Universitat de Girona

Comparing the effectiveness between fentanyl, remifentanil and propofol in preterm newborns undergoing INSURE procedure: a randomized controlled trial

End of Term Project

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1. ABBREVIATIONS

BPD	Bronchopulmonary dysplasia
DPPC	Dypalmitoil-phosphatidil-coline
ELBW	Extremely low birth weight
FiO_2	Inspired oxygen fraction
GA	Gestational age
GW	Gestational weeks
IVH	Intraventricular hemorrhage
INSURE	Intubation, surfactant administration, immediate extubation
LBW	Low birth weight
nCPAP	Nasal continuous positive airway pressure
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
PDA	Patent ductus arteriosus
PEEP	Positive end-expiratory pressure
PVL	Periventricular leukomalacia
RDS	Respiratory distress syndrome
ROP	Retinopathy of prematurity
SpO_2	Oxygen saturation
VLBW	Very low birth weight

2. ABSTRACT

Keywords: Pulmonary Surfactants; Respiratory Distress Syndrome, Newborn; Remifentanil; Fentanyl; Propofol; Infant, Very Low Birth Weight; Infant, premature;

Objectives: the aim of this study is to compare the effectiveness of fentanyl, remifentanil and propofol as a premedication for INSURE procedure, to treat preterm newborns suffering from respiratory distress syndrome (RDS).

Design: non-placebo controlled, double blind, randomized controlled clinical trial.

Setting: multicenter clinical trial, involving 22 centers from Catalonia and Madrid. Hospital Universitari Josep Trueta (Girona) will be the reference center.

Participants: preterm newborns, below 32 GW or 1,500 grams of birth weight, suffering from RDS.

Interventions: patients will be randomized in three groups and each group will receive one of the three premedications. Secondly they will undergo INSURE procedure, response and adverse effects will be recorded. Infants will be followed-up during two years, and long-term effects development will be collected.

Main outcome measures: primary outcome measure is the time to succesful extubation. Secondary outcome measures include appearance of complications during the procedure, and development long-term secondary effects.

3. INTRODUCTION

3.1. Definitions

Prematurity is defined as a birth occurring before completed 37 weeks of gestation[1]. It can be classified depending on gestational age (GA) or on birth weight.

	Gestational Age	Proportion[2]
Late preterm birth	32-37 GA	84.3%
Very preterm birth	28-32 GA	10.4%
Extremely preterm birth	<28 GA	5.2%

Table 1: Distribution of preterm birth according to Gestational Age. Adapted from: 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[2]

	Birth Weight (grams)	Proportion[3]
Low birth weight (LBW)	2.500-1.500g	88.5%
Very low birth weight (VLBW)	1.500-1.000g	11.5%
Extremely low birth weight (ELBW)	<1.000g	

Table 2: Distribution of preterm birth according to birth weight. Adapted from Euro-Peristat[3]

There also are adjusted weight percentile tables for premature newborns according to gestational age (annex 1).

3.2. Epidemiology

"About one in 20 babies born in Europe in 2010 weighted less than 2.500 grams at birth"[3]. 135 milion livebirths were estimated to occur in 2010 worlwide[2], with an estimated mean preterm birth rate of 11.1%. Preterm birth rates vary among countries, highest rates occuring in low-income ones. In Spain, there were 485.252 livebirths on 2010 (crude birth rate of 10.5%)[4], with a percentage of newborns weighting less than 2.500 grams that varies between 7.8%[5] to 8.8%[3] depending on the study[6].

Prematurity and its complications[7;8] are responsible for a 35% of 3.1 million annual neonatal deaths worldwide, specially due to neonatal infections, and long-term morbidities such as neurodevelopmental delay. Perinatal mortality rate of 11.7% was described in our country for newborns under 1.500 grams[9]. Mortality and risk of complications increase with increasing prematurity, it can be as high as 81% for newborns under 24 GA, 42.2% between 25-26 GA and 17.5% between 26-27 GA[9].

3.3. Short-term complications of prematurity

Apnea of prematurity[10]

Apnea is produced physiologically in preterm babies, known as periodical respiration. This respiratory pattern is irregular, with short pauses and no repercussions. We consider apnea as patological when it is longer than 20 seconds, having or not clinical repercussions, or when apnea causes cardiocirculatory alterations independently of its duration. Apnea appears in premature newborns, being more frequent as gestational age decreases.

General caring measures must be taken to decrease this episodes, such as temperature, oxigenation and posture. When apnea is secondary, for example due to neonatal sepsis, hipoglycemia or analgesia, treatment of the cause solves apnea. Primary apnea is treated with caffein, as it activates respiratory center. nCPAP can decrease episodes of apnea as it avoids airway collapse.

Intraventricular hemorrhage (IVH)[10]

IVH is the most frequent brain damage present in premature babies. About 20-30% of VLBW newborns suffer from IVH, and it is an important cause of future neurodevelopmental disorders. Smaller newborns are at more risk of severe IVH and posterior complications.

Diagnose can be obtained through cranial ultrasonography. IVH can be classified in 3 degrees depending on the severity and localization:

- Grade I: bleeding confined in germinal matrix (periventricular)
- Grade II: intraventricular bleeding occuping less than 50% of the ventricle

- Grade III: intraventricular bleeding occuping more than 50% of ventricle area, or distending the ventricle.
- Periventricular hemorrhagic infarction (PHI): PHI can occur when severe IVH progresses, by an obstruction of terminal veins and periventricular congestion, causing ischemia and infarction. It is present in 15% of IVH, usually in massive IVH, and a periventricular white matter hemorrhagic necrosis can be observed.

Prevention of IVH can be made by preventing premature birth. Esteroids have been shown to act in reduction of mortality and severity of IVH. Once it is established, the aim is to avoid its progression. Protective measures must be applied, such as preventing haemodynamic alterations, coagulation disorders, and protecting germinal matrix vessels.

Periventricular leukomalacia (PVL)[10]

PVL is defined as the necrosis of periventricular white matter, located dorsal and lateral to lateral ventricles. Commonly it appears associated with IVH, taking into account that situations causing perinatal ischemia can result to both of these conditions. Incidence of PVL increases as gestational age decreases.

PVL can be diagnosed, like IVH, through ultrasonography, appearing as a bilateral hiperechogenicity. It can be classified in 4 stages, depending on the severity and extension.

This ultrasonographic discovery is related to future neurodevelopmental disorders. Most common long-term complication resulting from PVL is spastic diplegia, often manifested in inferior limbs. Prevention of PVL can be made not only by preventing premature birth, but also preventing prenatal infections, insuring an early diagnose and antibiotic treatment, as prenatal infections have been shown to increase risk of having PVL.

Patent ductus arteriosus (PDA)[10]

Ductus arteriosus is a blood vessel that connects descendent aorta to pulmonary artery, and it is an essential vascular structure during fetal period, since it allows to bypass the fetal non-functioning lungs. Ductus is usually closed after birth, but in premature newborns, closure can be delayed more than a week. It is also associated with RDS. Incidence of PDA increases as gestational age decreases.

It is usually manifested by a systolic heart murmur, and we must suspect a PDA when a baby suffering from RDS, who was clinically improving, suddenly starts worsening. Diagnose is obtained through echocardiography. Prenatal steroids have been shown to be protective in the development of PDA, and treatment can vary, and might be conservative, pharmacological or surgical.

Sepsis[10]

Premature babies have more risk to develop neonatal sepsis because of the lack of maturity in their immunological system. Neonatal sepsis is the presence of microorganisms in a newborn's bloodstream, appearing in the first 28 days of living.

We can differenciate between vertical transmission, when colonization is produced before or during birth, and nosocomial infection when it is acquired in the NICU. Clinical manifestations are not specific, and diagnose is made by suspicion, when risk factors of vertical transmission are known, and also by clinical criteria. It is also common to find the microorganism responsible in the mother's vaginal culture. Cerebrospinal fluid must be analysed when it's possible, since 20-25% of neonatal sepsis can be associated with meningitis. Specific antibiotical treatment and suportive measures are needed to treat sepsis, as mortality is not infrequent in this condition.

Retinopathy of prematurity (ROP)[10]

Premature newborns usually develop ROP, a proliferative perripherical vitreorrepinopathy, due to immaturity of retina vessels. Vision might be affected,

specially in most severe cases, which can be completely lost. To prevent this situation, laser treatment in early stages is needed.

Necrotizing enterocolitis (NEC)[10]

NEC is a severe digestive disorder present during neonatal period. Ethiology is not well-stablished, a combination of factors result in a intestinal necrosis, and perforation in some cases. Incidence of NEC is around 7% in VLBW. Prematurity and enteral formula feeding have been shown to be the most important risk factors associated in the development of NEC.

Abdominal distention can be an early sign of NEC and must be evaluated in premature newborns. Diagnose is stablished considering the clinical manifestations (Bell staging), radiological findings, such as intestinal pneumatosis, and analytical findings, which are not specific but can manifest the severity.

Prevention of NEC is made by breastfeeding. Mortality remains between 15-30%, being higher in smaller newborns. NEC can result in digestive sequelae, like stenosis and short bowel syndrome, and treatment includes conservative measures and surgical procedures.

Hypoglycemia[10]

Hypoglycemia occurs when metabolic adaptation fails after birth. It is more common in premature newborns, incidence fails around 3.2-14.7% in this group. Glucose levels to define hypoglycemia are controversial, but generally, glycemia under 45 mg/dl is considered pathological.

Hypoglycemia is common in infants of diabetic mothers. Preterms are also at more risk because of the lack of glycogen reserves, immaturity of hormonal response and difficulties in alimentation. In this case, early feeding is needed and it should be frecuent. When hypoglycemia is persistent, intravenous glucose can be administered, and if it’s not corrected, intramuscular glucagon is avaliable.

3.3.1. Respiratory distress syndrome (RDS)[10-12]

Epidemiology and presentation

Respiratory distress syndrome (RDS), also know as hyaline membrane disease (HMD) almost exclusively affects preterm newborns, specially under 32 GW. The incidence increases as the gestational age and the birth weight decreases, observing a 50% frecuency in newborns between 26-28 GW[10], and a frecuency as high as 93% in VLBW infants independently of their gestational age[13]. RDS is still an important contributing cause to neonatal mortality and morbidity, despite the advances in prenatal diagnosis, prevention, respiratory suport and surfactant therapy.

It appears when a newborn suffering from surfactant defficit starts breathing, being the defficit not only biochemical, but also functional, as the pulmonary development has not been already completed. This immature lung cannot provide enough oxygenation and gas exchange, therefore breathlessness and cyanosis appear shortly after being born, insidiously progressing, getting more severe between 24-48 hours of living, and improving after 3 days alive.

Risk factors

Incidence of RDS can vary according to some perinatal risk factors. Incidence has been proven to be higher in male newborns, caesarean delivery, caucassic babies, second twins, perinatal asphyxia or other perinatal or postnatal complications. Thoracic malformations causing lung hypoplasia, such as diaphragmatic hernia, can result in an increased incidence of RDS.

