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THE ROLE OF LUNG ULTRASONOGRAPHY IN THE EVALUATION OF NEONATAL RESPIRATORY DISTRESS SYNDROME

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Doctoral thesis:

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Content

Resumen

Objetivo de nuestro estudio fue evaluar la capacidad diagnóstica de la ecografía pulmonar en el diagnóstico del Síndrome del Distrés Respiratorio (SDR), así como en el seguimiento de la respuesta al tratamiento.

La ecografía pulmonar se comparó con los rayos X como método estándar de diagnóstico y seguimiento de pacientes con SDR. La escala radiográfica de cuatro grados se comparó con perfiles de ultrasonido de tres grados.

El segundo objetivo era hacer un cálculo de las dosis recibidas debido a la radiografía de tórax en niños incluidos en el estudio.

También se compararon los hallazgos radiológicos con los datos clínicos y de laboratorio de los pacientes.

El estudio prospectivo incluyó 150 neonatos con diferentes edades gestacionales (≤ 35 semanas), examinados en la departamento de Unidad de Cuidado Intensivo Neonatales (UCIN) de la Centro Clínico Universitario, Sarajevo, Bosnia y Herzegovina.

Los exámenes se llevaron a cabo del año 2016 al 2019. Fueron realizados por radiólogo, utilizando una sonda de ultrasonido lineal de 7,5 y 10 MHz, en posición supina y en ambos decúbitos laterales, para valorar el área pulmonar anterior, lateral y posterior, en dirección caudo-craneal y con exploraciones sagitales y transversales.

La ecografía pulmonar se realizó después de una radiografía de tórax. La ecografía pulmonar repetida se ha realizado después de 24/36 o 48 horas respectivamente, de acuerdo con los signos clínicos y las condiciones clínicas del paciente y en algunos casos simultáneamente con radiografías de tórax repetidas.

Se calcularon dosis efectivas para los pacientes con el programa PCXMC.

Para las pruebas estadísticas el nivel considerado de significancia estadística fue $P<0.05$. MedCalc software ver. 19.0.3. (MedCalc Software, Mariakerke, Belgium) fue usado para todos los análisis estadísticos.

MANOVA no paramétrico se ha calculado dentro del software PAST ver. 3.25.

Discusion

La ecosografía pulmonar, a pesar de su amplio uso en entornos clínicos, todavía no forma parte de las recomendaciones o algoritmos oficiales en el manejo del paciente de diversas afecciones patológicas respiratorias y torácicas.

Nuestros resultados muestran una diferencia significativa entre la radiografía y el examen por ultrasonido en favor de la ecografía, con alta sensibilidad del ultrasonido en el diagnóstico del síndrome de distrés respiratorio en el prematuro.

En nuestro grupo de estudio encontramos la presencia de consolidaciones subpleurales, junto con líneas B confluentes y otros signos de SDR, con correlación estadísticamente significativa para este parámetro, en pacientes que tenían formas graves de RDS y peores resultados. Consideramos estos resultados como uno de los hallazgos más significativos de este estudio, e hicimos una propuesta de clasificación por ultrasonido modificada, que en nuestra opinión, servirá mejor en la evaluación de los hallazgos patológicos del síndrome del distrés respiratorio.

La ecografía pulmonar permite diagnosticar y dar seguimiento al síndrome del distrés respiratorio en neonatos prematuros.

Las dosis de radiación ionizante administradas a los pacientes prematuros son aceptables y muy por debajo del límite de las dosis publicadas.

La ecografía pulmonar puede reemplazar a un número considerable de exámenes de rayos X simple de tórax, disminuyendo así el número total de exámenes de rayos X y la administraron de dosis efectivas de radiación a los pacientes.

Debido al hecho de que el ultrasonido no es perjudicial para el paciente, se puede repetir tantas veces como sea necesario, contrariamente al examen de rayos X de tórax que utiliza radiación ionizante. Es indiscutible que el examen de rayos X tiene sus ventajas.

Para el diagnóstico del SDR la radiografía simple de tórax se puede hacer como examen inicial a la vez del examen de comprobación de inserción de tubos (en la mayoría de los casos, control de posición de catéteres umbilicales o tubos naso-gástricos).

1. Introduction

1.1 Embryology of the lungs

Lung development divides into three main stages: embryonic, fetal, and postnatal phase. As a part of complex embryologic development, at the end of the third week, the septum transversum (diaphragm) separates thoracic and abdominal cavities, but not entirely by leaving large openings on each side of the foregut called pericardio-peritoneal canals. Due to the rapid growth of the lungs and their expansion into the mesenchyme of the body wall, the mesoderm of the body wall form two components: the definitive wall of the thorax and the pleuropericardial membranes. Finally, the thoracic cavity divides into the two pleural cavities and the definite pericardial cavity.

At the fourth week of gestation, lung bud or respiratory diverticulum represents as an outgrowth from the ventral wall of the upper part of the foregut. This process manages by an increase in retinoic acid, which causes the upregulation of the TBX4-transcription factor that induces the formation of the lung bud and further development of the lungs.

In the beginning, there is open communication between lung buds and foregut. When lung bud expands caudally, two tracheoesophageal ridges fuse to form tracheoesophageal septum, separate from the foregut which divides into ventral portion- trachea and lung buds and a dorsal part- the esophagus.

In the fifth week of gestation, the bronchial buds form the right and left main bronchi. Then, the right main bronchi form three secondary bronchi and the left forms two secondary bronchi, thus in the eight weeks of gestation lungs are composed of the three lobes on the right side and the two lobes on the left side.

Pleuroperitoneal and pleuropericardial folds form the primitive pleural cavities by separating pericardioperitoneal canals from the peritoneal and pericardial cavities. The belonging mesoderm transforms into visceral and parietal pleura, and the space between those pleura is the pleural cavity. The visceral pleura extends between the lung lobes.

Secondary bronchi continue to divide in a dichotomous fashion, forming ten tertiary or segmental bronchi in the right lung and eight tertiary bronchi on the left side. By the end of 24 weeks of gestation, there have been 17 generations of subdivisions formed and during the postnatal period of life an additional six divisions. At the same time, simultaneously with the dividing of bronchi, lungs move caudally, to their final position.

Maturation of lungs divides into four periods:

1. Pseudoglandular period (5-16 week) – Branching of bronchi into terminal bronchioles, but respiration is not possible, because respiratory bronchioles and alveoli are not present.
2. Canalicular period (16-26 week) – Continuing branching of terminal bronchioles into respiratory bronchioles which divide into three to six alveolar ducts with an increase in vascular supply. At the end of this period, respiration is possible.
3. Terminal sac period (26 week to birth) – Terminal sacs (primitive alveoli) are formed, surrounded by flat alveolar cells. The number of terminal sacs is sufficient to permit the survival of the premature infant.
4. Alveolar period (8 months to childhood) – Most of the remaining dividing into alveolar ducts occurs within six months after birth. Mature alveoli have developed contact between epithelial and endothelial (capillary) cells.

During the terminal sac period, sacs are lined with type I alveolar epithelial cells with protrusion of capillaries into the alveolar sacs, and the close contact between epithelial and endothelial cells makes a blood-air barrier. Also, another type of cells develops at the end of this period called type II alveolar epithelial cells that produce surfactant which forms a phospholipid coat on the alveolar membranes, thus lowering surface tension at the air-alveolar interface/surface.[1]

Mature alveoli develop around 30 weeks of gestation. It is an estimation that only about one-sixth of the full complement of alveoli develop before birth; the rest of the alveoli develop after birth during the first eight years. Parallel with the process of alveolarisation; the capillary network is preparing to support gas exchange process.[2]

During the 34 weeks of gestation concentration of surfactant increase.

At the birth, with first respiration, most of the lung fluid is resorbed, and with air entering alveoli, surfactant prevents a collapse of the alveoli and enable gas exchange at cell membranes. Absent or insufficient surfactant, due to the collapse of the primitive alveoli, cause respiratory distress syndrome in premature babies.[1,3]

1.2 The Blood Supply of the Lungs

The blood supply of the lungs derives from the splanchnopleuric mesoderm (by the sixth arterial arch) that covers the lung bud. From the thoracic aorta arise the bronchial arteries, and a venous plexus surrounds the developing bronchial buds and drains to the left atrium.[1]

1.3. Anatomy and physiology of the lungs

The lungs are paired organs in the thoracic cavity. Heart and structures of the mediastinum divide the thoracic cavity into two anatomically distinct chambers. Double-layered serous membrane - pleural membrane covers and protects each lung. The superficial layer- parietal pleura, lines the wall of the thoracic cavity and the deep layer- visceral pleura, covers the lungs themselves. A pleural cavity is a small place, between the visceral and parietal pleurae which contain a small amount of fluid. This pleural fluid reduces friction between the membranes, allowing them to slide easily over one another during breathing. Pleural fluid also causes the two layers to adhere to one another- resulting in surface tension. Separate pleural cavities surround the left and right lungs.

The lungs extend from the diaphragm, slightly superior to the clavicles and lie against the ribs anteriorly and posteriorly. The inferior portion of the lung- the base, is concave and fits over the convex diaphragm. The superior part of the lung is the apex. The mediastinal (medial) surface of each lung contains the hilum with bronchi, pulmonary blood and lymphatic vessels, and also nerves. Due to the space occupied by the heart, the left lung is about 10% smaller than the right lung, and the diaphragm is higher on the right side, accommodating the liver inferiorly.

One or two fissures divides each lung into lobes. Both lungs have an oblique fissure, and the right lung also has a horizontal fissure. The oblique fissure in the left lung separates the superior lobe from the inferior lobe. In the right lung, the superior part of the oblique fissure separates the superior lobe from the inferior lobe; the inferior part of the oblique fissure separates the inferior lobe from the middle lobe, which is bordered superiorly by the horizontal fissure.

The branching of bronchi is segmental; thus, the right primary bronchus gives rise to three secondary (lobar) bronchi- superior, middle, and inferior bronchi. The left primary bronchus gives rise to superior and inferior secondary bronchi. Within the lung, the secondary bronchi further give rise to the tertiary (segmental) bronchi, so there are ten tertiary bronchi in each lung. The bronchopulmonary segment is a segment of lung tissue that each tertiary bronchus supplies and also contains a lymphatic vessel, an arteriole, and a venule.

Terminal bronchioles further subdivide into respiratory bronchioles, and they have alveoli budding from their walls. Alveoli participate in gas exchange.

Around the alveolar ducts, there are numerous alveoli and alveolar sacs. An alveolus is lined by simple squamous epithelium and supported by a thin basement membrane; an alveolar sac consists of two or more alveoli that share a joint opening. The walls of alveoli have two types of alveolar epithelial cells. The type I alveolar cells are simple squamous epithelial cells, and they are more numerous than type II alveolar cells which are between type I cells and they have rounded or cuboid epithelia and secrete alveolar fluid-surfactant which prevent the collapse of the alveoli. Type I cells are responsible for gas exchange through the respiratory membrane composed of four layers.[4]

Parasympathetic fibers cause bronchoconstriction, and sympathetic fibers stimulate bronchodilatation. Sensory fibers belong to the vagus nerve and thoracic ganglia (2-5). Branches from pulmonary plexus enter into lungs thru hilum, and they follow bronchial branching inside of lungs.[5]

Respiration is a process of drive airflow during inhalation and exhalation. Also, other factors affect the rate of airflow and the pulmonary ventilation: surface tension of the alveolar fluid, compliance of the lungs, and airway resistance.[4]

2. Definition of problem

2.1. Respiratory distress syndrome

According to the latest The Global Action Report on Preterm Birth for 2010, there are 15 million preterm births every year around the world and these numbers rising or 5-18% is the range of preterm birth rates across 184 countries of the world.[6]

More than 80% of preterm births occur between 32-37 weeks of gestation, and most of these babies can survive with essential newborn care.[6]

Depending on gestational age, premature divides into :

- extremely preterm (<28 weeks);
- very preterm (28–<32 weeks);
- moderate or late preterm (32–<37 completed weeks of gestation)[7]

According to their body, weight premature classifies as :

Extremely low birth weight (ELBW)	less than1000 g
Very low birth weight (VLBW)	less than 1500 g
Low birth weight (LBW)	less than 2500 g

In the CDC guidelines, it is defined as necessary to make an adjustment of premature growth in first 24 months of life, to follow up their development in an appropriate way.[8]

Respiratory distress syndrome (RDS), also known as hyaline membrane disease, is the most common clinical syndrome encountered among preterm infants born at less than 32 weeks of gestation and the complications of disease account for substantial mortality. Disease severity depends on low gestational age, perinatal asphyxia, hypothermia, male gender and absence of prenatally corticosteroid treatment to mother and also most probably cesarean section.[9]

According to Euro-peristat report, severity and incidence of RDS are in inverse correlation with gestational age with 92% of neonates born at 24–25 weeks affected, 88% at 26–27 weeks, 76% at 28–29 weeks and 57% at 30–31 weeks.[10]

Also, neonates with low gestational weight have more severe types of RDS.

