover (AB/BA) trial of surfactant treatment with LISA catheter with marked tip vs. LISA catheter with unmarked tip in a manikin simulating an extremely low birth weight infant.

Setting: the study will be conducted at the University Hospital of Padova as coordinating center (daniele.trevisanuto@unipd.it) and Fondazione Poliambulanza of Brescia as participating center (paolo.villani@poliambulanza.it).

Inclusion criteria: level III NICU consultants and residents will be eligible to participate in the study.

Exclusion criteria: there are no exclusion criteria for this study.

Randomization: all participants will be randomly assigned to AB or BA arms in a 1:1 ratio. Randomization will be performed using a computer-generated random assignment list. Arm assignments will be included in sealed opaque envelopes sequentially numbered.

Procedure: participants in AB arm will be assigned to perform the procedure with LISA catheter with marked tip, followed by the procedure with LISA catheter with unmarked tip. Participants in BA arm will be assigned to the reverse sequence. A washout period of 6 hours (one procedure in the morning and one in the afternoon) will be included to reduce any carryover effect.
During each simulation, an external observer will record the study outcomes.
After the first attempt, the positioning of the device will be evaluated by the external observer using a laryngoscope, and the procedure will be repeated if the device will not be in the trachea.
The maximum time allowed for each attempt will be 30 seconds. If the procedure would not be completed in 30 seconds, the participant will stop for 30 seconds and will perform another attempt. The procedure will be repeated until the device will be positioned in the trachea. If the device would reach the trachea during the attempt, the procedure will not be repeated in case of incorrect depth in the trachea. The total time of device positioning will be calculated as the sum of the times of all attempts needed to achieve the device positioning in the trachea.

Outcome measures: the primary outcome measure will be the positioning of the device at the correct depth in the trachea (as assessed by the external observer using a laryngoscope).
The secondary outcome measures will be the total time and the number of attempts for positioning the device in the trachea, and participant's opinion on

using the device (evaluated using a Likert scale). The time of device positioning was defined as the time elapsed from the positioning of the laryngoscope in the manikin mouth to the connection of the syringe to the catheter. As the procedure would be repeated in case of device not in the trachea, the total time of device positioning will be calculated as the sum of the time of device positioning in all attempts.

Sample size: a sample size of 32-66 participants will be required for a 80-90% chance of detecting an increase in the primary outcome from 70% to 90% at a 2-sided significance level of 5%. The calculation was based on unpublished data (paper under revision) and was performed using R 4.1 (R Foundation for Statistical Computing, Vienna, Austria).

Recruitment: written and oral information will be offered to the participants by a competent professional who is trained in neonatal resuscitation. Consent to use the data will be obtained by all participants.

Blinding: due to the characteristics of the intervention, neither caregivers nor outcome assessors will be masked to treatment allocation. However, the statistician performing data analysis will be masked to treatment allocation.

Guidelines for Management: before starting the study, the participants will join a meeting where all the details of the study protocol will be presented. During each simulation, an external observer will record the study outcomes.

Data collection: data will be recorded in a data sheet designed for this study and maintained in order to protect confidentiality before, during, and after the trial by the principal investigator in a personal computer protected by password. All data will be collected by an observer not involved in the simulation. The following information will be registered: randomization sequence, participant age and experience, study outcomes (as described before).

Statistical analysis: continuous data will be expressed as mean and standard deviation or median and interquartile range, and categorical data as number and percentage. The study will include a washout period that was chosen to reasonably prevent carryover effects. Since tests for carryover effect are generally underpowered, the inclusion of an adequate washout period is strongly recommended to prevent carryover effects. (7) The primary outcome measure will be compared between the two procedures using Mc Nemar test. The secondary outcome measure will be compared between the two procedures using Student t test and Mann-Whitney test. All tests will be 2-sided and a p-value less than 0.05

will be considered statistically significant. Statistical analysis will be performed using R 4.1 (R Foundation for Statistical Computing, Vienna, Austria).

Duration of study: after obtaining approval from the Ethics Committee, we expect to perform the study in two weeks.

Ethical considerations: the trial is being submitted to the Ethics Committees of the participating centers. All participants will provide written informed consent and all data will be anonymized.

Compliance to protocol: compliance will be defined as full adherence to protocol. Compliance with the protocol will be ensured by the principal investigator and the local collaborators; they will be responsible for local data collection.

Dissemination policy: the results of the trial are expected to be published in a scientific journal and to be presented in medical seminars and conferences. The final reporting will follow the CONSORT Report guidelines (http://www.consort-statement.org).

Abbreviations: LISA: less invasive surfactant administration; RDS: respiratory distress syndrome.

Competing interests: the authors declare that they have no competing interests.