Genetic disorders of surfactant production and metabolism have been demonstrated. It is a rare cause of severe RDS, present in term newborns, requiring lung transplantation in most cases. These mutations include surfactant protein B and C gene mutations, and mutations in ABCA3 gene. More than 100 ABCA3 gene mutations that cause surfactant disfunction have been described. Normal ABCA3 protein is found in the the lamellar bodies produced by alveolar type II cells as a transporter. Its principal function is to transport phospholipids to interact with surfactant proteins to form the surfactant. These mutations can

prevent the insertion of ABCA3 protein in lamellar bodies, or can inhibit its function. As a result, surfactant composition and function is abnormal.

Babies of mothers suffering from uncrontolled insulinodepenent diabetes or gestational diabetes, usually big for gestational age, have a higher risk of developing RDS. This risk can be reduced with a well medical control of mothers with diabetes. Also, maternal factors such as hypertension (chronical or during pregnancy) and placental abruption.

Physiopathology

Surfactant is an agregation of macromolecules of proteins (4 proteins have been described in surfactant: SP-A, SP-B, SP-C and SP-D), phospholipids (principally phosphatidil-colyne, most of it in form of DPPC or dypalmitoil-phosphatidil-coline) and carbohidrates. This components help reduce superficial tension between air and liquid.

Many factors can produce surfactant defficit. There's a possibility that surfactant production is decreased, or surfactant inactivation is increased, or surfactant quality might be altered. In either way, this defficit results in a loss of tense-active function that causes alveollar collapse. Consequently, there's ventilation difficulty, ventilation-perfussion mismatching and atelectasis. Lungs appear rigid, easier to collapse. Diaphragm and torax wall weakness provoques that more effort is needed to maintain enough ventilation, so it is insufficient.

Ventilation-perfussion mismatching causes hypoxemia, cianosis, CO_2 retention, mixed acidosis and increasing pulmonary vascular resistance, resulting in right-to-left shunting that at the same time, worsens hypoxemia. Pulmonary edema also inactivates surfactant, and more pressure is needed to open collapsed alveoli.

Surfactant therapy helps breathing in a way that less pressure is needed to open collapsed alveoli, contributing to a bettes oxygenation and gas exchange.

Clinical presentation

Newborn suffering from RDS, usually premature, has a shortness of breath, rapid and shallow breathing and appears to be cyanotic. Severity of RDS can be estimated with Silverman scoring system. It evaluates 5 parameters, each one rating from 0 to 2:

- Upper chest or torax-abdomen dissociation: in can be synchronized (0), abdominal movement with rigid torax (1) or see-saw breathing (2).
- Lower chest or retraction: absence of retraction (0), intercostal retraction (1) or marked, including intercostal, suprasternal and infrasternal retraction (2).
- Xiphoid retraction: absent (0), visible (1) or marked (2).
- Nasal alae flaring: absent (0), minimal (1) or marked (2).
- Expiratory grunt: absent (0), audible with stethoscope (1) or audible at naked ear (2).

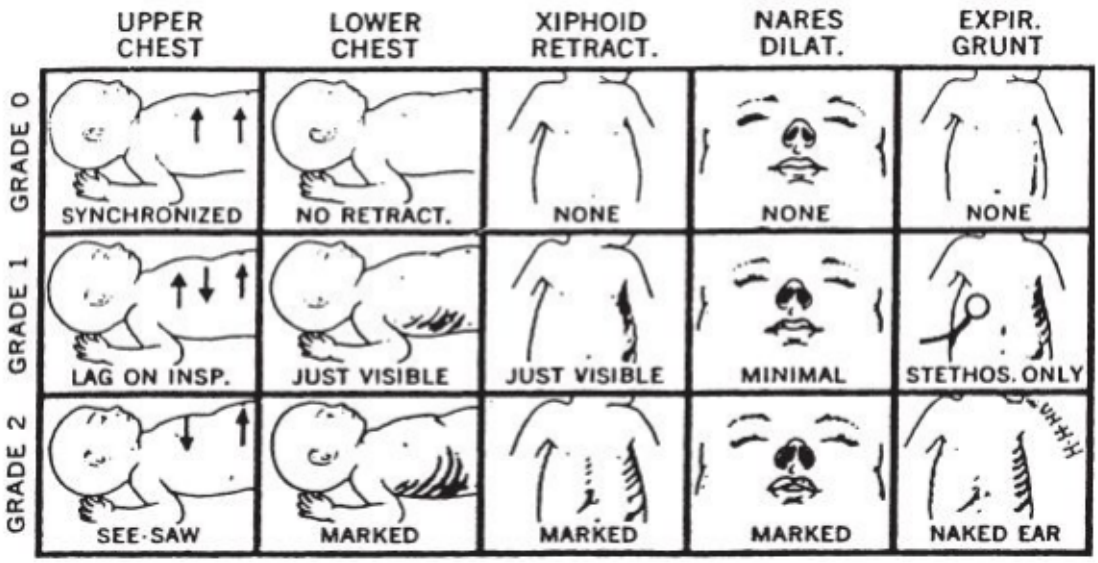

Figure 1: Silverman Scoring System. Extracted from: Silverman, W. and Anderson, D.: Pediatrics 17:1, 1956. American Academy of Pediatrics.

With this scoring system, RDS can be classified in:

- 0-2: no respiratory distress, or the distress is very mild.
- 3-4: moderate respiratory distress
- Equal or above 5: severe respiratory distress.

Usually, babies suffering from RDS grunt during expiration, due to glottis closure to maintain enough pulmonary volume. They need oxygenotherapy after being born, usually between 40-50% of concentration, and the need for oxygen increases, reaching a peak in the first 24-48 hours that can reach 100% needed concentration of O_2. Transiently, we can notice an improvement in oxygen needed when we treat concurring complications such as acidosis or hypothermia, but it deteriorates again.

Newborns with higher birth weight (usually from diabetic mothers) evolve slowly, needing less O2 and developing a generalised athelectasis in 48-72 hours.

Usually, an improvement can be observed after 48 hours of treatment when it's not complicated, and oxygenotherapy can be stopped a week after, except for VLBW babies, that demand higher requirements. Most premature infants, appart from suffering a more severe RDS, develop more frequently other complications of prematurity such as intraventricular hemorrhage (IVH), patent ductus arteriosus (PDA), air leak and infection.

Diagnosis

When a premature newborn is suffering from respiratory distress, the diagnosis is based on his clinical history and the toracic radiological findings, but keeping in mind that as the severity may not always be representated.

Arterial gasometry can help estimate the severity, commonly newborns with RDS develop mixed acidosis, hypoxemia and CO_2 retention. Oxygenation index is a parameter to stablish the gravity of RDS when the baby is under mechanical ventilation, considering a index above 15 indicates severe RDS. This parameter stablishes the relationship between FiO_2, mean airway pressure (MAP) and arterial pO_2:

$$OI= ((FiO_2 x MAP) \times 100) / PO_2$$

Radiological findings include a decrease on pulmonary volume (it can appear normal in the first hours), diffuse reticulogranular pattern, that can progress to

severe bilateral opacity (white-out), air bronchogram, atelectasis, and other complications such as emphysema, pneumothorax or BPD. These alterations are usually more noticeable on lung bases.

Prenatal prediction

It is possible to assess prenatal lung maturity examinating amniotic fluid obtained through amniocentesis (15-18 GW). The following tests are useful, but not commonly used in clinical practice to predict lung maturity, as they require an invasive procedure:

- Lecithin/shingomyelin (L/S) ratio: estimated with chromatography, taking into account that the results may be affected when the sample is contaminated with meconium or blood. The risk of RDS is considered very low when the L/S ratio is above 2, with some exceptions (infants of diabetic mothers, erythroblastosis fetalis and intrapartum asphyxia).
- TDx fetal lung maturity test II: by the use of fluorescent polarization it determines the surfactant to albumin ratio. Contaminated samples may affect the results. A ratio above 55 indicates lung maturity, taking into account the results are more precise when threshold according to gestational age are applied.
- Lamellar body counts: lamellar bodies increase with advancing gestational age in amniotic fluid. Obtaining a value above 50.000 lamellar bodies per microliter indicates lung maturity.
- Phosphatidylglycerol (PG): late predictor, when PG is found in amniotic fluid it correlates with lung maturity. Low sensivity is its major disadvantage, but this test can be applied even when the sample is contaminated.
- Foam stability index (FSI): amniotic fluid and ethanol are shaked in a test tube, secondly foam stability is measured. FSI is defined as the highest ethanol volume fraction that would allow the formation of stable foam. The apparition of this foam indicates presence of surfactant active material, and ethanol dilutions help determine the concentration.

Prevention[14]

Prevention of RDS is achieved with corticosteroid therapy. Corticostheroids induce surfactant production and accelerates lung maturation, reducing the incidence of RDS, concurring complications of prematurity (IVH, NEC) and mortality. With this therapy not only DPPC synthesis increases, but also pulmonary remodelation and maduration.

Indications for this treatment are pregnant women 24-34 GW at high risk of preterm delivery in the following week. Intact membranes or preterm rupture of membranes is required, chorioamnionitis should be absent.

Two doses of betamethasone (12 mg intramuscular) separated by 24 hours are required. Four doses of dexamethasone (6 mg intramuscular) separated by 12 hours are equaly effective. Incomplete corticostheroid therapy also improves outcome.

nCPAP early application may avoid surfactant inactivation, helping to mantain a correct alveolar volume and avoiding alveolar collapse. After surfactant therapy nCPAP also contributes to a clinical improvement by maintaining this alveolar volume.

Treatment[12;15-18]

RDS patients need to be treated in NICUs and monitorization is essential. Hypoxemia and acidosis should be prevented, and proper gas exchange should be achieved, minimizing lung injury and its complications.

Oxygenotherapy

Optimal pO_2 remains between 50-60 mmHg, according to that, FiO_2 should be adjusted. Higher pO_2 must be avoided as it can produce pulmonary injure and ROP. Targeted saturation remains between 88-92% in newborns below 30 GW, and 88-95% for older infants. Saturation will be monitorized continuously, and oxygen concentration must be checked every hour.

Arterial blood gases have to be checked frecuently, and 30 minutes after every intervention in respiratory therapy.

Ventilatory support[19]

Continuous positive airway pressure (CPAP) is a non-invasive ventilation method, usually applied with binasal cannula, used in the treatment of RDS. It is useful to avoid alveolar collapse, prevents atelectasis, minimizes lung injury, improves RDS evolution and allows rapid extubation of the patients after receiving surfactant therapy.

nCPAP might fail, specially in ELBW or in severe RDS, that require FiO_2 above 40-50% and have a $PaCO_2$ above 55-60 mmHg. In this case, they are intubated, mechanically ventilated and they receive surfactant therapy.

nCPAP is used connected to variable CPAP delivery devices, continuous-flow ventilator and “buble” CPAP are the most common. Continuous-flow ventilator is usually used, starting from a pressure of 5-7 cm H_2O, a flow of 5-10 L/minute, and then increasing pressure to a maximum of 8 cm H_2O.