Similar symptoms as respiratory distress syndrome have transient tachypnea, and sometimes it is difficult to differentiate between these two entities, especially in the first 24 hours. Beside x-ray imaging and clinical condition of the patient, differentiation among RDS and TTN is also possible with lung ultrasound.[11,12]

RDS is a result of surfactant deficiency and a manifestation of pulmonary immaturity. Surfactant usually coats the alveoli, and by lowering surface tension prevents atelectasis. In respiratory distress syndrome, there is a gradual development of the interstitial thickening and dilatation of the terminal alveoli with a disturbed/impaired process of gas exchange. As a result of impeded respiratory function, respiratory distress syndrome manifested with hypoventilation, hypoxemia, and respiratory acidosis.[13]

Risk factors for the development of RDS are mostly connect to immature neonate, including prematurity, pulmonary hemorrhage, infection, perinatal asphyxia, pulmonary hypoplasia, but also some pathological conditions of mothers can also be responsible for the development of RDS such as multiple pregnancies, gestational diabetes, elective cesarean section.[14]

Respiratory distress syndrome affects more severely premature children from diabetic mothers, and it is a familiar fact that fetal hyperinsulinemia interfere with glucocorticoid production which is responsible for lung maturation and production of surfactant.[15]

In several clinical trials have been concluded that antenatal glucocorticoid therapy reduces the incidence of RDS in premature children from 29-32 gestational weeks. In contrary, in premature infants from 24-28 gestational week, significant benefits form antenatal glucocorticoid therapy have not proved, but in these children, there have been reduced incidence of high grade intracranial hemorrhage.[16]

Without appropriate treatment, following symptoms such as grunting, cyanosis, nasal flaring tachypnea, and intercostal retractions occur shortly after birth and increase in severity in the first two days of life. After 48-72 hours, under treatment, it usually starts recovery, which is associated with diuresis and clearance of excess lung fluid and decreasing demands for ventilatory support.[9]

For the Vermont Oxford Network, an infant defines as having RDS when it has PaO2 less than 50 mmHg with room air, central cyanosis in room air, a necessity for additional oxygen to maintain a PaO2 higher than 50mmHg, or a blood oxygenation level over 85% within the first 24 hours of life.[17] Clinical staging correlates well with x-ray findings. On plain X-ray radiography, there is reticulogranular or ground-glass opacification, progressive hypoaeration, and air bronchograms. Symptoms and radiological signs progress during the first 6h of life, and in mild to moderate disease, the granular densities persist for 3-5 days, clearing from peripheral to central and upper to lower lungs.[18]

Although the CRIB (clinical risk index for babies) score developed as a tool for assessing initial neonatal mortality risk, also it was used as a predictor of morbidity in respiratory distress syndrome (RDS) and chronic lung disease.[19]

In diagnosing neonatal RDS, CRIB score has used in the correlation with established positive clinical signs, blood gas analysis such as hypercapnia and hypoxia and radiographic picture. Most prominent radiographic presentations are granular opacities and air bronchogram, and these findings are visible usually after the first 6 hours of life. In most cases, granular opacities are bilateral and symmetric, but also their distribution can be asymmetric.

2.2. Surfactant production

Pulmonary surfactant is lipoprotein, which is produced in the endoplasmic reticulum of the type II pneumocytes and then transported through the Golgi apparatus and concentrated into intracellular lamellar bodies that migrate to the cell surface. On the cell surface contents of these bodies are expressed onto the alveolar luminal surface. The primary surface-active material in surfactant is the phospholipid, dipalmitoylphosphatidylcholine (DPPC) which combined with four surfactant proteins A, B, C, and D, that are also produced by the type II pneumocytes, to form a complex lattice called tubular myelin. Surfactant is made up of 70% to 80% phospholipids, approximately 10% protein and 10% neutral lipids, mainly cholesterol. Among four surfactant proteins, two of them (SP-B and SP-C) are hydrophobic, while the other two (SP-A and SP-D) are hydrophilic polypeptides.[20,21]

Without the elevated alveolar surface tension, there is a resultant collapse of the alveoli.

Production of surfactant starts at 16 weeks; one of the surfactant components – Lecithin starts between 18-20 gestational week, and the highest concentration is around 35-36 gestational week. Phosphatidylglycerol and sphyringomyelin produce right after lecithin with the highest level at 36 weeks, respectively with a small peak between 28 and 30 weeks. If we know the mutual relation between some of the surfactant components, then it is clear the reason why is used L/S (lecithin/sphyringomyelin) ratio for the evaluation of lung maturity from amniotic fluid.[22–24]

During fetal growth, plasma adrenal cortical hormones stimulate the synthesis of surfactant, and in contrary hyperinsulinemia inhibits it. That is the main reason why is a synthesis of surfactant lower in fetuses with high levels of insulin, and consequently, newborns from diabetic mothers have 5-6 times higher incidence of respiratory distress syndrome compared to standard rates.[25,26]

In newborns, especially preterm metabolism of surfactant is slower, than in adults.

Pulmonary surfactant helps in lowering surface tension and preventing the collapse of the alveoli and generally lowers surface tension to <6 dynes/cm with a content that could divide into an intra-alveolar and an intracellular pool.[27]

The total surfactant pool size is not equivalent to the amount of active surfactant.[28]

In respiratory distress syndrome, there is an impaired process of surfactant metabolism. The total amount of surfactant reduced to less than 10 mg/kg surfactant. Term infants have an estimated pool size of 100 mg/kg surfactant.[29]

Therapeutic doses of surfactants are between 10–20 times the average pool sizes during surfactant replacement therapy which approximates the pool size in term infants.[30]

Defects in surfactant metabolism can be inherited, and consequently can also lead to high morbidity and mortality due to respiratory deficiency as a result of respiratory distress syndrome in premature. There are several gene defects described :

1. Hereditary SP-B deficiency, inherited as autosomal recessive disease, with mutation of Human SP-B gene located on chromosome 2, cause lethal RDS.31
2. Hereditary SP-C Associated disorder- inherited as an autosomal dominant disorder, due to the mutation of Human SP-C gene located on chromosome 8, can lead to acute and chronic lung disease.[31,32]
3. ABCA3- transporter gene mutation, inherited as an autosomal recessive disorder with gene mutation on chromosome 16. This gene is highly expressed in Type II epithelial cell, and its mutation can result in ARDS in infants.[33]

Lack of surfactant results in alveoli collapses, pulmonary atelectasis, hypoventilation, reduction of pulmonary ventilation/perfusion ratio, and with normal blood flow, this causes metabolic acidosis. Fetal circulation could persist due to pulmonary arterial spasm, vascular resistance, the persistence of foramen ovale and patent ductus arteriosus and consequently right to left shunt. As a result of decreased pulmonary perfusion, alveoli become more porous with the presence of interstitial edema, but also with exudation of fibrin which collects in alveoli walls and thus forms hyaline membranes. These hyaline membranes enable gas exchange thru alveoli, increasing hypoxia, and acidosis that further inhibits surfactant production. Without adequate treatment, this circle goes around again; the infant has a more severe impaired respiratory function and severe clinical condition. [29]

2.3. Antenatal corticosteroid therapy

In 1972, Liggins and Howie published for the first time, results of their research regarding prenatal use of corticosteroid therapy in pregnant women and improved outcome in premature neonates, less severe RDS and reduced mortality of newborns.[34]

Nowadays, antenatal use of corticosteroid therapy is widely adopted, especially since consensus conference held by the National Institutes of Health in 1994 and numerous trials and papers have proven its beneficial use with reduced complications and less severe respiratory manifestations in premature babies.[35] Although the most efficient use has been shown in premature from 29-32 gestational week, in lower gestational age (24-28 week) incidence of complications, such as intracranial hemorrhage was reduced. In the study of Bennerman et al., late preterm neonates, from mothers who received corticosteroid therapy (antenatal), had transitional hypoglycemia and less severe forms of RDS.[35]

Since 1999, the use of a single dose of corticosteroids to mothers at risk for preterm delivery from 24 weeks of gestation has established in the UK, and since the protective role of corticosteroids lasts approximately seven days, the repeated dose was given every 7-10 days. The Royal College of Obstetricians and Gynaecologists' guidelines recommend prophylactic use of corticosteroids up until 36 weeks' gestation.[36]

The common practice is the administration of two doses of corticosteroids during 24 h hours.[37]

There are no significant adverse effects observed of corticosteroids use for the mothers or neonates or long term consequences on infants.

Suspected chorioamnionitis is the absolute contraindication for antenatal corticosteroid use.

Recommended regime for antenatal corticosteroids, according to WHO guidelines are 24 mg of corticosteroid (betamethasone or dexamethasone) divided into two doses, preferably within 48 hours or respectively 24 hours before delivery.[38]

According to ACOG guidelines, both betamethasone and dexamethasone are acceptable for use in women at risk for preterm delivery.[39]

2.4. Respiratory distress therapy

Treatment of respiratory distress syndrome is based on surfactant administration because of the lack of surfactant, or its insufficient amount is the primary pathophysiology mechanism of immature lung disease.

Surfactant administration results in: decreased alveolar surface tension, dramatically and fast improvement in gas exchange reduced need for high O2 concentration and ventilator support and improved lung compliance.[40,41]

An optimal strategy for the treatment of RDS is based on several decisions: adequate surfactant replacement therapy, right time and method for surfactant administration, number of doses, and also type of support ventilatory strategy.[42,43] Treatment of RDS depends on gestational age, but it is not recommended to administer surfactant before the onset if RDS symptoms, as prophylactic therapy. [38] Some studies suggest treatment of premature with CPAP in the delivery room, and these patients prove to have better outcome.[44] Premature born < 30 weeks of gestation with a severe form of respiratory distress syndrome and substantial need for mechanical ventilation should receive surfactant, right after delivery.[45]

To reduce the need for mechanical ventilation surfactant can be administered by INSURE technique (intubate–surfactant–extubate-to CPAP).[46]

In updated European guidelines for RDS treatment, it recommended using a less invasive procedure for surfactant administration (LISA) technique with specially designed catheters which reduce the need for mechanical ventilation with a more gentle approach to very small babies. [47]

The requirements for mechanical ventilation can be appraised depending on applied technique for surfactant administration.[48]

In mild RDS, increased (inspiratory) O2 concentration can be sufficient for the optimal level of arterial O2. Even though desaturation in RDS is caused due to the inability to maintain sufficient and adequate functional residual capacity, consequently, the tendency of atelectasis increases and usually there is a need for artificial ventilation.

Today, there are different modalities of ventilation under pressure, such as :

- CPAP (continuous positive air pressure)
- BIPAP (nasal positive pressure ventilation)
- IPPV (intermittent positive pressure ventilation)
- IMV (intermittent mandatory ventilation)
- patient triggered ventilation (PTV)
- volume-limited ventilation
- VGV (volume guaranteed ventilation)
- PSV (pressure support ventilation)
- HFOV (high-frequency oscillatory ventilation)[9]

Each of these ventilation modalities has its place in the acute phase of the disease, but also during the separation process from mechanical ventilation, with different advantages and disadvantages.

Beside the surfactant therapy, O2 support therapy and ventilation under positive pressure, treatment of RDS requests some supportive measures which includes : regulation of body temperature, adequate fluid intake, maintenance of ABS and mineral balance (pH under 7,3), satisfying energy intake, monitoring and eventually support of cardio-circulatory function, but also strategy for prevention and treatment of infection.[9]

2.5. Bronchopulmonary dysplasia (BPD)

BPD is defined as a prolonged need for supplementary O2 support between 28- 30 days of life or in 36 postmenstrual weeks.[49]

BPD can be classified as mild, moderate, or severe disease.