13. APPENDIX 2 – CFR OF THE STUDY.

CASE REPORT FORM

TITLE: Tracheal positioning of LISA catheter with marked vs. unmarked tip in extremely low birth weight infants with RDS: a crossover randomized controlled manikin trial.

Informazioni sul partecipante
Nome _____ Cognome _____ Medico: a) specialista b) specializzando Età _____ Anni di esperienza in TIN _____ Numero di somministrazioni di surfattante con tubo endotracheale: a) <5 b) 5-10 c) 10-20 d) >20 Numero di somministrazioni di surfattante con LISA (catetere rigido): a) <5 b) 5-10 c) 10-20 d) >20 Numero di somministrazioni di surfattante con LISA (catetere morbido): a) <5 b) 5-10 c) 10-20 d) >20

Procedura 1 **indicare il device usato: □ Catetere marcato □ Catetere non marcato**		
Outcome		
Outcome	**Definizione**	**Risultato**
Raggiungimento della corretta profondità in trachea	È stata raggiunta la corretta profondità in trachea della punta?	
Tempo totale di posizionamento (in secondi)	Tempo dall'inizio della laringoscopia al corretto posizionamento in trachea. Il tempo limite per ciascun tentativo è di 30 secondi. Se sono necessari più tentativi, va sommato il tempo di ciascun tentativo.	
Numero di tentativi (n.)	È stato ottenuto il corretto posizionamento in trachea al primo tentativo o successivi?	

Procedura 1 **indicare il device usato: □ Catetere marcato □ Catetere non marcato**		
Soddisfazione del partecipante		
Aspetto	**Definizione**	**Risposta**
Maneggiare il device	Hai sperimentato difficoltà nel maneggiare il device?	□ 1 per nulla □ 2 un po' □ 3 abbastanza □ 4 molto □ 5 moltissimo
Visualizzare la glottide	Hai sperimentato difficoltà nel visualizzare la glottide?	□ 1 per nulla □ 2 un po' □ 3 abbastanza □ 4 molto □ 5 moltissimo
Inserimento del device in trachea	Hai sperimentato difficoltà nell'inserire il device in trachea?	□ 1 per nulla □ 2 un po' □ 3 abbastanza □ 4 molto □ 5 moltissimo
Profondità corretta	Hai sperimentato difficoltà nel posizionare il device alla corretta profondità?	□ 1 per nulla □ 2 un po' □ 3 abbastanza □ 4 molto □ 5 moltissimo
Difficoltà complessiva	Qual è stata la difficoltà complessiva che hai sperimentato nell'usare il device?	□ 1 nessuna difficoltà □ 2 lieve difficoltà □ 3 moderata difficoltà □ 4 molta difficoltà □ 5 elevata difficoltà

Procedura 2 **indicare il device usato: □ Catetere marcato □ Catetere non marcato**		
Outcome		
Outcome	**Definizione**	**Risultato**
Raggiungimento della corretta profondità in trachea	È stata raggiunta la corretta profondità in trachea della punta?	
Tempo totale di posizionamento (in secondi)	Tempo dall'inizio della laringoscopia al corretto posizionamento in trachea. Il tempo limite per ciascun tentativo è di 30 secondi. Se sono necessari più tentativi, va sommato il tempo di ciascun tentativo.	
Numero di tentativi (n.)	È stato ottenuto il corretto posizionamento in trachea al primo tentativo o successivi?	

Procedura 2 **indicare il device usato: □ Catetere marcato □ Catetere non marcato**		
Soddisfazione del partecipante		
Aspetto	**Definizione**	**Risposta**
Maneggiare il device	Hai sperimentato difficoltà nel maneggiare il device?	□ 1 per nulla □ 2 un po' □ 3 abbastanza □ 4 molto □ 5 moltissimo
Visualizzare la glottide	Hai sperimentato difficoltà nel visualizzare la glottide?	□ 1 per nulla □ 2 un po' □ 3 abbastanza □ 4 molto □ 5 moltissimo
Inserimento del device in trachea	Hai sperimentato difficoltà nell'inserire il device in trachea?	□ 1 per nulla □ 2 un po' □ 3 abbastanza □ 4 molto □ 5 moltissimo
Profondità corretta	Hai sperimentato difficoltà nel posizionare il device alla corretta profondità?	□ 1 per nulla □ 2 un po' □ 3 abbastanza □ 4 molto □ 5 moltissimo
Difficoltà complessiva	Qual è stata la difficoltà complessiva che hai sperimentato nell'usare il device?	□ 1 nessuna difficoltà □ 2 lieve difficoltà □ 3 moderata difficoltà □ 4 molta difficoltà □ 5 elevata difficoltà

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