CPAP may need to be reduced when hypercapnia is present, and in the case pulmonary vascular resistance may be raised, promoting right-to-left shunting, when positive pressure is transmitted in pulmonary vessels.

Weaning from nCPAP must be done as soon as the baby improves, usually when FiO_2 is below 30% and there’s no distress, whilst monitorizing oxygen saturation. In this case, lowering of the distending pressure is needed and if the infant remains stable, nCPAP discontinuation can be attempted.

Surfactant therapy[20]

By the administration of endotracheal exogenous surfactant the infant rapidly improves[21], residual functional capacity and pulmonary distensibility also increase, resulting in less oxygen and ventilatory support dependance. Incidence of complications such as emphysema and pneumotorax decreases.

Early rescue is usually preffered over delayed treatment. Surfactant can be obtained from natural orygin or synthetical. Most used surfactant in our environment is porcine[22], Curosurf (annex 2). Initial dose required is 2.5 mL/kg (200 mg/kg phospholipid), and readministrations of 1.25 mL/kg every 12 hours can be made if the patient requires it, up to two total doses. The dose is repeated when the baby still requires mechanical ventilation, at FiO_2 higher than 30% and airway pressure above 7 cm H_2O. Repeated dosages have shown to provide a better outcome in RDS treatment, however, giving more doses than reccommended (ore than three in the case of curosurf) does not add any benefit.

During administration, desaturation, bradycardia and apnea are frequent adverse effects. They often suffer from transient hypoxemia and need additional oxygen. Surfactant administration must be careful, according to the baby's tolerance. To avoid apnea, respiratory rate must be higher than 30 breaths per minute. Surfactant administration technique will be commented when INSURE method is explained.

Response to surfactant theraphy can vary depending on patient factors (concurrent illnesses, lung maturity achieved, excessive fluid administration and inadequate ventilation). Newborns suffering from RDS usually improve with surfactant therapy, but 20% of them do not respond. In this case, other pathologies, such as pneumonia, hypoplasia, pulmonary hypertension or congenital heart disease, must be considerated.

Pulmonary hemorrhage can result from surfactant therapy, but it is very rare, and occurs more commonly in ELBW male infants, or babies suffering from PDA. Surfactant therapy has not shown to prevent neurodevelopmental disorders and alterations in physical growth.

Mechanical ventilation

Mechanical ventilation must be started when we are in a situation of respiratory acidosis with $PaCO_2$ or rapidly rising, when PaO_2 falls below 50 mmHg or

oxygen saturation falls below 90% with FiO_2 above 50%, or in case of severe apnea. These indications may vary depending on the gestational age of the infant and the course of the disease.

Many modes of pressure-limited ventilation can be used for this purpose. Preferred one is synchronized inttermitent mechanical ventilation (SIMV), that synchronizes with the infant's own breathing, but assist-control, pressure support and volume-guarantee are also avaliable. High-frecuency oscillatory ventilation needs to be used in the most severe cases to minimize lung injury. This setting is also useful to treat infants who are still hypoxemic after regular mechanical ventilation, because of atelectasis, that produces shunting. Ventilator used in all cases must be continuous-flow, pressure limited, time cycled ventilator, in order to adjust independently pressure, inspiratory and expiratory durations.

Before connecting the infant to a ventilator, it is usually useful to ventilate the newborn manually. After that, mechanical ventilation is usually started at stablished initial settings, that are peak inspiratory pressure between 20-25 cm H_2O, PEEP of 5-6 cm H_2O, respiratory rate of 25-30 breaths per minute, inspiratory duration of 0.3-0.4 seconds at required FiO_2 (usually 50-100%). This settings might be adjusted depending on the clinical evaluation of the infant (color, chest motion, respiratory effort, breath sounds, oxygen saturation) and arterial blood gas results.

The aim is to maintain $PaCO_2$ between 45-50 mmHg, rising levels usually indicate an underlying complication. A minimal hypercapnia is accepted to minimize lung injury, but metabolic acidosis must be controlled in order not to worsen RDS.

During mechanical ventilation the newborn must be kept in a NICU, monitorizing his vital signs and evaluating frecuently his clinical condition. Constant evaluation of ventilator settings must be checked, and arterial blood gases need

to be checked every 4 to 6 hours, more frecuently if it's required, and 30 minutes after every intervention in ventilator settings.

As soon as the infant starts improving, weaning can be attempted. Extubation success will depend on the characteristics of the baby, such as size, blood gases, clinical stage, and response to treatment. Newborns lighter than 2000 grams must be weaned starting to decrease respiratory rate to 20 breaths per minute, and if he tolerates it and is stable at FiO_2 below 30%, the infant can be extubated. Heavier babies tolerate extubation at higher settings. nCPAP is commonly used after extubation to stabilize the infant.

INSURE

Treatment of RDS consists mainly of respiratory support, including nCPAP or mechanical ventilation, and surfactant therapy. Although, mechanical ventilation is quite an invasive procedure and can cause lung injury, and might increase BPD incidence[23]. Infants treated with nCPAP after surfactant administration have shown to have a better outcome than those who received mechanical ventilation after surfactant therapy.

INSURE method, that consists on IN-tubation, SUR-factant and E-xtubation, was proposed with the aim to reduce the need for mechanical ventilation and improve RDS outcome. It consists of performing an endotracheal intubation of babies previously undergoing nCPAP, only for surfactant administration through endotracheal tube, and early extubation and put on nCPAP again. It has shown to reduce mechanical ventilation needs, reduce the duration of respiratory support and need for surfactant[23], as well as reducing the incidence of air leak syndromes[24].

However, not all infants are candidats to enter an INSURE procedure. INSURE failure has been continuously documented, with wide ranges of 9-50%, according to different populations included and criteria used to define failure. Risk factors that could predict such failure in determinate groups of patients are still being analysed[25].

INSURE method consists of the following steps. Infants being ventilated with nCPAP receive intravenous premedication. The drug used and dose vary among clinical trials reviewed. Secondly, intubation is performed orally, and tube position is evaluated by chest auscultation. Surfactant, usually Curosurf at 200 mg/kg, is then administered as a bolus by tracheal instillation[26]. In some cases, depending on premedication used, most commonly when using morphine, naloxone is needed, at 0.01 mg/kg, to revert opioid induced respiratory depression. Usually, infants are mechanically ventilated between 10-15 minutes[27]. Early extubation is performed once the infant's respiratory rate, heart rate, and SpO_2 are satisfactory enough.

Whilst performing intubation, many studies have recommended the use of premedication, although there is no consensus about what premedication to use, and that's the aim of our study. Short duration of action is necessary in order to perform extubation within minutes[24;28-31].

Complications of RDS

Sudden worsening of the infant's clinical condition might indicate some of the following complications. PDA usually worsens RDS, and it appears when pulmonary vascular pressures start to decrease. Treatment of PDA must be considered, specially if they are VLBW infants or PDA is sympthomatic. When a baby suffering from RDS deteriorates, air leak must be suspected, usually presenting hypotension, apnea, bradycardia and acidosis. In this case, we will look for radiological findings indicating pneumotorax, pneumomediastinum, pneumopericardium or emphysema.

Infants having RDS are also at more risk of infection, due to their condition, their low gestational age and immanture immunological system, and the facility microorganisms have to access their bloodstream through instrumentation used during treatment. Intracranial hemorrhage is also more frequent in this group of patients.

Other complications of prematurity are also frequently present, such as ROP and neurodevelopmental impairment. Most important long-term complication is BPD, which will be commented below.

3.4. Long-term complications of prematurity[10]

Most common long-term complications include respiratory disorders, such as respiratory infections, taking into account that respiratory syncytial virus is the most common, asthma and BPD. Premature infants are also at higher risk of sudden infant death syndrome.

Usually premature-born kids have growth impairment, compared with those born full-term. ELBW infants commonly appear to be shorter, lighter, with lower body mass and lower head perimeter. They also manifest more health problems during adulthood, including insulin resistance and high blood pressure.

Neurodevelopmental impairment is common, specially as the gestational age decreases. Premature infants in the future can develop impaired cognitive skills, motor defficits, including cerebral palsy, vision and hearing losses, behavioral and psychological problems.

3.4.1. Bronchopulmonary dysplasia (BPD)[10;12]

Also known as chronic lung disease of prematurity, BPD is defined as:

- Infants born below 32 GW: need for supplemental oxygen during the first 28 days, at 36 GW, corrected age.
- Infants born later than 32 GW: requirement of oxygen for the first 28 days. Severity is classified depending on the oxygen needs that the infant requires at 56 days of living.

Level of severity can be classified depending on:

	< 32 GW	> 32 GW
Moment of evaluation	36 GW	Between 28-56 days of living
Mild BPD	Ambient air	Ambient air
Moderate BPD	FiO_2 <30%	FiO_2 <30%
Severe BPD	FiO_2 >30%, nCPAP or mechanical ventilation	FiO_2 >30%, nCPAP or mechanical ventilation

Table 3: Diagnose and Severity of BPD. Adapted from AEPED: Neonathology protocols[10]

Epidemiology

Taking into account that criteria to define BPD is controversial, incidence may vary a lot among different neonatal units. Generally, incidence is higher in infants with lower gestational age and lower birth weight, being around 40% in newborns between 25-27 GW. Female and African-American newborns are at lower risk to end up deleloping BPD.

Pathogenesis

Firstly, some factors have been shown to be associated to BPD development:

1. Immature lung, as before alveolar septation is produced, premature lung is more susceptible to damage.
2. Inadequate antioxidant enzyme activity might predispose the lung to oxygen toxicity and damage.
3. Excessive early intravenous fluid adminitration might contribute to pulmonary edema.
4. Persistent shunting and late PDA closure are related to BPD development.
5. Intrauterine or perinatal infection releases cytokines and contributes to BPD's ethiology.