Despite the evident progress in the treatment of premature infants including antenatal corticosteroid therapy and administration of surfactant, the incidence of BPD is still high and it is directly connected with the survival of extremely premature babies in a range between 4,5-36%. Also, it is connected with bad pulmonary and neurological outcomes.[50,51]

Previously, bronchopulmonary dysplasia has been considered as a result of RDS in extremely premature infant, but today it is also known that perinatal factors that influence lung maturation (especially chronic low-grade chorioamnionitis) probably have an important role in the pathogenesis of BPD.[52] Presence of Ureaplasma urealiticum in amniotic fluid is associated with the occurrence of bronchopulmonary dysplasia.[53]

The pathogenesis of BPD is multifactorial, representing a combination of the immature lung parenchyma, insufficient number of alveoli, lack of surfactant and exposure to prolonged O2 ventilatory support, pre and postnatal infection, patent ductus arteriosus. Also, preeclampsia is one of the predisposing factors for the development of BPD because antiangiogenesis interrupts lung development and surfactant production.[54] As a result of all these factors, bronchopulmonary dysplasia represents with necrotizing bronchiolitis and alveolar septal thickening with classical radiographic appearance of “honeycomb” or “bubbly” lungs in most severe stages.[16]

Parenchymal and interstitial changes of lungs in neonates can be evaluated with x-ray, but also with CT scan.[55] In a neonate, CT can in details show areas of air trapping, hypoaerisation but also enable evaluation of bronchial branches sizes.[56]

3. Dosimetry/radiation protection

Patient dosimetry is the obligation of all professionals involved in patient care when using ionizing radiation. In 1970 in the USA was first introduced systematic nationwide surveys, later in the 1980s in the UK for patients' dose measuring. In 1996 first was introduced the term -Dose reference level /DRL published in the ICRP recommendations; these regulations cover different aspects of dosimetry and radiation protection. Dose reference levels (DRL) are well defined and established in published recommendations, updated version ICRP 135.[57] DRLs are not marking for dose limits, because dose limits do not apply for medical exposures and dose reference levels should be established according to data from clinical practice. For x-ray examinations, DRLs should be estimated according to dose values that patients received during the examination.[57]

Previously, in this process of dose measurement, different phantoms were used, but clinical data from patients, enable the perspective of the distribution of these data, which was hard to obtain with phantoms.

It is important to emphasize that DRLs are not marking for dose limits and they should not be used for individuals, though for a group of patients and as a start point for the process of the optimization of protocols (with ionizing radiation) and through that for the radiation protection.

In ICRP recommendations is clearly stated which physical quantities should be used, so for the different procedures are defined as follows:

- Radiography: PKA (primary quantity) and Ka,e (useful additional quantity)
- Fluoroscopy: PKA (primary quantity), Ka,r, fluoroscopy time and number of images (useful additional quantities)
- Computed tomography: CTDIvol and DLP, determined for a 32 cm phantom (all body CT examinations: chest, abdomen, trunk, and spine) and for a 16 cm phantom (head CT examinations); besides CTDIvol, when available, SSDE can be used for all body CT examinations
- Interventional radiology: PKA (primary quantity), Ka,r, fluoroscopy time and a number of images (useful additional quantities). [58,59]

For the children in the ICRP 185 recommendations grouping of patients should be carried out with intervals according to their body weight and age for the head exam as follows:

- Weight groups for body exams: < 5 kg, 5 - < 15 kg, 15 - < 30 kg, 30 - < 50 kg, 50 - < 80 kg.

The recommended weight group (< 5 kg or neonates) applies to newborn babies but does not apply to those in incubators.

- Age groups for head exams: 0 - < 3 months, 3 months - < 1 y, 1 - < 6 y, ≥ 6 y [60]

For the children, it is also recommended that obtained data can be represented as a DRL curve by expressing the DRL quantity as a continuous function of the grouping parameter, so the collected data can enable an analysis of the relationship between patient doses and grouping parameter. This could be helpful in an unsymmetrical patient cohort with difficulties in matching adequate patient doses records with specific patient groups.

In our study, we calculated Ka, e, KAP value, the recommended value for x-ray examinations and also effective dose, but also we measured scattered radiation because in the patient ward there are three incubators, so theoretically radiation exposure of one patient is potentially significant for the other. Simulation of the scattered radiation has been done with cylindrical phantoms and calculation based on the number of x rays for the total dose, expressed in mGy. Ionizing radiation has a cumulative effect so, the importance of multiple exposures can be a significant factor for the patient itself, but also surrounding patients. Despite the fact that medical exposure has no proposed limits, it is essential to evaluate average number of chest x-ray examinations in population (in our cohort patients with respiratory distress syndrome), so the numbers can be evaluated in the course of their possible decrease and also potential replacement with methods that are not using ionizing radiation such as ultrasound.

Radiation dosimetry is based on the quantitative measurement of energy delivered to the patient by direct or indirect radiation.

Kerma is used as a definition for delivered kinetic energy (photons and neutrons/ indirect ionizing radiation) per unit mass. The unit of Kerma is joule per kilogram. Name of the unit is Gy.[61]

1 Gy = 1 J/kg

Air Kerma is defined ad released kinetic energy that traveling through air. Air Kerma or Ka,e is expressed in Gy or mGy.

KAP represents an average air Kerma, which is multiplied by a corresponding x-ray beam cross-sectional area (cm^2). KAP is not a surrogate for patient dose, and it is directly proportional to organ doses for a fixed x-ray beam area. KAP is expressed in Gycm2.[62]

The effective dose is considered as a measurement of actually dose that patients receive during the radiological examination. KAP is converted with conversion factors into an effective dose that is expressed in mSv. [63]

KAP can be used for the assessment of different types of radiological examinations in terms of evaluation if an appropriate amount of radiation has been used. Values of KAP distribution can also be used for identification the third-quartile value (75%) which is important for establishing “diagnostic reference level “ and thru that review the process of optimization process.[64]

4. Radiological and ultrasound analysis

Lung ultrasound is using in clinical practice for more than three decades.[65] This method has been used fist in adult patients, mostly in emergency settings. As a result of that approach, specific protocols were created for different pathological intrathoracic conditions.[66–68] Lichtenstein et al. first described ultrasound artifacts and characteristic ultrasound profiles for lung pathology, including alveolar interstitial syndrome, atelectasis, consolidation, effusion, followed with many other authors and publications regarding lung ultrasound use in clinical practice.[69–72]

The use of lung ultrasound in children is even more comfortable to perform, since the body size and weight of children allow a detail evaluation of thorax, especially in the neonatal period. Lung ultrasound has been used in the assessment and monitoring of a variety of pathological conditions, including respiratory distress syndrome as one of the most frequent pathologies in neonates.[59,73–76]

Normal transthoracic lung ultrasound (LUS): On the interface where parietal and visceral pleura has a contact there is a reflexing surface, and pleura is seen as a smooth, hyperechoic, horizontal line, which is moving during the respiratory cycle (lung sliding sign). Pleura thickness considers normal ≤ 5 mm.[77]

Lungs are filled with air, and due to high acoustic impedance between the visceral pleura and the lung, visualization of lungs parenchyma in normal condition is difficult, so the interpretation of ultrasound findings is based on the analysis of two types of artifacts that appear below pleural line called A and B lines, which are perpendicular to each other.[78]

A-lines are horizontal lines with equal mutual distance, parallel with the pleural line, and they represent normal aeration of lung parenchyma.

B lines are vertically oriented lines, propagate from pleural line distally. In pathological conditions, B lines erase A-lines, but usually few B-lines that not reach the end of the screen can be found. B lines can be with more or less dense distribution, depending on the underlying pathological condition. The abundance of B-lines reflects the extent of pulmonary edema.[65,79,80] The presence of B-profile with present lung sliding is a sign of interstitial edema. In contrary to this, finding the abolition of lung sliding with B profile and consolidation of lung tissue is characteristic of pneumonia due to inflammatory adherence and the presence of exudate effusion.[80]

Terms that are used in lung ultrasonography, beside A and B lines are lung sliding sign, parenchymal consolidation, alveolar- interstitial syndrome, lung point, double lung point, white lungs and for M mode - “stratosphere” sign and “sandy beach” pattern in pneumothorax diagnosing.

Lung ultrasound is based on the interpretation of the artifacts. In the healthy neonate with normally aerated lungs, there is a prevalence of A-lines.

LUS finding of RDS: when the lung parenchymal disease propagates to the pleura, an acoustic window is formed, and that enables evaluation of lung tissue. The pathological finding is presented with the presence of B lines; they erase the A-lines and move with 'lung sliding.' They are a result of the accumulation of fluid in the subpleural interlobular septa surrounded by air. B-lines can be seen as individual or multiple lines with a trend become confluent or fuse together, and distance between lines can be from 3-5 mm.[26]

If the distance between B lines is 7 mm it is considered as interstitial edema (on CT findings they are seen as thickened interlobular septa).

If the distance between B lines is ≤ 3 mm, it is considered as alveolar edema (ground-glass opacities on CT exam).[81]

RDS was diagnosed by Raimondi et al. with the simultaneous presence of three ultrasound findings: abnormalities of the pleural line, white lung image, and absence of spared areas in all lung fields.82 After a cesarean section, the presence of a few B lines is considered normal, due to resides of fluid in the lungs. Lung consolidation is described as subpleural or tissue-like areas with blurred margins or wedge-shape borders. Dynamic air bronchogram is hyperechoic linear lines that appear within the hypoechoic consolidated lung. On the contrary, in the atelectatic lung parenchyma, static bronchogram is present. The lung pulse sign-vertical motion of the pleural line is seen in lung atelectasis.[26,83]

All ultrasound findings are classified into three profiles :

Type 1- a full hyperechoic image of the lung fields or „white lung";
Type 2- prevalence of B-lines, lung sliding sign present :
Type 3- A-lines predominance, lung sliding sign present

Lung ultrasound is using equally in the adult population for the diagnosing of different pathological conditions, including ARDS (adult respiratory distress syndrome).[84,85]

It is essential to differentiate respiratory distress syndrome from some other pathological conditions in neonates, such as transient tachypnea. Ultrasound shows excellent results in the evaluation and differentiation between these two entities.[12,86]

During the period of conducting patient in our study, we also evaluate neonates with transient tachypnea trying to differentiate between RDS and TTN patients only based on ultrasound findings, blinded to clinical data. The most crucial ultrasound finding in these patients was the presence of a double-lung point sign. This sign is showing the spared upper parts of the lungs and the presence of interstitial fluid in the lower part of the lungs. These results are not included in final statistical analysis because this is an ongoing study, created as an idea during the examination of patients with respiratory distress syndrome in our study.

The typical radiographic picture differs, depending on the severity of RDS. Usually, as a result of hypoaeriation, on chest x-ray air bronchogram is present and granular opacities, which are the most commonly diffuse, bilateral and symmetric. Radiographic presentation is a consequence of atelectasis, and later a result of edema and interstitial fluid, sometimes with the consolidation of parenchyma/pneumonia and hemorrhage.[3]

Radiographic findings can be classified as follows :

Stage I- Fine homogenous ground glass shadowing;
Stage II- Bilateral widespread air bronchogram;
Stage III- Confluent alveolar shadowing;
Stage IV- Alveolar shadowing obscuring cardiac border[87]

A radiographic picture is changing according to the clinical status of patients and therapy (applied), especially if a patient received surfactant. Also, the radiographic picture is fully recognizable after 6-24 h and depending on treatment, so different radiographic presentations have to be interpreted together with all other clinical and lab.data.

5. Aim of the study

To evaluate the diagnostic ability of lung ultrasound in the detection of pulmonary manifestations of respiratory distress syndrome as well as in the monitoring of the response to treatment.

Lung ultrasound was compared to x-ray as a standard method of diagnosing and follow up of RDS patients. The four-grade radiographic scale was compared to three-grade ultrasound profiles.

Radiological findings were also compared to clinical and laboratory data of patients.

Also, one of the aims is to calculate received doses after chest x-ray in children included in the study based on parameters (tube voltage and tube current values).

6. Hypothesis

Primary hypothesis: Lung ultrasound will enable the diagnosing of respiratory distress syndrome in neonates in correlation with chest x-ray and clinical signs (positive for impaired respiratory function in RDS) which is considered as a standard way of diagnosing RDS. Lung ultrasound will reduce the use of chest x-ray examinations.

Secondary hypothesis: Lung ultrasound will reduce the use of chest x-ray examinations.

7. Patients and methods

The prospective study included 150 neonates with different gestational age (≤ 35 weeks), examined in the NICU department of the University Clinical Center, Sarajevo, Bosnia and Herzegovina.

Examination was conducted from 2016-2019, performed by radiologist, using 7,5 and 10 MHz linear probe in supine and both lateral decubitus positions of the anterior lung area (between the sternum and anterior axillary line), lateral lung area (between anterior and posterior axillary lines) and posterior lung area (between the posterior axillary line and the spine) in caudo-cranial direction.

Longitudinal and transverse scans were included. A complete evaluation of both lungs was required.[81]

Ultrasound scans were classified into three profiles, grading based on the severity of respiratory distress syndrome, exponentially from most severe (profile 1) to mild forms or resolution of disease (Profile 3).

Lung ultrasound was performed after a chest x-ray. Repeated lung ultrasound has been performed after 24/36 or 48 hours, respectively, according to clinical signs and clinical conditions of the patient and in some cases, simultaneously with a repeated chest x-ray.

Radiographic findings were classified into four stages, and the ultrasound findings classify according to 3 profile gradations. These gradation scales have inverse relation, meaning that Ultrasound profile 1 corresponds to X-ray grades 3 and 4, which are the most severe forms of RDS. Initially, the statistical analysis compared exiting grading scales. Results did not show a significant difference between grade 3 and 4, so these grades were fused, and in further analysis inverse modified 3-grade x-ray scale was used.

Chest radiography at NICU was performed with mobile x-ray machines. In our study, it was used a single x-ray unit, GE TMX+ (General Electric, Boston, MA, USA) and Agfa CR30-X computed radiography (CR) imaging system (Agfa-Gevaert, Mortsel, Belgium). They were operated by different radiographers using the same exposure technique and technical parameters (Table 1.) :

Projection - Anterior - Posterior	AP
Tube potential (kV)	53
Tube loading (mAs)	3.2
Filtration (mmAl)	3
Focal spot size (mm)	0.8
Radiation output,Y (μSv mAs m^2)	52.3
Focus-skin distance (cm)	87.7
CR detector size (cm^2)	18x24

Table 1. Tecnical parameters for a chest x-ray

Effective doses for the patients were calculated with the PCXMC program.

PCXMC is a computer program for the calculation of patient organ doses and the estimation of effective doses during radiography and fluoroscopy investigations.[88]

Doses are calculated for 29 organs and tissues; an effective dose is estimated according to currently valid weight factors from ICRP publication 103 and weight factors from publication 60. [89,90] Program supports models for pediatric and adult patients, and also change their size. This program enables recognition of applied radiographic technique.

Organ doses can be used for the estimation of cancer risk caused after exposing to ionizing radiation. The program is based on the Monte Carlo simulation. Picture 1. shows the main screen window of the PCXMC program where can choose different parameters including human fantom, height, weight, focus distance from the x-ray tube, parameters of the beam and also maximum radiation energy which will be used in simulation and number of simulations.

Fantom data that softer is using are based on hermafrodit fantom, which is defined and specified by Cristy and Eckerman in 1987.[91] There are options between child fantom aged from 0,1,10 and 15 years and adult fantom. Softver enables corrections of height and weight for adult fantom.

An effective dose is a result of simulation defined by weight tissue factors from ICRP reports 103, air kerma and field surface and other parameters.

Parameters for the Monte Carlo simulation shown in Table 2.

Measurements of scatter radiation was made with a cylindrical water phantom similar in size to a neonatal patient (height: 28 cm, diameter 8 cm) with an appropriate instrument (RTI Piranha Dose Probe, Molndal, Sweden), calibrated in terms of ambient dose equivalent at 10 mm phantom depth, denoted as H*(10). Measured H*(10) at 1 m distance from the phantom center at exposure parameters used for imaging was 0.061 μSv per patient. This value was used as the effective dose, E, in afterward calculations.

The applied backscatter factor (BSF) was 1.1.58

Ka,e alone could be used as a relevant dose descriptor.

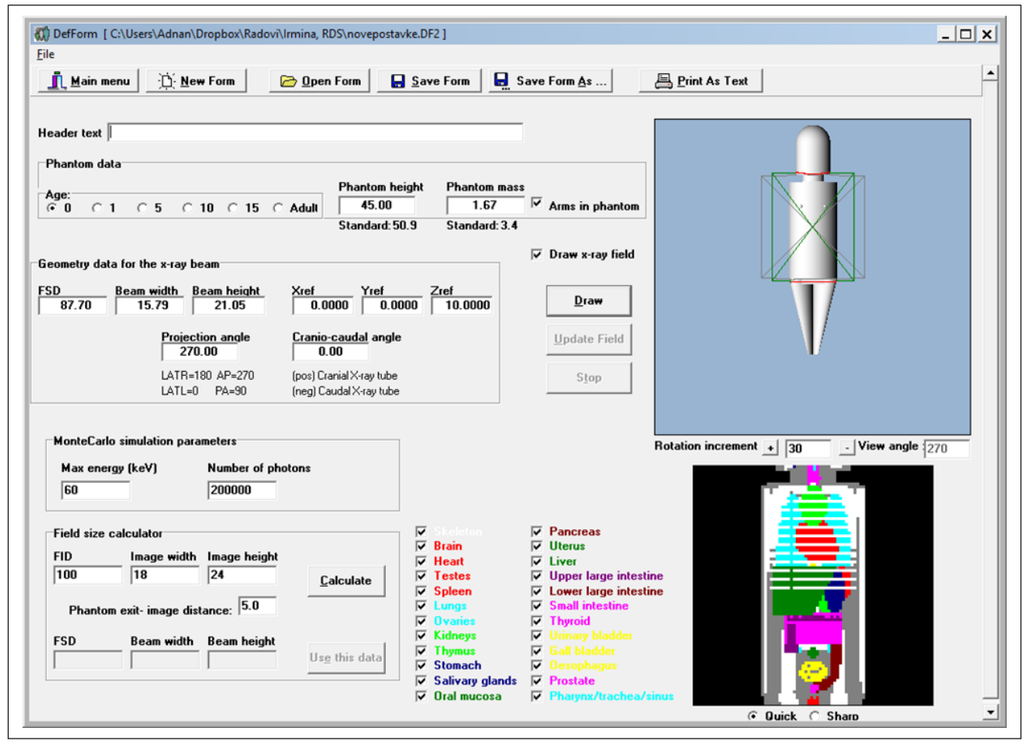
Parameter	
Fantom	
Age	0
Height	45
Weight	1,67
Beam	
Distance focus-skin	87,7
Beam width	15,79
Beam height	21,05
Xref	0
Yref	0
Zref	10

Number of simulation	
Maksimum energy	60
Fotons number	200000
Radiation energy	
Anode voltage	53
Filtration	3
Anode angle	16
Dozimetric value	
Ka,e	Reported value

Table 2. Parametres for Monte Carlo simulation-softver PCXMC 2.0.1.4

We calculated values of Kerma in air Ka, with tube current value 53 kV and tube voltage 3,2 mAs with distance from skin surface 87,7 cm. Kerma in the air was calculated according to the following formula :

Skin dose with BSF 1,1 (factor of reverse dispersion) Ds,e = 1,06 x BSF x Ka[92]



Picture 1. PCXMC program 2.0.1.4. screen window

7.1. Inclusion criteria

Neonates with clinical and radiographic signs of neonatal respiratory distress syndrome within the first 24h of life.

7.2. Exclusion criteria:

Neonates with congenital anomalies, intrauterine growth retardation, or severe underlying pathological condition.

7.3. Patient preparation

No special preparation, sedation, food, or fluid restrictions were needed.

8. Statistical analysis

Biostatistics methods

To estimate descriptive indicators, we have calculated frequency, lowest value, highest value, arithmetic mean, median, variance, standard deviation, standard error, as well as deviation from a normal distribution.

Test for Normal distribution was made using the Kolmogorov-Smirnov approach, with the considered level of statistical significance $P < 0.05$.

To assess the difference between sex ratio, we have used $\chi 2$ test. For the estimation of differences in gestational age and weight between males and females, independent samples t-test was used.

In the assessment of the pairwise difference between Ultrasound day-1, Ultrasound day-2, and between results of Ultrasound day-1 (initial study) and x-ray (initial study) we implemented Wilcoxon test (paired samples) test. Correlation test (Spearman's rho) was used to predict the relationship between Respiratory distress syndrome (RDS), and other clinical parameters (APGAR score, number of days on CPAP). In the case of gestational age and weight, we have used a one-way analysis of variance (ANOVA) to assess any difference according to RDS. Logistic regression was implemented to estimate the relationship between x-ray, ultrasound day-1 and other clinical parameters with patient outcome.

Since parameters such as a number of days on CPAP, O2, and MV (mechanical ventilation) variables were not within normal distribution, the Mann-Whitney U test (independent samples) was used for the comparison according to x-ray and US (ultrasound) subgroup. Subgroup differentiation was made based on ultrasound findings into two subgroups: group 1- patients that had subpleural consolidation and group 2- patients without consolidations. The same test was used when we compared x-ray values according to clinical data such as type of delivery, antenatal corticosteroid therapy, and premature rupture of membranes (PROM). To estimate the relationship between x-ray grades (3 and 4) and patient outcome, surfactant therapy, premature rupture of membranes (PROM) we have used Fisher's exact test. To predict the relationship between the degree of RDS and PROM $\chi 2$ test was implemented.

Receiver Operating Characteristic (ROC) analysis was applied to assess the cut-off point of the observed parameters (gestational age and weight) relative to the outcome (dead or alive/cured). AUC (Area Under the Curve) is calculated for estimating the differentiation character in terms of outcome without taking into account the specificity and sensitivity parameters.

In order to estimate the interobserver agreement between x-ray and ultrasound, a weighted Kappa test has been applied.

NPMANOVA (Non-Parametric MANOVA) was used to estimate the significant difference between two or more groups.

In total patient cohort,? The percentage of patients were twins, and to test some specific parameters, we evaluate twin patients, additionally in separate subgroups.

In twins study, we have observed APGAR score 1 (first minute), APGAR scores 5 (fifth minute), weight, and outcome. Wilcoxon test (paired samples) was used to compare APGAR score 1, as well as APGAR score 5 between each pair of twins. Also, Spearman's rho correlation coefficient was calculated. In the case of patient weight, paired-samples t-test was implemented as well as Pearson's correlation coefficient. Fisher's exact test was calculated to estimate the significance of the birth of twins in means of grade of RDS with the outcome.

For all the above mentioned statistical tests considered the level of statistical significance was P< 0.05. MedCalc software ver. 19.0.3. (MedCalc Software, Mariakerke, Belgium) was used for all statistical analyses except for Non-Parametric MANOVA when we used PAST ver. 3.25 software.[93]

9. Results

9.1. Descriptive analysis

Descriptive statistics analyzed overall statistical data regarding the gender, birth weight, gestational age, Apgar scores, type of delivery, antenatal dexamethasone therapy, premature rupture of membranes, regardless of RDS grade. Also, it shows results for applied therapy CPAP, surfactant, mechanical ventilation or Oxigen therapy as an overall estimation.

There is no statistical significant difference in proportion of female (74; 49,7%) and male (75; 50,3%) patients (Fisher's exact test P = 0,9347) (Figure 1).

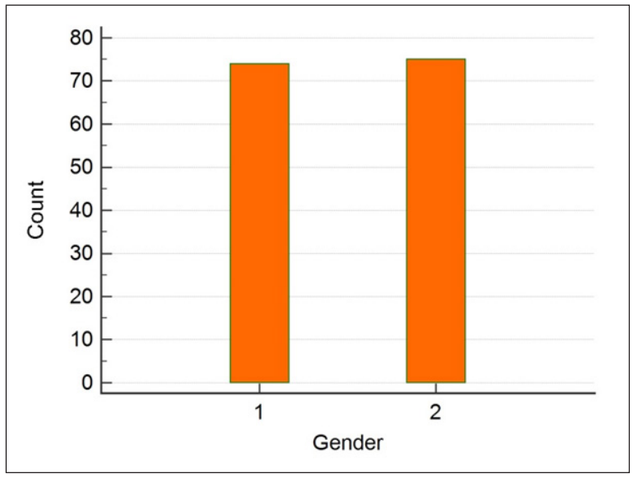


Figure 1. Proportion of male and female patients

Descriptive statistical parameters for gestational age shown in tables Table 3., Figure 2. The lowest noticed value was 24 weeks and the highest 36 weeks, with an average of 31,0537.

Sample size	149
Lowest value	24,0000
Highest value	36,0000
Arithmetic mean	31,0537
95% CI for the Arithmetic mean	30,6654 to 31,4420
Median	31,0000
95% CI for the median	31,0000 to 32,0000
Variance	5,7539
Standard deviation	2,3987
Standard error of the mean	0,1965

Table 3. Descriptive statistical parameters for gestational age

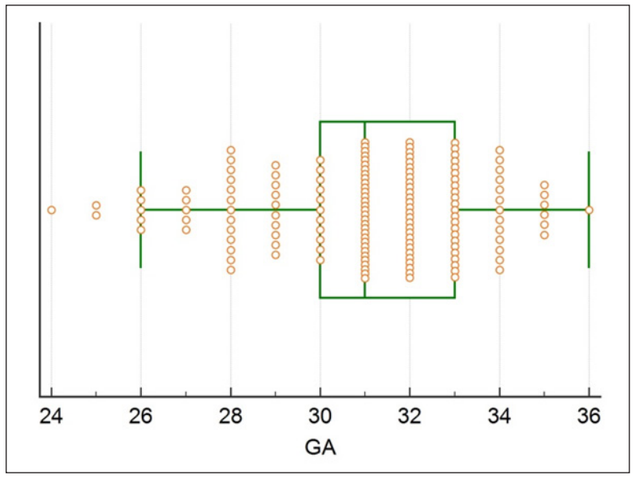


Figure 2. Graphical display of descriptive statistical parameters for gestational age

In Table 4. and Figure 3., we can see that the lowest noticed value was 560 g and highest 2910 g with an average of 1660 g. The other descriptive statistical parameters are shown on the same table.