Acute phase of BPD can be explained in the following diagram:

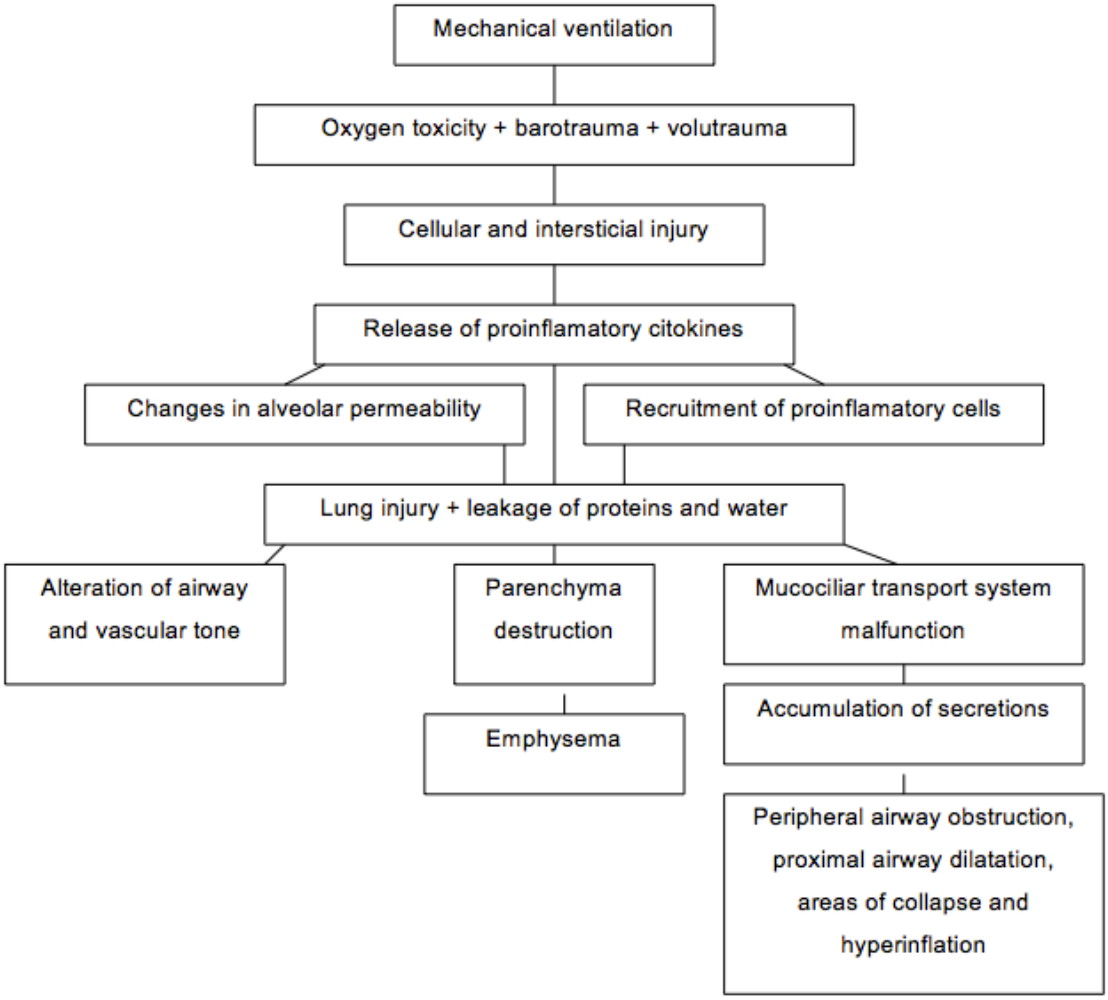

Figure 2: Pathogenesis of BPD

Excessive release of growth factors and cytokines produces an insufficient repair, that leads to fibrosis. Disruption of intersticial fluid clearance causes pulmonary fluid retention. Overall, muscularization and hyperreactivity are increased, lung compliance decreases, airway resistance is bigger, gas exchange is impaired, there's a mismatch in ventilation-perfusion and air trapping appears.

Clinical presentation

Infants usually manifest tachypnea, respiratory retraction and respiratory rales. On arterial blood gases usually hypoxemia and hypercapnia appear, with respiratory acidosis, usually compensated.

Radiological findings of BPD are unspecific, and include diffuse opacification when BPD is mild, and hyperinsuflation, with more noticeable densities when the stage is more severe. BPD can be classified into four stages depending on the severity and radiological findings.

Prevention

Some measures can be adopted in order to avoid BPD. Preventing a premature birth or retarding to administer esteroids will help accelerate lung maturity, reduce incidence, severity and mortality caused by BPD. Treatment of RDS syndrome with surfactant therapy doesn't reduce BPD incidence, but profilactic treatment after birth does. Infants suffering from RDS that require mechanical ventilation are at risk to develop future BPD. To avoid this situation, mechanical ventilation should be optimized. Volume-guarantee and high frecuency oscillatory ventilation are two modalities that have shown to be safe and useful in this case.

Other measures have shown to be useful to prevent BPD, such as early control of infections, avoiding aggresive measures to revive the newborn, early use of nCPAP, avoiding PDA, and conducting fluid and sodium restriction to avoid pulmonary edema that might complicate underlying disorders.

Treatment

During the infant's stay at the NICU, our goal must be to minimize lung injury, maximize nutrition and diminish oxygen needs. Arterial blood gases must be checked to evaluate proper gas exchange, and should be compared to results from capillary blood gas, useful to monitor pH and pCO_2. Pulso oxymetry is used constantly.

Mechanical ventilation

In acute phase, airway pressures and tidal volume must be minimized, insuring an appropiate gas exchange. The aim is to avoid hyperventilation, keeping saturation levels between 90-95% and PaO_2 around 60-80 mmHg. During

chronical phase settings must be kept at a level that maintains $PaCO_2$ below 65 mmHg, waiting for a steady weight gain that allows extubation.

Oxygenation

PaO_2 must be maintained above 55 mmHg. To achieve this, saturation levels must be kept at 85-93% in infants below 32 GW, and 87-97% for older babies.

Fluid and nutrition management

Infants suffering form BPD need to have fluid intake limited, as low as 130 mL/kg/day to maintain a proper urine output, and giving enough high-density caloric nutrients to promote growing. Fluid intake must be checked frequently, and as respiratory state stabilizes, less limitation is needed.

Metabolic rate appears to be increase, although intake is low. To maximize caloric intake we increase lipid administration as it's more efficient. Also, energy expenditure must be reduced as much as possible. Normally, prolonged parenteral nutrition is needed, and when enteral nutrition is possible, it should be started through orogastric or nasogastric tube. Supplementation might be helpful to promote repair, specially with vitamin A, that also minimizes fibrosis.

Medication used

In some cases, diuretics might be used to treat fluid retention. Although diuretics have not been shown to diminish duration of ventilator dependence, hospital stay and bettering long-term outcome, they can atenuate symptoms of BPD. Furosemide and chlorotiazide are usually used for this purpose, although chlorotiazide is preferred to avoid furosemide toxicities. Combination of both may reduce furosemide toxicity as the dose is lower. Diuretic chronic treatment may cause hyponatremia, hypokalemia and hypochloremmia, in this case, diuretic dose must be reduced or we must iniciate supplementation with NaCl and KCl.

Infants suffering from BPD can respond to bronchodilatador therapy. Their airway tone might be increased or bronchospasm may appear, resulting in

obstructive episodes that can be treated using nebulized β-adrenergic agonists and/or muscarinic agents.

Postnatal corticosteroids are only reserved to infants with progressive respiratory failure refractory to other therapies, because of the pottencial harm steroids can produce and unknown long-term benefits.

Pain management is also important in this group of patients when they show signs of discomfort. Use of oral sucrose, morphine sulfate or fentanyl, short-acting benzodiazepines or chloral hydrate might be useful for this purpose, but we must take into account that it can interfere with ventilation and oxygenation technique.

Complications

BPD can be associated with many of the following complications:

- Upper airway obstruction: it is common to produce trauma to the nasal septum, larynx, trachea or bronchi due to intubation and suctioning. Postextubation edema may also cause stridor. Abnormalities may manifest when the infant catches an upper respiratory tract infection.
- Pulmonary hypertension: echocardiogram must be obtained from babies with BPD at 36-37 GW who still require assisted ventilation or FiO_2 above 30% or have a $PaCO_2$ higher than 60 mmHg.
- Systemic hypertension: sometimes accompanied by left ventricular hypertrophy.
- Left-to-right shunting: due to collateral circulation. This complication might be facilitated by chest tube placement, thoracic surgery and pleural inflammation.
- Infection: chronic illness and malnourishment can predispose to suffer infection. In severe clinical stages, we might identify *Ureaplasma* sp. and *Mycoplasma hominis*, that must be treated.
- Central nervous system dysfunction.
- Hearing loss: due to the use of ototoxic drugs
- ROP: infants with BPD are at higher risk

- Nephrocalcinosis: linked to the use of furosemide
- Osteopenia: due to inadequate calcium and phosphorum retention, prolonged immobilization and calcium loss due to diuretics. Vitamin D, calcium and phosphorus must be supplemented.
- Gastroesophageal reflux: treatment of reflux is needed when it descompensates pulmonary condition or feeding process.
- Inguinal hernia: surgery must be delayed until respiratory stage improves, when the hernia is reductible.
- Early growth failure: due to inappropiate intake and excessive energy expenditure.

Outcome

Mortality in this group is around 10-20% during the first year of age, usually caused by infection. Risk for long-term morbidities is also increased. Underlying affected pulmonary function may persist, there's more risk to suffer reactive airway disease, bronchiolitis and pneumonia.

Children with BPD have higher rates of cognitive, educational and behavioral impairments, but it is not a clearly independent predictor of afverse neurologic outcome. Growth failure is usually affected by the severity and duration of BPD.

3.4.2. Neurodevelopmental outcomes[10;12]

Among VLBW infants that previously developed neonatal complications such as BPD, brain injury and severe ROP, a high percentatge of them has poor neurosensory outcomes posteriorly, in form of cerebral palsy, cognitive delay, severe hearing loss or bilateral blindness, abnormal motor development, functional and social developmental problems.

Neurodevelopmental delay is defined as a delay in more that two standard deviations during the firsts 5 years of living, in one or more of the following areas: gross and fine motricity, language and cognition, social expression and daily life activities.

- Neuromotor problems: cerebral palsy has an incidence of 7-12% among VLBW infants and 11-15% in ELBW infants, and the most common form is spastic diplegia. Also, they have more risk to develop motor coordination problems and motor planning. Early diagnose and referral to a specialist is key in the treatment of this condition, that can range from orthotic treatment, botulinum-A toxin and baclofen.
- Cognitive delay: progress is usually evaluated using intelligence quotient (IQ) scales. Usually VLBW infants obtain lower scores than full-term infants, although they usually stay within the normal range. A high percentage of them will require some type of special education during the future. Learning abilities usually affected are related to visuospatial and visuomotor abilities, written output and verbal functioning. Social and communication development may also be affected, prematurity has been shown to be a risk factor for autism.
- Emotional and behavioral health: sleep problems are more common in this group of patients. Also, risk to develop behavioral problems is increased, usually related to hyperactivity and attention defficit. Commonly they become less socially competent than full-term children.

To detect neurodevelopmental delay screening tests are used to detect children with developmental abnormalities. Those scales are not useful to stablish the severity of the delay nor the cause. They are useful to alert the pediatrician to refer children that could benefit from detecting and giving treatment to a possible neurodevelopmental delay. In our environment, most used scale is Haizea-Llevant and Denver scale (annex 3).

3.5. Justification

INSURE[32] method have been proven to reduce the need for mechanical ventilation, improve RDS outcome, reduce the duration of respiratory support and need for surfactant and reduce the incidence of air leak syndromes[23;24]. Many studies have been carried on to prove INSURE's effectivity, but the range can vary a lot depending on the population included and criteria defining failure. Risk factors are still being analysed to predict INSURE failure[25].

Many studies have recommended the use of premedication[33] when a patient is about to be treated with INSURE therapy. It has been proven that intubation conditions are more optimal with the use of premedication, needing less attempts and shorter time[29]. That's why we chose not to have a control group for this study, as it would not be ethical to have a group of patients that would not benefit with these advantatges. Eitherway, optimal premedication strategy has still not been found.