Sample size	149
Lowest value	560,0000
Highest value	2910,0000
Arithmetic mean	1660,1812
95% CI for the Arithmetic mean	1577,4822 to 1742,8803
Median	1690,0000
95% CI for the median	1560,5683 to 1730,0000
Variance	260951,3791
Standard deviation	510,8340
Standard error of the mean	41,8492

Table 4.Descriptive statistical parameters for birth weight

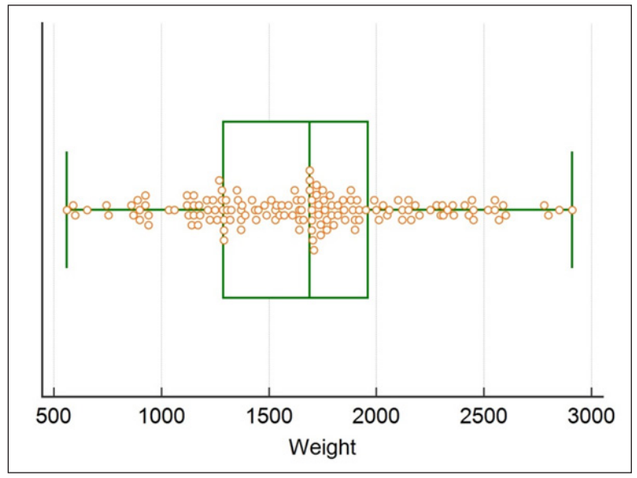


Figure 3. Graphic display of descriptive statistical parameters for birth weight

Descriptive statistical parameters for APGAR score in 1st minute were shown in Table 5 since for APGAR score in 5th minute in Table 6.

Sample size	144
Lowest value	1,0000
Highest value	10,0000
Arithmetic mean	6,8472
95% CI for the Arithmetic mean	6,5347 to 7,1597
Median	7,0000
95% CI for the median	7,0000 to 7,2404
Variance	3,5989
Standard deviation	1,8971
Standard error of the mean	0,1581

Table 5. Descriptive statistical parameters for -apgar score (first minute)

Sample size	134
Lowest value	2,0000
Highest value	10,0000
Arithmetic mean	7,8657
95% CI for the Arithmetic mean	7,6383 to 8,0931
Median	8,0000
95% CI for the median	8,0000 to 8,0000
Variance	1,7713
Standard deviation	1,3309
Standard error of the mean	0,1150

Table 6. Descriptive statistical parameters for apgar score in fifth minute

Table 7., 8. and 9. shows statistical parameters for CPAP, MV, and O2.

Sample size	108
Lowest value	1,0000
Highest value	25,0000
Arithmetic mean	3,5648
95% CI for the Arithmetic mean	2,8698 to 4,2599
Median	2,0000
95% CI for the median	2,0000 to 3,0000
Variance	13,2761
Standard deviation	3,6436
Standard error of the mean	0,3506

Table 7. Descriptive statistical parameters for cpap

Sample size	48
Lowest value	1,0000
Highest value	38,0000
Arithmetic mean	6,6667
95% CI for the Arithmetic mean	4,4893 to 8,8440
Median	4,0000
95% CI for the median	3,0000 to 5,2559
Variance	56,2270
Standard deviation	7,4985
Standard error of the mean	1,0823

Table 8. Descriptive statistical parameters for mechanical ventilation

Sample size	122
Lowest value	1,0000
Highest value	55,0000
Arithmetic mean	7,7541
95% CI for the Arithmetic mean	5,9855 to 9,5227
Median	5,0000
95% CI for the median	4,0000 to 5,0000
Variance	97,3605
Standard deviation	9,8671
Standard error of the mean	0,8933

Table 9. Descriptive statistical parameters for o2

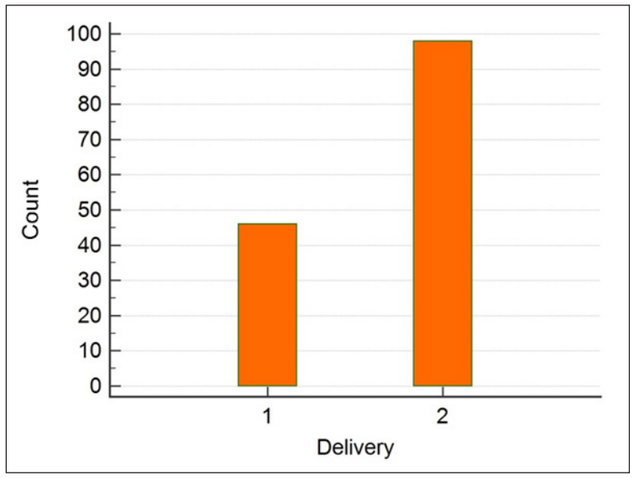


Figure 4. Absolute frequency of the type of delivery within observed group of patients (1-spontaneous; 2-cesarean section)

There is a statistically significant difference in the proportion of two different types of delivery within the observed group of patients (1= spontaneous; 2= cesarean section) (χ^2 test = 18,778

P < 0,0001) (Figure 4.), when was observed much more cases of cesarean section (68,1%).

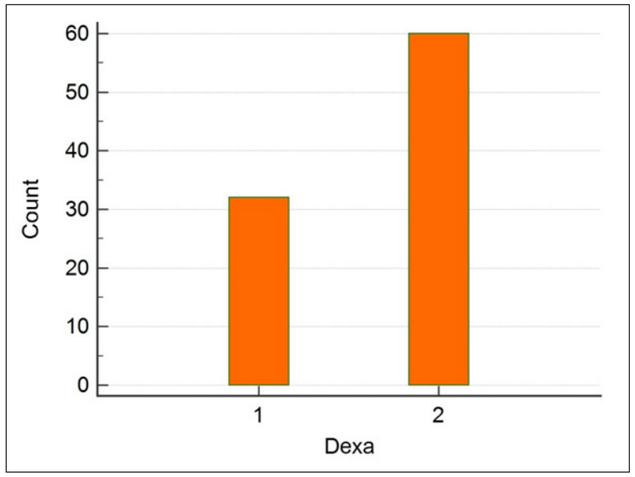


Figure 5. Absolute frequency of antenatal dexamethasone therapy within the observed group of patients (1-no;2-yes)

Also, there is a statistically significant difference in the proportion of frequency of using antenatal dexamethasone within an observed group of patients (1=NO; 2=YES) (χ^2 test = 8,522, P = 0,0035) (Figure 5.), when was observed much more cases of antenatal dexamethasone (65,2%).

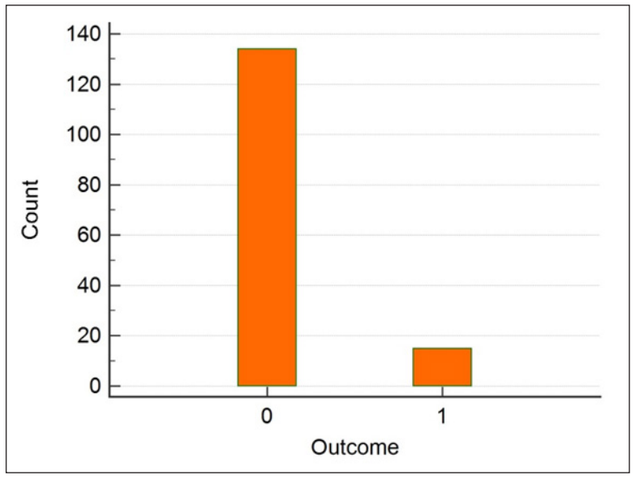


Figure 6. Absolute frequency of outcome within observed group of patients (0-live;1-death)

We have found that a much higher number of patients have survived (89,9%) (χ^2 test = 95,040, P<0,000) (Figure 6.).

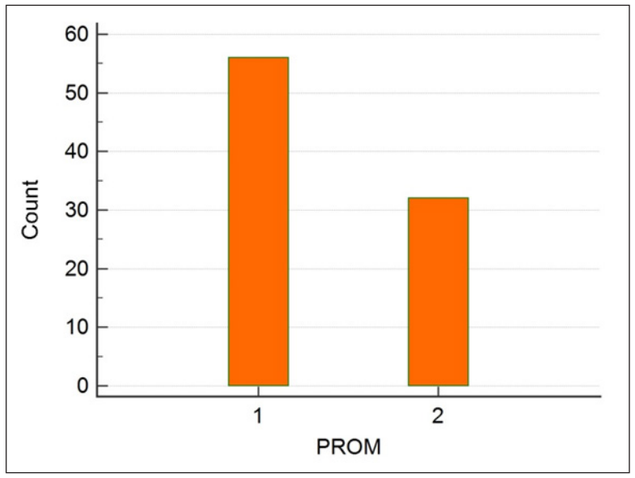


Figure 7. Absolute frequency of premature rupture of membranes (prom) within observed group of patients (1-no; 2-yes)

We had noticed a statistically significant difference in the proportion of frequency of premature rupture of membranes (PROM) within the observed group of patients (1=NO; 2=YES) (χ^2 test = 6,545, P = 0,0105) (Figure 7.) when was observed more cases without (PROM) - (63,6%).

Also, we had noticed a slightly statistically significant difference in the proportion of frequency of surfactant use within the observed group of patients (1=NO; 2=YES) (χ^2 test = 4,174, P = 0,0411) (Figure 8.), when was observed more cases without surfactant therapy (58,7%).

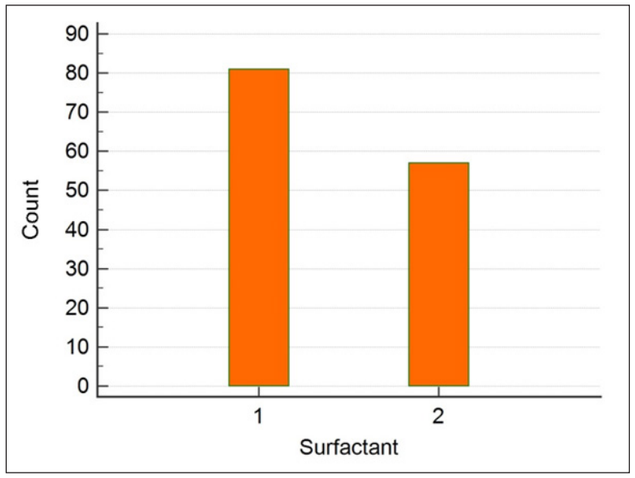


Figure 8. Absolute frequency of surfactant use within observed group of patients (1-no; 2-yes

Comparison between ultrasound and x-ray scale

When we compared Ultrasound findings in the first day and X-ray (4 grades scale), Wilcoxon paired samples test showed a statistically significant difference (large sample test statistics Z=-4,085795; P < 0,0001, Figure 13., Table 10).

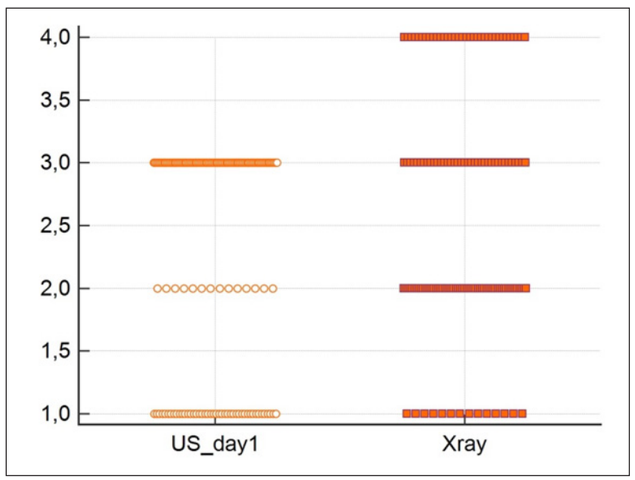


Figure 13. Comparison between ultrasound-day 1 and x-ray

Wilcoxon test (paired samples)

Sample 1	US_day1
Sample 2	Xray

	Sample 1	Sample 2
Sample size	142	142
Lowest value	1,0000	1,0000
Highest value	3,0000	4,0000
Median	3,0000	2,5000
95% CI for the median	3,0000 to 3,0000	2,0000 to 3,0000
Interquartile range	1,0000 to 3,0000	2,0000 to 3,0000

Hodges-Lehmann median difference	0,5000
95% Confidence interval	0,0000 to 0,5000

Wilcoxon test (paired samples)

Number of positive differences	71
Number of negative differences	26
Large sample test statistic Z	-4,085795
Two-tailed probability	P < 0,0001

Table 10. Comparison between ultrasound day 1 and x-ray (4-grades)

9.2. General considerations

Some variables were not within normal distribution (Table 11.), and parametric statistics could not be implemented. Therefore, values were converted to the rank type of data, and nonparametric statistics were applied.