The aim of this study is to compare three diferent therapeutical aproaches based on previous studies that show that remifentanil, fentanyl and propofol could be optimal as a premedication for INSURE[24]. No comparative studies have been made about these premedications together, and not with such a big sample, that's why our clinical trial will have such an important transcendence in making clinical decisions.

Another inconvenience we're facing is the lack of studies regarding premedication dosages. We decided to chose more studied dose regimens, that have proven to be effective and incidence of adverse effects is minimal. Considering the dose chosen will not always be effective for sedation purposes, a second dose will always be prepared in case it is needed. More studies are needed to stablish the best dose regimen for these premedications, and a design to optimize the sedative dose in this age group has already been made[34].

Important clinical implications will take place with this clinical trial, considering that if INSURE's time is optimized, meaning less time undergoing mechanical ventilation, and less adverse effects occur, the infant will be less exposed to develop complications such as lung injury, BPD and future adverse neurodevelopmental outcomes.

4. QUESTION, OBJECTIVES AND HYPOTHESIS

4.1. Question

Which is the most suitable drug (between Propofol, Fentanyl and Remifentanil) to use in the intubation of a patient undergoing the INSURE procedure for treating RDS?

4.2. Objectives

4.2.1. Primary objective

Compare the effectiveness, in terms of shortening the duration of mechanical ventilation, of Propofol, Remifentanil and Fentanyl in preterm newborns undergoing the INSURE procedure to treat RDS.

4.2.2. Secondary objectives

- To evaluate the safety of Propofol, Remifentanil and Fentanyl and the appearance of adverse effects during INSURE procedure.
- To evaluate the incidence of long-term effects (BPD, neurological sequelae) after treating patients suffering from RDS with premedication followed by INSURE.

4.3. Hypothesis

Remifentanil is the most suitable drug in the intubation of newborns undergoing the INSURE procedure, because of its short duration of action and less incidence of short-term and long-term adverse effects. It has a more favourable risk-benefit ratio than fentanyl and propofol.

5. METHODS

5.1. Study design

This is a multicenter, non-placebo controlled, double blind, randomized controlled clinical trial to compare the effectiveness and safety of Propofol, Remifentanil and Fentanyl, in preterm newborns that require surfactant therapy to treat RDS, using the INSURE method. The patients will be followed up from the inclusion to the study, during their stay in the hospital, and during 2 years after to evaluate the incidence of long-term adverse effects. Subjects will be randomized in a 1:1:1 ratio in three groups, whether they receive treatment with Propofol, Remifentanil or Fentanyl.

The study will be conducted in the NICUs of 22 tertiary centers from Catalonia and Madrid that dispose of NICU. Based on data obtained form SEN1500[9] 2013, 1,462 infants suffered from RDS in Spain in 2013 in the centers studied. If we pick hospitals from Catalonia and Madrid, we obtain 528 patients per year, so our expectation is that enough patients will be included in the study in two years.

Hospital Universitari Josep Trueta will be the reference center. Each of the hospitals participating in the study will have a principal investigator assigned. The centers that will be asked to participate in the study are the following:

Catalonia	Madrid
Hospital Germans Trias I Pujol	Hospital La Paz
Hospital Clínic de Barcelona	Hospital Clínico San Carlos
Hospital Vall d'Hebron	Hospital Gregorio Marañón
Scias Hospital de Barcelona	Hospital de Getafe
Hospital Sant Joan de Déu	Hospital Severo Ochoa
Corporació Parc Taulí	Hospital 12 de Octubre
Institut Dexeus	Hospital de Madrid-Torrelodones
Hospital de la Santa Creu I Sant Pau	Hospital Puerta de Hierro
Hospital General de Catalunya	Hospital Fuenlabrada
Clínica Corachán	

Hospital Universitari Dr. Josep Trueta Hospital Universitari Arnau de Vilanova de Lleida Hospital Juan XXIII	

Table 4: Centers that will be requested to join our clinical trial

5.2. Termination standard

We consider that our trial should be stopped if significant differences between the three groups in terms of intubation time and apparition of adverse effects are found when performing a mid-term analysis.

5.3. Study population

5.3.1. Inclusion criteria

- Newborns under 32+0 gestational age and/or below 1.500 grams of birth weight
- Requirement of nCPAP oxigen therapy with FiO_2 above or equal 40% to maintain SpO_2 90-95% with PEEP 6 cm H_2O.

5.3.2. Exclusion criteria

- Severe malformations
- Immediate need for intubation at delivery room
- Cyanotical congenital heart disease
- Pneumonia
- Pneumotorax

5.4. Sample

5.4.1. Sample size

To calculate the sample size for our principal variable (time for extubation), power calculator GRANMO was used. We accepted an alpha risk of 5% and a beta risk of 20%, in a two-sided test, anticipating a drop out rate of 0%, as the principal variable does not require any follow-up of the patients. A study group

of 1,008 patients is needed, 336 subjects will be included in each group (randomization 1:1:1) to recognize as statistically significant a minimum difference of 50% between each pair of groups.

For the secondary variables, with this sample size we estimated the statistical power for each one:

Secondary dependent variable	Statistical Power for obtained sample
Number of intubation attempts	100%
Haemodynamic adverse effects	100%
Chest wall rigidity incidence	100%
Level of sedation (N-PASS)	94%
Need for second premedication dose	100%
INSURE failure	100%
Incidence of long-term complications	74%

Table 5: Statistical Power calculated for each secondary variable

5.4.2. Estimated time of recruitment

We calculated 1,008 patients that need to be recruited for the purpose of this study. By analysing data collected from SEN1500 in the year 2013[9], around 1,462 infants suffered from RDS in all the hospitals included in SEN1500. We chose to include the same hospitals, only from Catalonia and Madrid to this study. In 2013, 528 total patients were obtained in such centers, so we estimate to obtain enough sample in two years.

5.4.3. Enrollment

The parents of newborns that match our inclusion criteria will be asked to participate in the study. They will be informed about the aim of the study, RDS, the INSURE procedure, premedication options and the risks associated. Once they are informed by giving an information sheet (annex 4 and 5), and have signed the informed consent (annex 6 and 7) newborns will be enrolled in the study.

As it's not predictable when a newborn patient will suffer from RDS since it is an acute pathology, there's no sampling frame. Patients will be enrolled in the clinical trial by consecutive sampling.

5.4.4. Randomization procedures

Randomization process will be performed by the main investigator by simple randomization computer-generated, distributing the patients in three groups by a 1:1:1 ratio, whether they receive Propofol, Remifentanil or Fentanyl.

Hospital pharmacy will be provided with a list that includes number of the patient and what group they belong to. Each time a dose is needed to proceed with INSURE, the neonathologist in charge of the procedure will call the hospital pharmacy to ask for premedication, informing of the weight of the patient in order to calculate the dose. Then pharmacy workers will prepare 2 dosages of the premedication previously randomized as specified on the list, the second one just in case a second dose is needed because a good level of sedation was not achieved with the first dosage. Pharmacy will prepare precharged dosages with opaque tubes, because propofol has a different coloration than fentanyl and remifentanil.

5.4.5. Blinding

Double blind clinical trial. Neonathologists and nurses attending the patient will not know which one of the premedications available is being used, as well of the parents or legal tutors from the infant. Only the hospital pharmacy will be aware of the contents of each preparation.

5.5. Study treatment groups

The patients who match our criteria and are enrolled in the study will receive one of the following dose regimens before undergoing INSURE procedure:

- GROUP A: propofol 1 mg/kg IV
- GROUP B: remifentanil 2 µg/kg IV
- GROUP C: fentanyl 2 µg/kg IV

5.5.1. Study intervention

Each center participating in our study will previously receive a formation course about INSURE procedure to minimize confusion variables related to differences in applying the method (for example, orally administrating the endotracheal tube versus nasally).

Previous to the intubation all patients will receive a dose of Atropin (0.1 mg/kg) and a dose of the analgesic drug provided by pharmacy. Then, N-PASS will be applied to se if a GSS is achieved. If it is, we can continue with the procedure, if not, N-PASS will be applied 3 and 5 minutes after, and if GSS is not achieved, a second premedication dose might be needed.

Infants will be disconnected from nCPAP temporarily. Then, intubation is carried out orally, evaluating tube position through auscultation. Surfactant is administered through endotracheal tube. Usually, infants are mechanically ventilated between 10-15 minutes[27]. Early extubation is performed once the infant's respiratory rate, heart rate, and SpO_2 are satisfactory enough.

If during the process the patient suffers from chest wall rigidity, a dose of rocuronium will be administered. Each patient will receive a dose of caffeine (10 mg) previous to the extubation. All patients will be treated with nCPAP after the extubation process. Naloxone (0.1 mg/kg) should be avaliable in case the effects of sedation need to be reversed.

Therapeutic strategy is resumed in the following scheme:

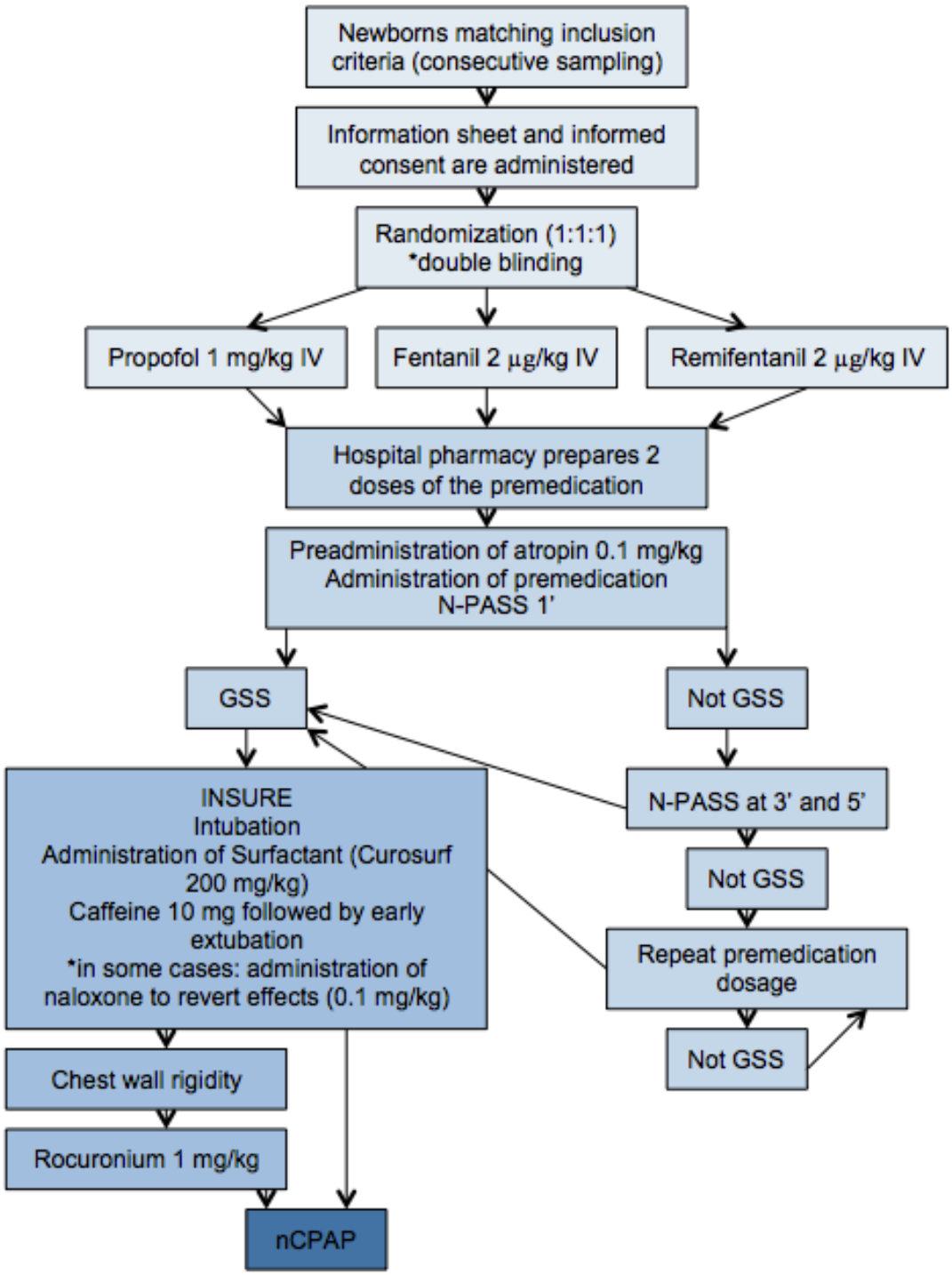

Figure 3: Therapeutic strategy

5.5.2. Readministration of premedication

Second dose of premedication should be administered when the baby doesn't achieve a GSS (punctuation between -7 and -3 in N-PASS scale)[35] in 1, 3, nor 5 minutes after giving a first dosage. More dosages could be administered if the infant requires it, and it is necessary to register number of premedication doses administered.

5.5.3. INSURE failure

We determine INSURE failure when the patient requires reintubation in the following week after being treated[24].

5.5.4. Resurfactation process

Maximum two more additional dosages can be administered, separated by intervals of 12 hours, when the baby is still requiring intubation and a FiO_2 higher than 30%[10;12]. Curosurf (poractant alfa) is readministered in doses of 1.25 mL/kg, up to two additional doses administered 12 hours appart.

5.5.5. Follow-up

Infants will be followed-up during their stay in the hospital to analyse the possible development of BPD, defined as a requirement of oxygen or respiratory suport at 28 days of life. Patients will be followed-up during the first 2 years of life in pediatrician routinary visits for premature infants, to analyse their neurodevelopmental outcome. Data about long-term adverse effects will be collected in our database.

5.6. Study variables

5.6.1. Independent variable

Independent variable of this study is each of the three groups depending on the medication given (fentanyl, propofol or remifentanil). It will be defined as a cathegorical nominal variable.

5.6.2. Dependent variable

Primary dependent variable

Primary outcome will be measured taking into account the following variable:

- Time for extubation: we will measure minutes since start of mechanical ventilation to start of spontaneous breathing with nCPAP. It will be defined as a quantitative continuous variable, expressed in minutes.

Secondary dependent variables

- Number of intubation attempts: each intubation will be supervised, successful or failing intubations will be collected and classified in one attempt, two, ore more than two. It is defined as a quantitative discrete variable.
- Incidence of acute adverse effects:
    - Haemodynamic adverse effects: every infant will be continuously monitorized during the procedure, responsible data collector will be in charge of taking notes. Results will be expressed as quantitative discrete variables.
        - SpO_2: oxygen saturation will be collected one minute previous to premedication administration, and monitorized during the following 30 minutes (or longer if the procedure requires more time). We will consider desaturation as a decrease below 85%.
        - Arterial hypotension: changes in blood pressure and significant arterial hypertension will be registered, considering it as a mean blood pressure below 25 mmHg.
        - Heart rate: through continuous monitorization we will register changes in heart rate, and also, aparition of bradycardia (below 100 beats per minute).
    - Chest wall rigidity: acute onset of stiffness despite applying adequate positive airway pressure ventilation. It has shown to be associated with the use of certain premedications[20], and it will be expressed as a qualitative dichotomic nominal variable (yes / no).

- Level of sedation achieved: we will rely on N-PASS scale (neonatal pain, agitation and sedation scale)[35]. It can be used to evaluate pain or sedation, for the aim of the study we have to evaluate the level of sedation achieved in newborns undergoing INSURE procedure. It consists of 5 criteria, and each item is graded from 0/1/2 for pain or agitation, or from -2/-1/0 for sedation (2 corresponding to highest infant discomfort and -2 to highest sedation). It can range from -10 to 10. A Good Sedation State (GSS) is defined as a score from -7 to -3. The following lists explains the five criteria that this scale evaluates:
    - Crying or irritability: as it is a sign of distress.
    - Behavioural state: body movements, such as arching or kicking, and the ability to rest and sleep. When an infant is well-sedated, there's a lack of body movements and a decrease to arousal to stimuli.
    - Facial expression: when an infant is well sedated their facial expression decreases in response to stimuli.
    - Extremity tone: sedated infants lack of grasp reflex and their muscle tone is decreased.
    - Vital signs changes: well sedated newborns manifests low variability of vital signs with stimulation, with depressed ventilatory effort.

  These five criteria will be evaluated a minute after premedication administration. If GSS is achieved, we can iniciate INSURE procedure. If not, N-PASS will be applied at 3 and 5 minutes, and if GSS is not achieved, a second dose of premedication will be administered until we can assure a GSS. Mean score obtained at 1 minute will be registered for every group of treatment and expressed as a quantitative discrete variable.
- Need for second dose of premedication: it will be expressed as a qualitative dichotomic nominal variable.
- INSURE failure: qualitative dichotomic nominal variable defined as a need to reintubate the patient in the following week after undergoing INSURE therapy.

- Incidence of long-term complications, expressed as qualitative dichotomic nominal variables:
    - BPD: incidence of BPD is defined as the need for supplemental oxygen or positive pressure support during the first 28 days.
    - Neurodevelopmental outcome: neurodevelopmental delay is a long-term neurological complication due to the time of intubation and alterations in cerebral blood flow. Screening will be carried out in routinary pediatric visits, by using validated scales for this purpose (Haizea-Llevant scale) (annex 3).

Covariables

- Gender: male / female newborn
- Gestational age at birth: defined as the first day of the mother's last normal menstrual period, confirmed by obstetric ultrasound in Gynecology and Obstetrics Department of the corresponding center.
- Birth weight: digital pediatric scale will be used for this purpose. Nursing staff will perform this measurement with the naked baby in supine position. It will be expressed in grams.
- Age at INSURE application: determined by the number of days of living when the method is applied.
- Infants of diabetic mothers: expressed as a qualitative dichotomic nominal variable (yes or no).

5.7. Measure instruments

Measure instruments required for this study include:

- Chronometer, to measure duration from start of the procedure to final extubation
- Neonatal pulse oxymetry, to measure oxygen saturation and heart rate during the procedure
- Blood pressure will be measured directly through arterial catheter, taking into account that infants in the NICU often have this kind of access. In the case they don't, blood pressure will be measured through non-invasive measures, like cuffs.

- N-PASS scale[19] will be applied to measure neonatal pain and sedation when giving premedication to perform INSURE method.
- Arterial blood gas measurements (PaO_2 and $PaCO_2$), usually through arterial catheter, to adjust ventilatory support.

5.8. Data collection

Data will be collected in medical records, and during the procedure, a responsible data collector will be in charge of taking notes regarding haemodynamic changes, adverse effects, and response.

Homogenity in data collection must be ensured between centers involved in our study. Data will be classified by centers, to analyse data for each center separately, in order to prevent bias and it will be introduced to our database.

6. STATISTICAL ANALYSIS

Univariate analysis

The results are expressed as percentages for cathegorical variables, and as means and standard deviations (mean+/-SD) for quantitative variables.

Bivariate analysis

Independent variable (treatment groups) is cathegorical with three components, and primary dependent variable, the time, is a continuous variable. Comparison between these two principal variables will be carried out in a bivariate analysis by using Kruskal-Wallis test, as well as comparation with secondary dependent discrete variables. Secondary dependent nominal variables will be analysed using Chi-square (X^2) test.

Multivariate analysis

To adjust confounding factors a multivariant analysis will be carried out. A general linear model (GLM) will be performed in order to associate the principal variables adjusting to the covariables that could represent a cofounding factor.

Data analysis will be carried out using Statistical Package for the Social Sciences (SPSS Windows), and management and recording of data will be performed using Microsoft Excel.

7. ETHICAL ASPECTS

Prior to starting the study, this protocol should be evaluated and approved by the Clinical Research Ethics Comittee (CEIC) of each center involved in the study. The clinical trial must be approved by AEMPS, conducting the EudraCT application. All the investigators will have to declare no conflict of interest.

An information sheet will be distributed to the parents, with comprehensive language to ensure a proper informed decision. Written informed consent must be obtained from parents in order to include their child to the study (annex 4-7).

This clinical trial is designed in accordance with World Medical Association *Declaration of Helsinki for Ethical Principles for Medial Research involving human subjects*(last revision October 2013).

All data collected regarding each patient will be analysed and kept confidential, guaranteeing the anonymity of the patient enrolled in the clinical trial according to *Ley Orgánica 15/1999, 13 de Diciembre, Protección de Datos de Carácter Personal*. It will also be conducted under the following normative framework:

- *RD 1/2015, 24 de Julio, ley de garantías y uso racional de los medicamentos y productos sanitarios*
- *Ley 14/2007, 3 de Julio, de investigación biomédica*
- *RD 223/2004, 6 de Febrero, ensayos clínicos con medicamentos*
- *RD 1591/2009, 16 de Octubre, y 1616/2009, 26 de Octubre, investigacion con productos sanitarios*.

Placebo group was not included in this study after conducting an ethical consideration, as the benefits of using premedication have been demonstrated whilst conducting INSURE procedure, and having a control group was not considered to be ethical in this case.

8. ADVERSE EFFECTS

Presence of adverse effects before, during or after the INSURE procedure will be recorded and reported by the investigator. If an adverse effect occurs, our first concern will be the patient's safety and providing optimal treatment if it's required. Unexpected adverse effects are considered to have not been described in previous experiences. Information will be recorded in the patient's clinical record and included in a report in the end of the trial.

Our study will have important clinical implications because of its high potency, as it will be carried out taking a big sample (1,008) and incidence of adverse effects would be able to be recorded in this large group. We consider that there's a lack of studies that include such a large population from premature

newborns with our inclusion criteria, that's why our study will be innovative comparing to previous ones carried out.

Fentanyl

Synthetic opioid analgesic that has rapid effect and short duration of action. It acts as a agonist of μ-opioid receptors, and its use is often associated with benzodiazepines. We decided to use a dose of 2 μg/kg intravenous, based on previous studies, that can be repeated if proper sedation is not achieved.

- Respiratory depression can occur at anesthetic doses (more than 5 μg/kg)[24].
- Chest wall rigidity, usually asociated with laryngospasm, in 4% of neonates receiving 2.2-6.5 μg/kg[36;37]. Naloxone can be used to reverse this effect
- When it is used at continuous infusions (not the aim of the study), infants can develop urinary retention, tolerance and withdrawal symptoms.

Remifentanil

Synthetic opioid analgesic that acts as a agonist of μ-opioid receptors. Remifentanil has a similar pharmacodynamic profile than fentanil, with higher power but same efficacy. We know that remifentanil clearance rate is fast, but complete pharmacokinetics in newborns are not clearly known[38]. Some studies have declared that less intubations are needed with remifentanil compared to fentanyl[38].

It has the same adverse effects than fentanil, with faster onset and shorter duration of action. It also has a faster onset and time to reach equilibrium between the plasma level and brain concentrations[38]. These characteristics make remifentanil it suitable for INSURE procedure[39;40]. That's why our hypothesis claims that remifentanil might be the most optimal premedication to use in INSURE procedure. Remifentanil has also considered to be safe to use as an indication for the INSURE[38].

We might observate a mild decrease in mean blood pressure during the procedure, and it is usually spontaneously normalized, and very rarely, patients might develop significant arterial hypotension (mean blood pressure below 25 mmHg), usually treated with bolus of isotonic saline[41]. Bradycardia might also appear after remifentanil infusion, but generally it has no clinical repercussions[38].

Like fentanyl, remifentanil can cause respiratory depression. Some studies have described the wakening and struggling of the newborns during the procedure[41], that was solved with a second dosage of premedication. We expect to avoid this situation by making sure we achieve GSS before undergoing INSURE procedure.

Remifentanil might also cause chest wall rigidity that difficults oxygenation, that needs treatment. Some clinical trials that we reviewed used succinylcholine for this purpose[42], in our study, we decided to treat chest wall rigidity with rocuronium.

Propofol

Short-acting anesthetic drug, that results in a decreased consciousness. Main problem when using propofol for the purpose of this study is the development of significant arterial hypotension[24;43;44]. Hypotension is treated with intravascular fluids, and in more severe cases, dobutamine can be used. One of the reviewed clinical trials[26] had to be stopped ahead of time because of clinically significant problems with arterial hypotension.

Other adverse effects have been described associated with the use of propofol, such as mild respiratory depression[24;45], cutaneous rash, difficulty to ventilate, bradycardia[43], insufficient sedation and percentage of slow responders[44].

9. LIMITATIONS OF THE STUDY

Taking into account that this study consists of a multicentric clinical trial, some advantatges and disadvantatges must be evaluated. First of all, the wide range of population studied (1,008) in several centers of Catalonia and Madrid, makes the results of this study valuable to apply in newborns of characteristics contemplated in our inclusion criteria, that match the majority of newborns suffering from RDS. Second of all, as we included 22 centers, there's a risk that differences in treatment application exist. We solved this problem by protocol standarization that will be applied at the begining of the trial. Lack of coordination might also be a problem for multicentric trials, but meetings are stablished to take place every 6 months to promote coordination between centers.

Our study will have important clinical implications, as no comparative studies have been made to evaluate the efficacy of premedications used in INSURE method. We consider the price is quite expensive like majority of multicentric clinical trials are, but we must take into account that results of this study will compensate sanitary costs related to prematurity complications.

Only three premedications have been included for the purpose of this study. More drugs could have been included, but we consider it will make the study even more expensive and difficult to carry on, and the three premedications chosen are the ones who have previously shown more probability to be more optimal for INSURE method[24].

Another inconvenience is that proper dosages for these premedications are unknown because of the lack of studies that analyse optimal dosage in this age group. The doses in our study have been chosen taking into account previous studies' dosages that have shown to be effective with minimal adverse effects. We chose to have a second dose of premedication avaliable in case one is not enough. A design to optimize the sedative dose in this age group has been already made but not carried on[34].

Another limitation is the lack of control group. We consider it's not ethical to include a control group that undergoes through INSURE method without previously receiving premedication, as it has been shown that the administration of premedication is benefitial for intubation procedure in this age group[24;28-30].

Confounding variables that might difficult stablishment of the relationship between main variables have been controlled by performing a multivariate analysis, randomization procedure, stablished inclusion criteria and double blinding methods.

We predict to have some losses due to acute RDS mortality during the stay in the NICU. Losses during the follow-up are expected to be minimal, as pediatrician visits for premature newborns are routinary and no additional testing is required.

10. WORK PLAN

Personnel involved in the research team will be composed by the investigator coordinator, a neonathologist and a nurse in each of the centers involved, hospital pharmacy and a statistical specialist. Responsible data manager will be the neonathologist assignated in each center.

This trial is planned to be developed in 4 phases:

Stage 1: Coordination phase (2 months)

In this phase, design of the protocol will be made. Principal investigator will contact each of the centers to ask for their enrollment in the study. Staff (neonathologist and nurse) will be asigned for each center, among with hospital pharmacy. To guarantee the professionality of the members participating in the study, we will ask for certain qualifications, skills and experience regarding the techniques we will be applying (neonatal intubation).

Organizational meetings will start to take place in this phase, each 6 months. First one has the aim to discuss the protocol and make sure there is an agreement between all centers. Timeline of the project and main tasks will also be planned, and personnel training will take place. Ethical approval from CEIC of each center will be required.

Stage 2: Field research (48 months)

We require 1,008 patients to be part of the study, and according to SEM1500 2013's data, with the centers we have selected we will obtain enough sample in 23 months. To enroll these patients in the study, once they match our inclusion criteria, parents or legal tutors will be given the information sheet (annex 4 and 5) and they will have to sign the informed consent (annex 6 and 7). Patients included will be randomized to belong to a treatment group.

INSURE procedure will be started giving the treatment from the group that the patient belongs. A responsible note taker will be asigned during the procedure to collect data regarding the procedure, vital signs and adverse effects. Patients who need multiple doses will be treated accordingly.

They will be followed-up for two years in pediatric rutinary visits to detect long-term adverse effects (mainly, BPD and neurodevelopmental outcomes). This visits are stablished to take place when the baby is 1 month, 2 months, 4 months, 6 months, 9 months, 12 months, 15 months, 18 months and two years. Data will be entered in the database every 2 months.

Stage 3: Data analysis and interpretation of the results (2 months)

Data collected will be analysed by the statistical analyst each 6 months and when stage 2 ends. It will be performed accordingly as specified in statistical analysis paragraph. Interpretation of the results will also take place during this period.

Stage 4: Publication and dissemination of the results (4 months)

Interpretation of the results will be made and an article will be written accordingly. Article will be published and will be presented in AEP congress.

Timeline of the project is avaliable in annex 8.

11. BUDGET

			Quantity	Cost	Total
Personnel costs	Statistical consultant		150 hours	40 €/h	€ 6.000,00
Meeting expenses	Transportation (8 trips)	From Madrid	9 (x 16)	120 €	€ 8.640,00
		From Catalonia	12(x16)	26 €	€ 2.704,00
	Diet and others		22	100 €	€ 2.200,00
Material expenses	INSURE therapy	Curosurf 240 mg	840	480 €	€ 403.351,20
		Expected resurfactations	45	480 €	€ 21.600,00
		Propofol 10 mg/mL	672	10,77 €	€ 7.237,44
		Remifentanil polvo 1mg	672	15,46 €	€ 2.077,82
		Fentanil 0.05 mg/mL	672	2,02 €	€ 271,49
		0.9% saline	1088	0,15	€ 163,20
	Printing	Information sheet	2016	0,04 €	€ 80,64
		Informed consent	2016	0,04 €	€ 80,64
Insurance				25.000 €	€ 25.000,00
Presentation	Article publication			2.500 €	€ 2.500,00
	Presentation to AEP	Inscription	2	667 €	€ 1.334,00
		Travel	2	100 €	€ 200,00
		Accomodation	2	100 €	€ 200,00
TOTAL					€ 483.640,43

Table 6: Budget

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13. ANNEXES

13.1. Annex 1: Percentiles of birth weight by gestational age

GW	p5	p10	p50	p90
24	435	498	674	977
25	480	558	779	1138
26	529	625	899	1362
27	591	702	1035	1635
28	670	798	1196	1977
29	772	925	1394	2361
30	910	1085	1637	2710
31	1088	1278	1918	2986
32	1294	1495	2203	3200
33	1513	1725	2458	3370
34	1735	1950	2667	3502
35	1950	2159	2831	3596
36	2156	2354	2974	3668
37	2357	2541	3117	3755
38	2543	2714	3263	3867
39	2685	2852	3400	3980
40	2761	2929	3495	4060
41	2777	2948	3527	4094
42	2764	2935	3522	4098

Table 7: Percentiles of birth weight by gestational age. Adapted from: A United States national reference for fetal growth[46]

13.2. Annex 2: Surfactant products avaliable

Name	Active ingredient	Source	Dosing	Phospholipid concentration	Protein concentration
Survanta	Beractant	Bovine lung extract	4 mL/kg (100 mg/kg phospholipid) divided into four quarter doses. Can use up to four doses, given no more frequently than every 6 hours	25 mg/mL	<1 mg/mL (SP-B and SP-C; does not contain SP-A)
Infasurf	Calfactant	Calf lung lavage fluid	3 mL/kg (105 mg/kg phospholipid). Can use up to three doses, given 12 hours apart	35 mg/mL	0.7 mg/mL (SP-B and SP-C; does not contain SP-A)
Curosurf	Poractant alfa	Porcine lung extract	Initial dose of 2.5 mL/kg (200 mg/kg phospholipid). Can use up to two subsequent doses of 1.25 mL/kg administered 12 hours apart (maximum volume 5 mL/kg)	76 mg/mL	1 mg/mL (SP-B and SP-C; does not contain SP-A)

Table 8: Surfactant products. Adapted from Cloherty's Manual of Neonatal Care[12]