Variable	Kolmogorov-Smirnov test for Normal distribution
CPAP	D=0,2407 reject Normality (P<0,0001)
MV	D=0,2531 reject Normality (P<0,0001)
O2	D=0,2518 reject Normality (P<0,0001)

Table 11. Variables which were not within normal distribution

Mann-Whitney test for independent samples showed no difference in RDS when we stratify groups according to a type of delivery (Mann-Whitney U= 2076,50; P = 0,9392, Figure 14).

Figure 14. Comparison of rds according to the type of delivery

The same as in the previous case, there is no difference in RDS when we stratify groups according to antenatal Dexamethasone therapy (Mann-Whitney U= 791,50; P = 0,3399, Figure 15).

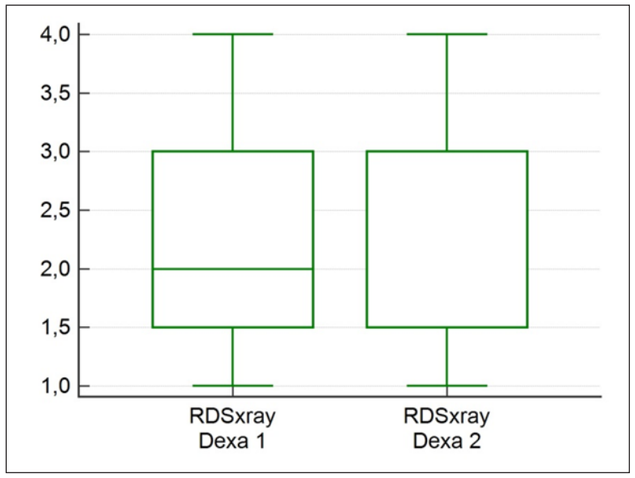


Figure 15. Comparison of rds according to antenatal dexamethasone

Logistic regression showed that gestational age contributes significantly to the prediction of the outcome (regression coefficient = -0,78357, P<0,0001). It means that a higher value of gestational age has a better prognosis of survival.

No difference in RDS noticed when we stratify groups according to PROM (Mann-Whitney U= 746,50; P = 0,4658, Figure 16).

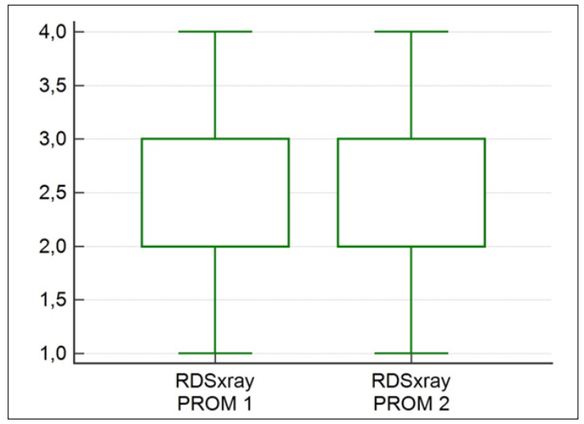


Figure 16. Comparison of rds according to presence of premature rupture of membranes

We have found a weak, but statistically significant correlation between CPAP and RDS (Spearman's rho=0,263, P=0,0075, 95% CI for rho=0,0723 to 0,435). Similar result is between Oxygen therapy (O2) and RDS (Spearman's rho=0,312, P=0,0006, 95% CI for rho=0,138 to 0,467). On the other hand, we did not have the same result for correlation analyses between mechanical ventilation (MV) and RDS (Spearman's rho=0,215, P=0,1476, 95% CI for rho=-0,0774 to 0,473).

Logistic regression showed that RDS contributes significantly to the prediction of the outcome (regression coefficient = 0,79783, P=0,0167). It means that a higher value of RDS has a worse prognosis of survival.

9.3. X-RAY GROUPS/ STAGES of RDS

As we can see from Figure 12., most patients had grade 1 when we have analyzed different grades of the X-ray method.

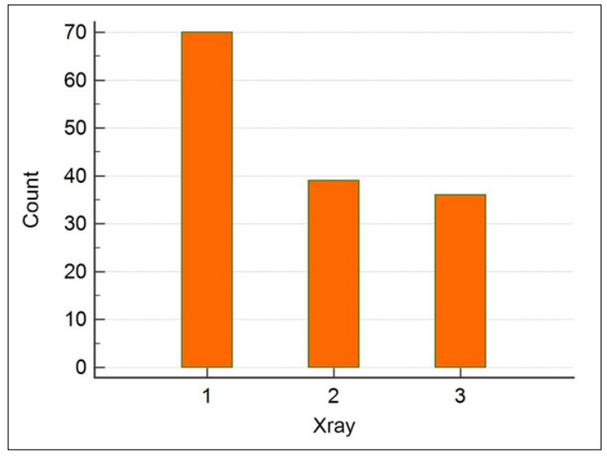


Figure 12. Absolute frequency of patients with different grades on x-ray

Clinical parameters and applied therapy were compared to grades of RDS.

No statistical significant difference found in CPAP regarding RDS grade 1 and 2 (Mann-Whitney U= 299,50; P = 0,1193, Figure 17).

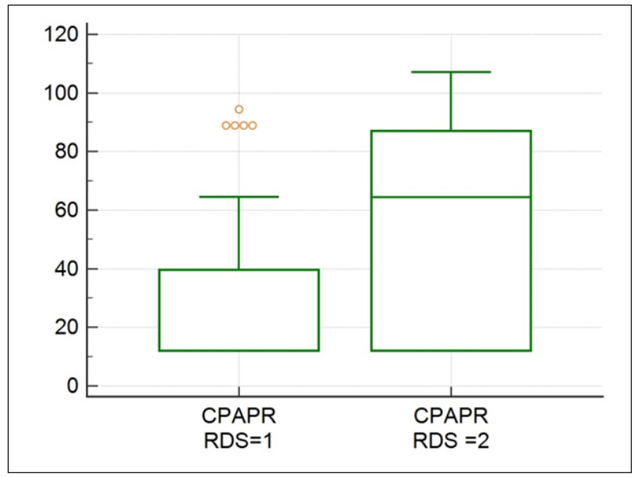


Figure 17. Comparison of cpap between rds grades 1 and 2

Also, no statistical significant difference found in CPAP between RDS stage 3 and 4 (Mann-Whitney U= 115,50; P = 0,5151, Figure 18).

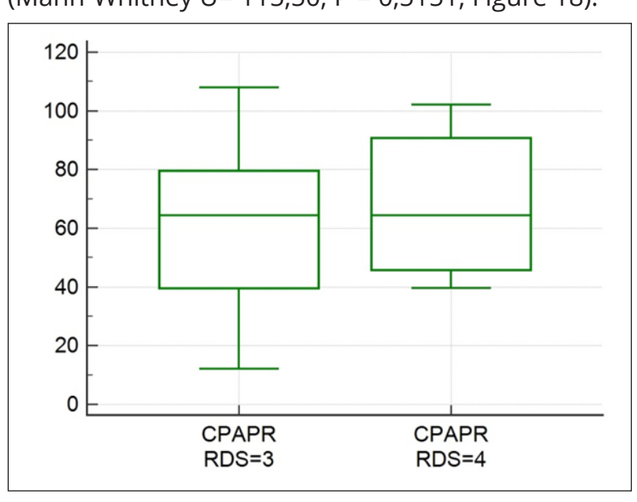


Figure 18. Comparison of cpap between rds stage 3 and 4

There is no statistically significant relationship between RDS (grades 1 and 2) and type of delivery (Fisher's exact test P = 0,605998664) (Figure 19).

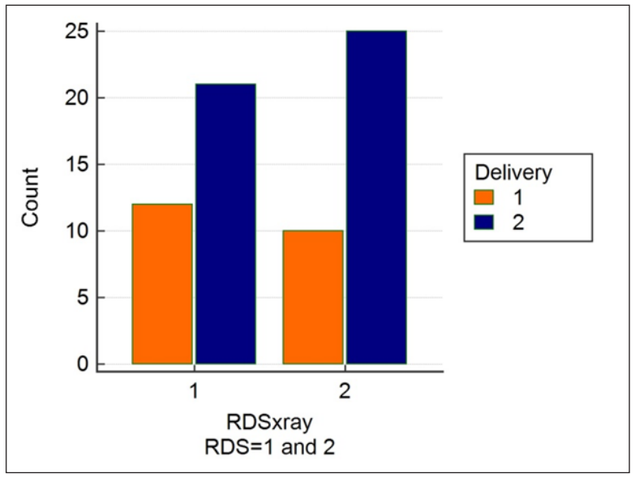


Figure 19. Absolute frequency of delivery type (1-spontaneous;2-cesarean section) within the observed rds grades 1 and 2

Also, there is no statistically significant relationship between RDS (3 and 4) and type of delivery (Fisher's exact test P = 0,525475915) (Figure 20).

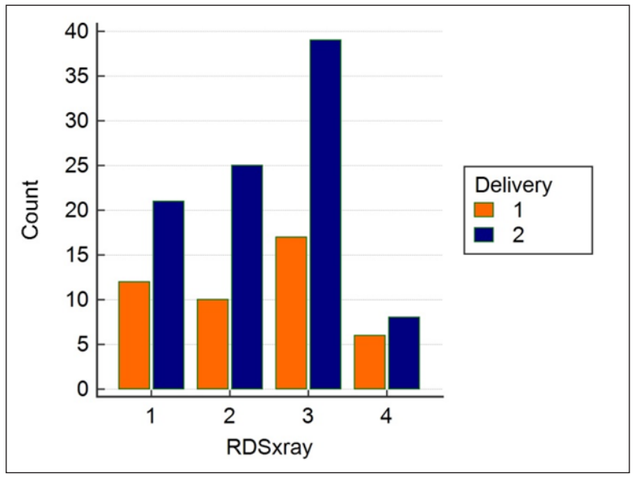


Figure 20. Absolute frequency of delivery type within observed rds grades 3 and 4 (1-spontaneous;2-cesarean section)

There is no statistically significant relationship between RDS (grades 1 and 2) and DEXA (Fisher's exact test P = 0,549897072) (Figure 21).

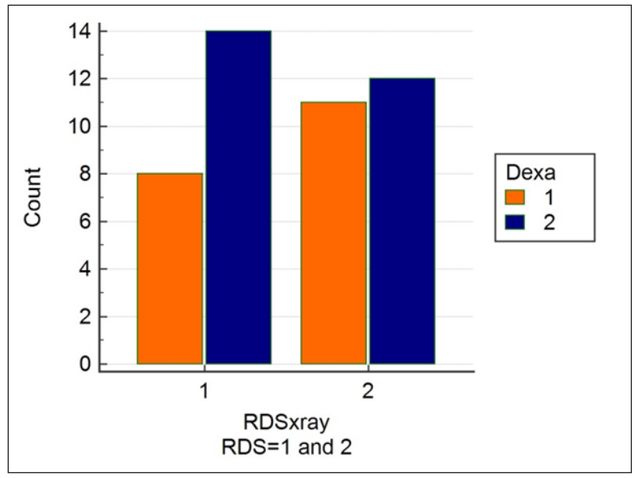


Figure 21. Absolute frequency of antenatal dexamethason within rds grades 1 and 2

There is no statistically significant relationship between RDS grades (3 and 4) and antenatal Dexamethasone therapy (Fisher's exact test P = 0,649371586) (Figure 22).

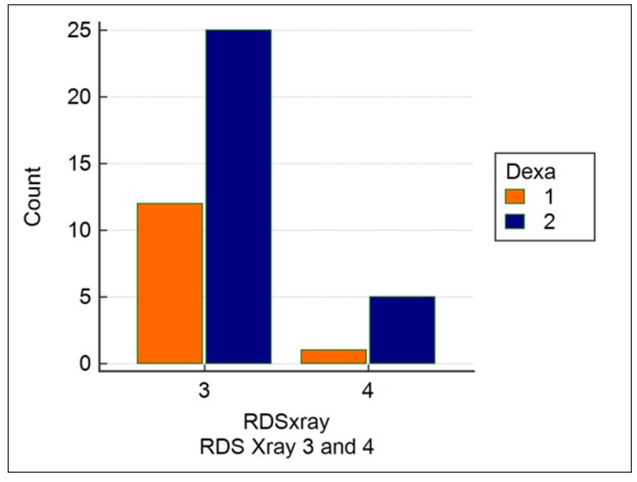


Figure 22. Absolute frequency of antenatal dexamethasone within rds grade 3 and 4 (1-no;2-yes)

We have not noticed statistical significant difference in gestational age between RDS grade 1 and 2 (t=-0,796, P = 0,4287, 95% CI of difference = -1,3507 to 0,5801). Arithmetic mean for grade 1 was 31,6286 (SD=1,8643; SE=0,3151) and for grade 2 31,2432 (SD=2,2162; SE=0,3643) (Figure 23).

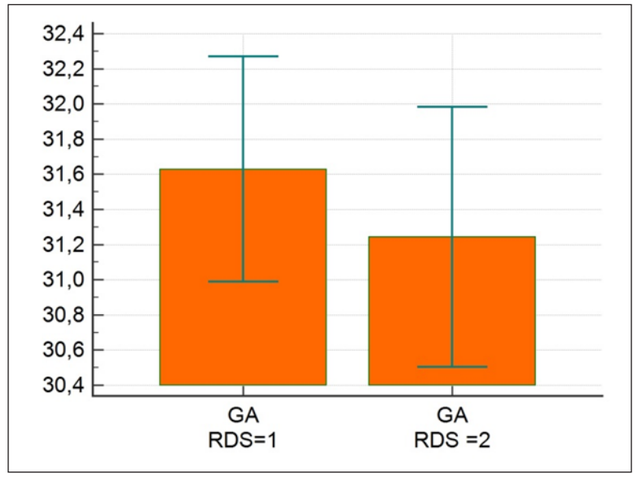


Figure 23. Comparison of gestational age for rds grade 1 and 2

On contrary to grades 1 and 2 , we have noticed statistical significant difference in gestational age between RDS stage 3 and 4 (t=-2,701, P = 0,0087, 95% CI of difference = -3,6319 to -0,5460) where arithmetic mean for stage 3 was 31,0175 (SD=2,4676; SE=0,3268) and for stage 4 28,9286 (SD=3,0751; SE=0,8218) (Figure 24).

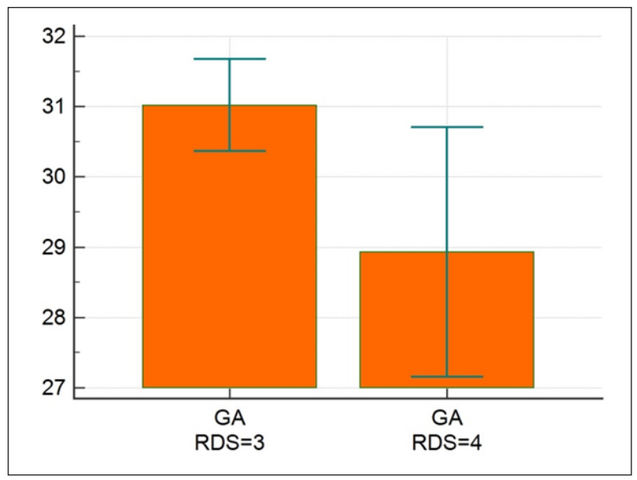


Figure 24. Comparison of gestational age between rds grade 3 and 4

No difference was detected in mechanical ventilation according to RDS grade 1 and 2 (Mann-Whitney U= 14,50; P = 0,6249, Figure 25) and no difference was detected in mechanical ventilation according to RDS stage 3 and 4 (Mann-Whitney U= 121,50; P = 0,7082, Figure 26).

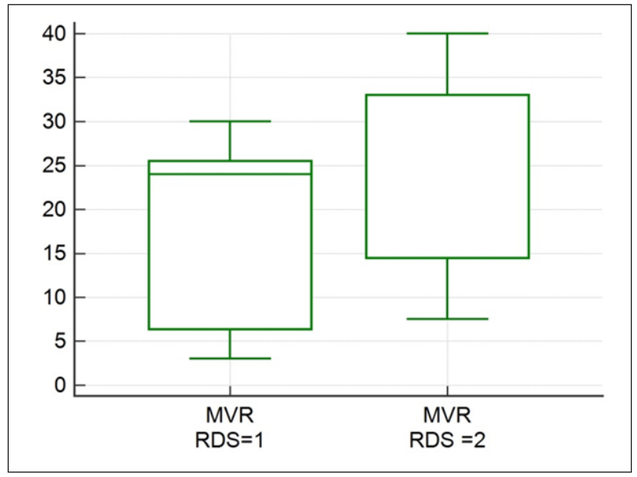


Figure 25. Comparison of mechanical ventilation between rds grade 1 and 2

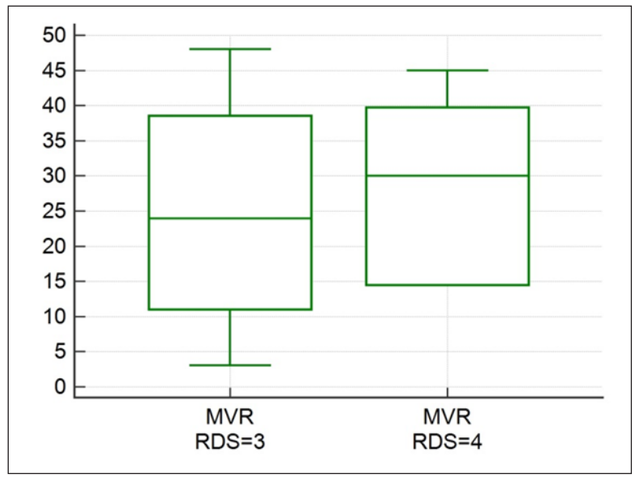


Figure 26. Comparison of mechanical ventilation between rds grade 3 and 4

We have found no statistically significant difference in Oxygen therapy (O2) regarding RDS stage 1 and 2 (Mann-Whitney U= 358,00; P = 0,4925, Figure 27).

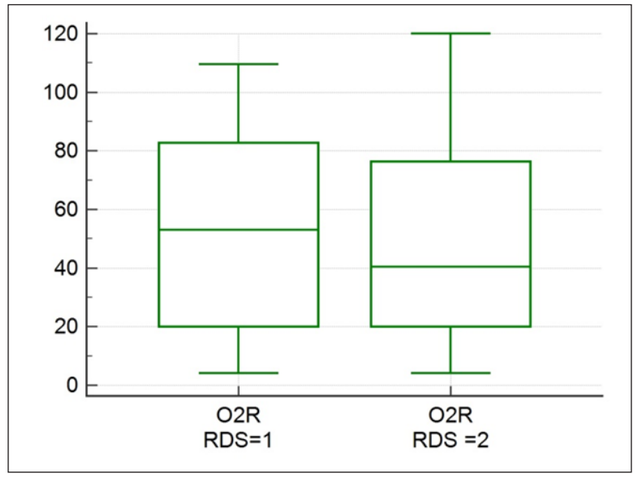


Figure 27. Comparison of o2 therapy between rds grade 1 and 2

We have found a statistical significant difference in Oxygen therapy (O2) according to RDS stage 3 and 4 (Mann-Whitney U= 117,00; P = 0,0035, Figure 28) in means of higher value in RDS stage 4.

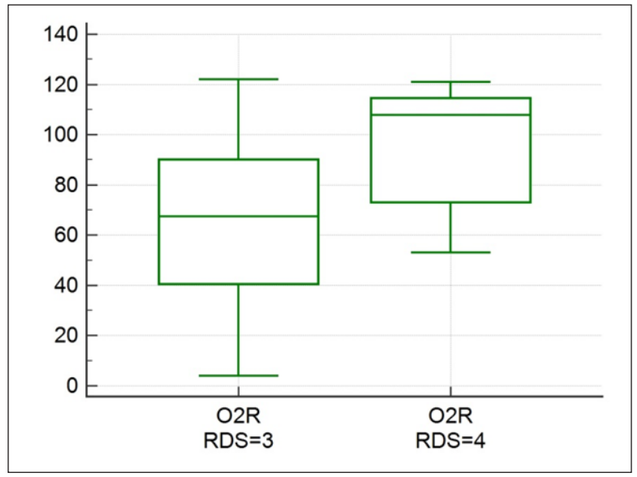


Figure 28. Comparison of o2 therapy between rds grade 3 and 4

There is no statistically significant relationship between RDS (grades 1 and 2) and OUTCOME (Fisher's exact test P > 0,99) (Figure 29).

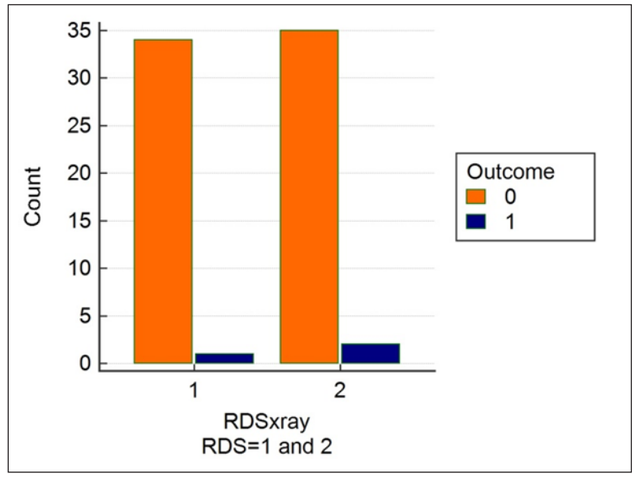


Figure 29. Absolute frequency of outcome (0-live;1-death) within rds grade 1 and 2

Logistic regression showed that RDS grades 1 and 2 do not contribute significantly to the prediction of the outcome (regression coefficient = 0,66416, P=0,5947).

There is no statistically significant relationship between RDS grades 3 and 4 and outcome (Fisher's exact test P = 0,693061348) (Figure 30).

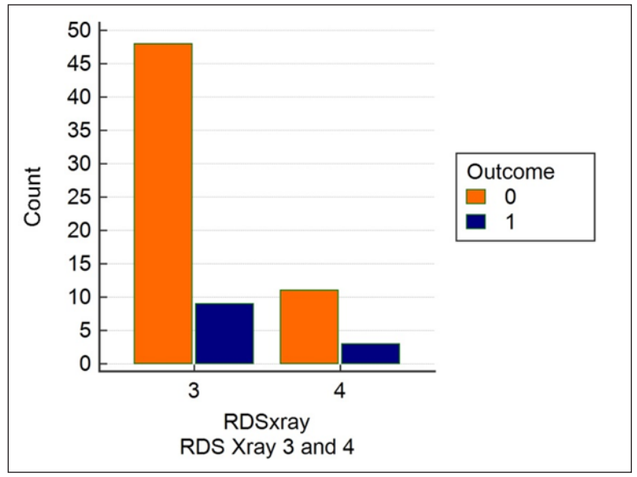


Figure 30. Absolute frequency of outcome between rds grade 3 and 4 (0-live;1-death)

Logistic regression showed that different RDS stages 3 and 4 do not contribute significantly to the prediction of the outcome (regression coefficient = 0,37469, 0,6154).

There is no statistically significant relationship between RDS (grades 1 and 2) and premature rupture of membranes-PROM (Fisher's exact test P > 0,99) (Figure 31).

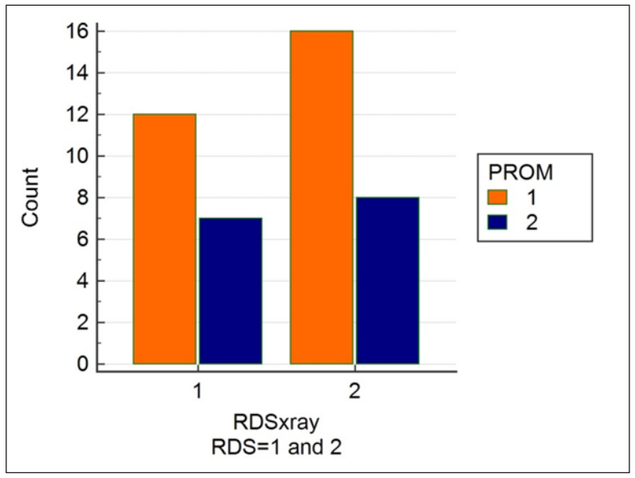


Figure 31. Absolute frequency of prom (1-no;2-yes) within rds grade 1 and 2

There is no statistically significant relationship between RDS 3 and 4 and premature rupture of membranes -PROM (Fisher's exact test P = 0,089082170) (Figure 32).

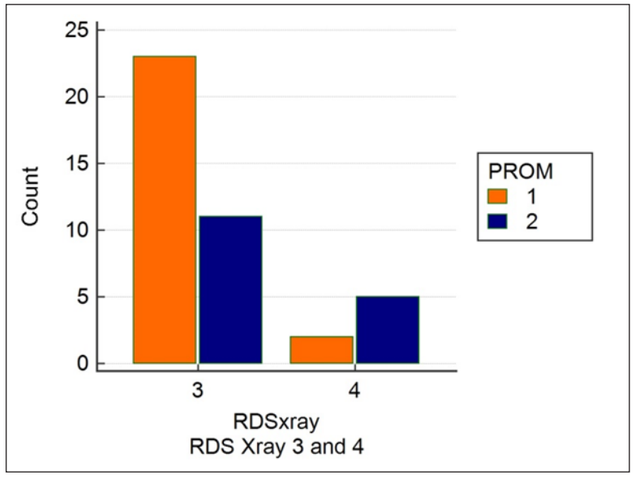


Figure 32. Absolute frequency of prom within rds grade 3 and 4 (1-no;2-yes)

There is no statistically significant relationship between RDS grade 1 and 2 and surfactant therapy (Fisher's exact test P =0,202191253) (Figure 33).