13.3. Annex 3: Haizea-Llevant developmental screening test

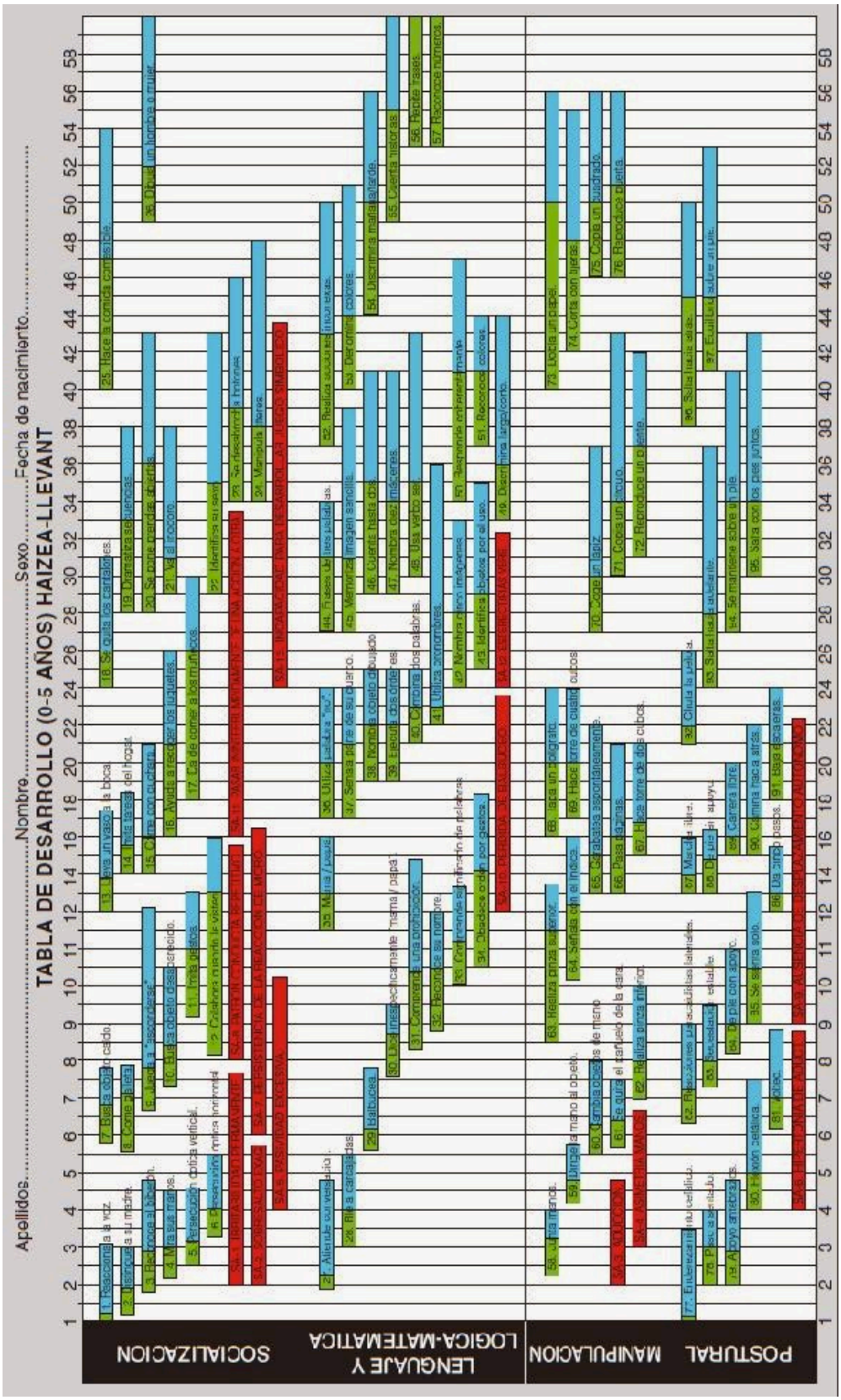

Figure 4: Haizea-Llevant screening test. Extracted from Neurología pediátrica

13.4. Annex 4: Information Sheet for parents or legal tutors (English)

Comparing the effectiveness between fentanyl, remifentanil and propofol in preterm newborns undergoing INSURE procedure: a randomized controlled trial

Hospital Universitari de Girona Doctor Josep Trueta

Please, do read this information carefully:

- Code of the study:
- Coordinator investigator:
- Center:

We inform you that a clinical trial is being conducted, in the aim of finding the best type of sedation when INSURE method is applied to treat an infant suffering from respiratory distress syndrome, in terms of less duration of mechanical ventilation and minimize adverse effects.

As a parent or legal tutor of the infant, we inform you that this study has been approved by the Clinical Research and Ethics Comitee and by the Comitees required in each center that the study is being conducted.

In this study, 22 centers from our country that dispose of a NICU have been included. We expect to include at least 1.008 newborns below 32 gestational weeks or 1.500 grams of weight, that require this treatment.

This study randomizes patients in three groups to receive any of the three medications that our study is including (fentanil, remifentanil or propofol), and we collect data according to the baby's response to the treatment received. Children will also be followed up for two additional years to evaluate long-term effects, in routinary pediatrician visits for preterm newborns. Our aim is to discover if there are significant differences between this drugs to optimize the treatment in this group of patients.

Realization of this study does not require any additional testing or any more visits than usual. Following of the baby will be carried out as routine visits of full-

term babies. Doctor responsible of your infant is the one who has access to his clinical history and his data to evaluate the results. We are not expecting that realization of this study supose any additional risk for your infant's health.

Confidenciality

According to *Ley Orgánica 15/1999, 13 de Diciembre, Protección de Datos de Carácter Personal*, data collected will not include identification data such as name, number of clinical history and any personal information, merely required clinical data will be collected. This data will be related to a code number that will prevent data from your infant to be known, and this relation will only be known by the doctor responsible.

This data will not be accessible by anyone that is not the professional responsible of the baby, and it would not be spread by any professional, preserving anonymity.

In order to reevaluate the results found, sanitary authorities and regulators will have access to data, having the obligation to maintain the patients anonymity.

Any event or question that might arise while the study is being conducted and after this sheet has been read, feel free to contact:

Responsible doctor: _____

Telephone: _____

13.5. Annex 5: Hoja de información para padres y tutores legales (Español)

Comparing the effectiveness between fentanyl, remifentanil and propofol in preterm newborns undergoing INSURE procedure: a randomized controlled trial

Hospital Universitari de Girona Doctor Josep Trueta

Por favor, lea la siguiente información con atención:

- Código del estudio:
- Investigador coordinador:
- Centro:

Le informamos que un estudio clínico se está llevando a cabo con el objetivo de encontrar el mejor tipo de sedación durante el método INSURE, en el tratamiento de neonatos con síndrome de distrés respiratorio, consiguiendo que la ventilación mecánica sea más corta y los efectos secundarios sean mínimos.

Como padre, madre o tutor legal del niño/a, le informamos que este estudio ha sido aprovado por el Comité de Ética E Investigación Clínica, y de los comités requeridos en cada centro participante del estudio.

En este estudio han sido incluidos 22 centros del país que disponen de UCI neonatal. Esperamos incluir al menos 1.008 neonatos inferiores a 32 semanas de gestación o 1.500 gramos de peso al nacer que requieran este tipo de tratamiento.

Este estudio aleatoriza los pacientes en tres grupos para recibir uno de los tres fármacos incluidos en el estudio (fentanilo, remifentanilo o propofol), y se recoje información acerca de la respuesta al tratamiento dado. Los niños/as también serán seguidos durante dos años adicionales para evaluar los efectos a largo terminio, en las visitas pediátricas rutinarias. Nuestro objetivo es descubrir diferencias significativas entre los tres grupos para optimizar el tratamiento en este grupo de pacientes.

La realización de este estudio no requiere ninguna prueba ni visita adicional. El seguimiento de los niños/as será llevado a cabo por las visitas rutinarias del recién nacido inmaduro de su pediatra. El médico responsable de su hijo/a es el único con acceso a la historia clínica y su información para evaluar los resultados. No se espera que la realización de este estudio comporte ningún riesgo para la salud de su hijo/a.

Confidencialidad

Acorde a la *Ley Orgánica 15/1999, 13 de Diciembre, Protección de Datos de Carácter Personal*, la información recogida no incluirá ningún tipo de información que permita identificar al niño/a, como nombre, número de historia clínica, ni ninguna información personal, sólo la información clínica necesaria sera recojida. Esta información estará vinculada a un código, para prevenir que la información de su hijo/a se conozca. Este vínculo sólo lo conocerá su médico responsable.

Esta información no será accesible por nadie que no sea su médico responsable, y no será difundida en ninguna forma, preservando la anonimidad.

Para reevaluar los resultados del estudio, las autoridades sanitarias y reguladoras podrán acceder a la información, teniendo la obligación de mantener la anonimidad de los pacientes.

Cualquier evento o pregunta que surja durante la realización del estudio y después de leer esta hoja de información, puede contactar con:

Médico responsable: _____
Teléfono: _____

13.6. Annex 6: Informed consent for parents or legal tutors (English)

Comparing the effectiveness between fentanyl, remifentanil and propofol in preterm newborns undergoing INSURE procedure: a randomized controlled trial

Hospital Universitari de Girona Doctor Josep Trueta

Please, do read this information carefully:

- Code of the study:
- Coordinator investigator:
- Center:

I, _____
As parent or legal tutor of the infant _____
Confirm that:

I have carefuly read the information sheet given.
I have been able to ask questions about the clinical trial.
My questions have been answered properly.
Enough information about the study has been given.

I got to talk with (name of the investigator/responsible neonathologist): _____

I comprehend that enrollment in this study is voluntary and that I'm free to retire from the study at any time, whithout any repercussion on the medical care of my baby and without having to explain my motives.

Consequently,

I consent that my infant is enrolled in this study.

☐ YES ☐ NO

Signature of the father, mother or legal tutor:

Signature of the investigator:

Date:

Date:

13.7. Annex 7: Consentimiento informado para padres y tutores legales (Español)

Comparing the effectiveness between fentanyl, remifentanil and propofol in preterm newborns undergoing INSURE procedure: a randomized controlled trial

Hospital Universitari de Girona Doctor Josep Trueta

Por favor, lea la siguiente información atentamente:

- Código del estudio:
- Investigador coordinador:
- Centro:

Yo, _____
Como padre, madre, o tutor/a legal de _____
Confirmo que:

He leído atentamente la hoja de información administrada.
He tenido la oportunidad de hacer preguntas acerca del estudio.
Mis preguntas han obtenido respuestas satisfactorias.
He obtenido suficiente información acerca del estudio.

He tenido la oportunidad de hablar con (nombre del investigador/neonatólogo responsable):
_____

Comprendo que la participación en este estudio es voluntaria y soy libre de retirarme en qualquier momento, sin que esto repercuta en el tratamiento de mi hijo/a y sin que se me pida una explicación al respeto.

Consecuentemente,

Consiento que mi hijo/a sea incluido en el estudio.

☐ SI ☐ NO

Firma del padre, madre o tutor legal:

Firma del investigador:

Fecha:

Fecha:

13.8. Annex 8: Work plan

STAGE	Activity	2015	2016				2017		2018		2019		2020		
		DEC	JAN	FEB	MAR-JUN	JUL-DEC	JAN-DEC	JAN-FEB	MAR-DEC	JAN-DEC	JAN-FEB	MAR-APR	MAY	JUN
STAGE 0	Literature review and protocol design													
STAGE 1: COORDINATION PHASE	Enrollment of selected centers and personnel													
	First meeting, discussion of the final protocol													
	Ask approval by CEIC of every center													
	Training personnel													
	Team meetings				EVERY SIX MONTHS									
STAGE 2: FIELD RESEARCH	Recruitment													
	Follow-up													
	Data collection and processing database					EVERY TWO MONTHS								
STAGE 3: DATA ANALYSIS	Data analysis					EVERY SIX MONTHS								
	Interpretation of the results													
STAGE 4: PUBLICATION	Article writing													
	Publication and dissemination of findings													

Table 8: Timeline of the project