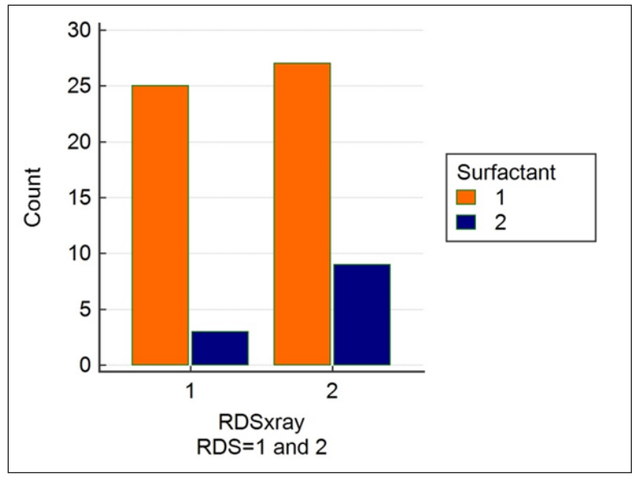


Figure 33. Absolute frequency of surfactant therapy within rds grade 1 and 2 (1-no;2-yes)

There is no statistically significant relationship between RDS grades 3 and 4 and surfactant (Fisher's exact test P = 0,192479720) (Figure 34).

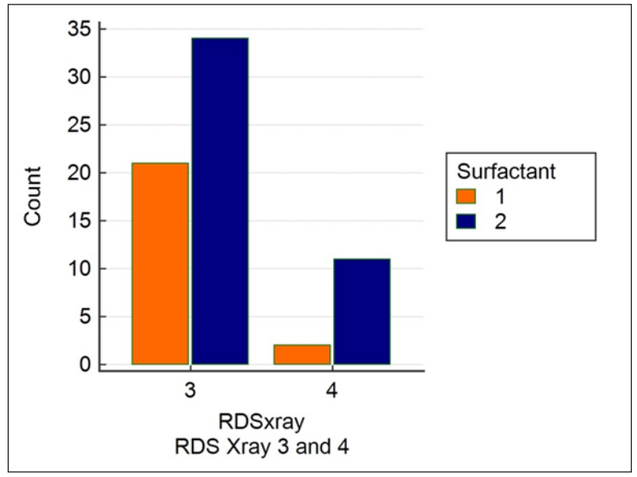


Figure 34. Absolute frequency of surfactant therapy within rds grade 3 and 4 (1-no;2-yes)

It has not been detected that birth weight is statistically significant higher (t=-0,292, P = 0,7715, 95% CI of difference=-245,4223 to 182,82, Figure 35) in RDS grade 1 (arithmetic mean=1722,5714; SD=440,2278; SE=74,4121) in comparison with grade 2 (arithmetic mean =1691,2703; SD=469,1105; SE=77,1213) and vice versa.

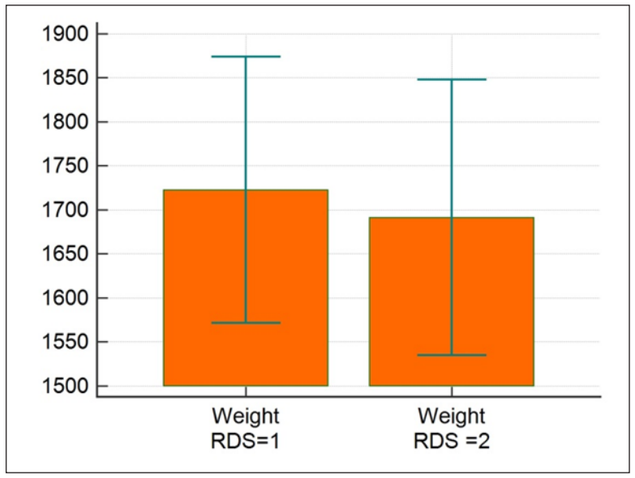


Figure 35. Comparison of birth weight between rds grade 1 and 2

It has been detected that birth weight is statistical significant higher in RDS stage 3 (arithmetic mean=1697,4561; SD=555,5331; SE=73,5822) in comparison with stage 4 (arithmetic mean =1251,4286; SD=558,2784; 149,2062) (t=-2,689, P = 0,0090, 95% CI of difference=-776,9100 to -115,1452, Figure 36).

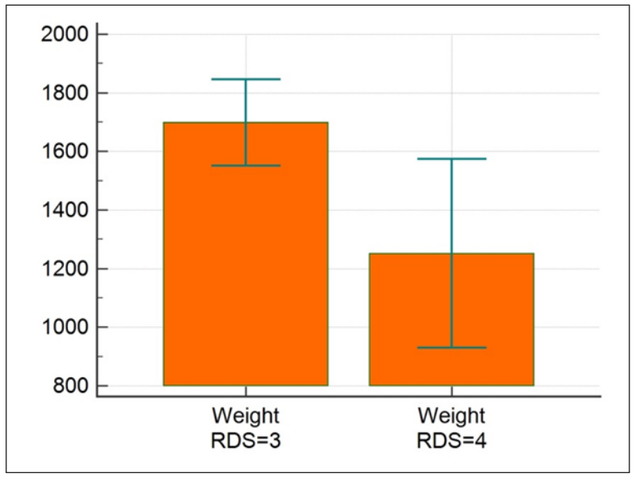


Figure 36. Comparison of birth weight between rds stage 3 and 4

9.4. Ultrasound grades (US)

When we analyzed the Ultrasound findings on the first day as a diagnostic method, the highest number of patients have had grade 2 (Figure 9.).

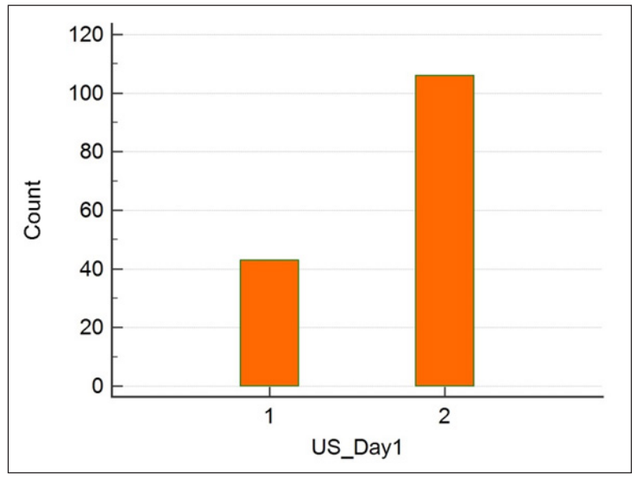


Figure 9. Absolute frequency of patients with different grades-ultrasound day 1

Grade 2 has had the most patients in ultrasound findings on the second day (Figure 10).

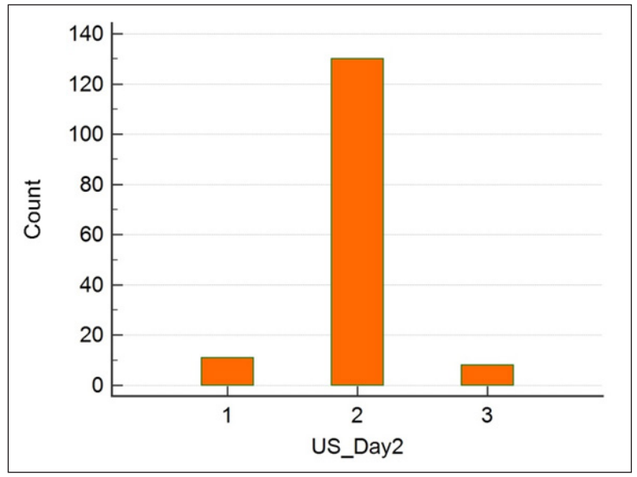


Figure 10. Absolute frequency of patients with different grades -ultrasound day 2

Most of the patients had had grade 2 when we analyzed the US third day (Figure 11.).

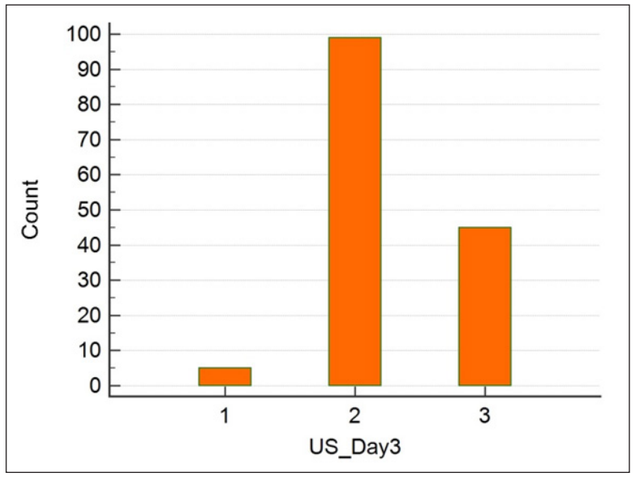


Figure 11. Absolute frequency of patients with different grades - ultrasound day 3

9.4.1. Ultrasound subgroups

Based on the presence of subpleural consolidations, further differentiation of ultrasound profiles were made into subgroup 1 and subgroup 2, where subgroup 1 represent findings with existing consolidation.

There is statistical significant difference in gestational age between Ultrasound day 1-subgroup 1 and subgroup 2 (t=2,876, P = 0,0064, 95% CI of difference = 0,8507 to 4,8743) where arithmetic mean for Ultrasound subgroup 1 was 27,7000 (SD=3,0203; SE=0,9551) and for Ultrasound subgroup 2 was 30,5625 (SD=2,6632; SE=0,4708) (Figure 37).

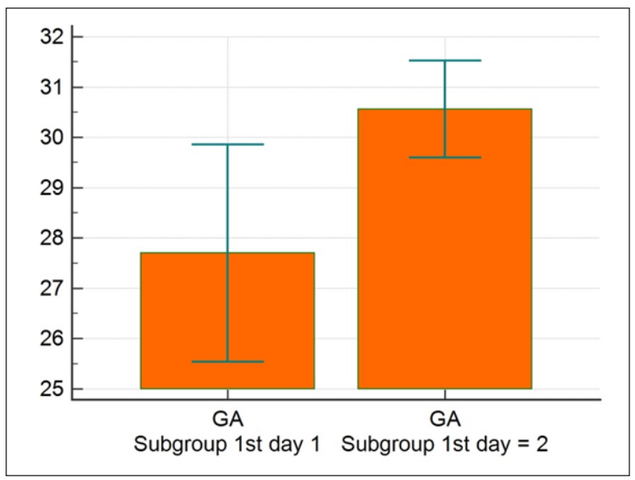


Figure 37. Comparison of gestational age within ultrasound subgroup 1 and 2

There is statistical significant relationship between Ultrasound day 1- subgroup 1 and outcome (Fisher's exact test P = 0,040039739) (Figure 38).

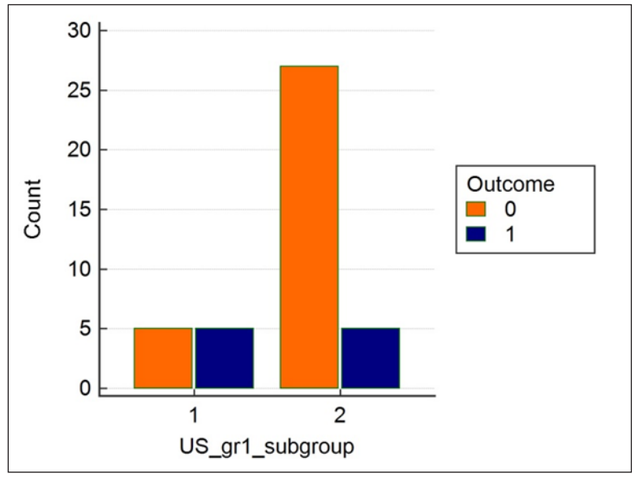


Figure 38. Absolute frequency within ultrasound subgroup 1 outcome (0-live;1-dead)

We have found statistical significant difference in weight between Ultrasound day 1 subgroup-1 and 2 (t=2,051, P = 0,0468, 95% CI of difference = 6,1258 to 823,8742) where arithmetic mean for subgroup-1 was 1101,0000 (SD=556,2114; SE=175,8895) and for subgroup-2 was 1516,0000 (SD=559,0540; SE=98,8277) (Figure 39).

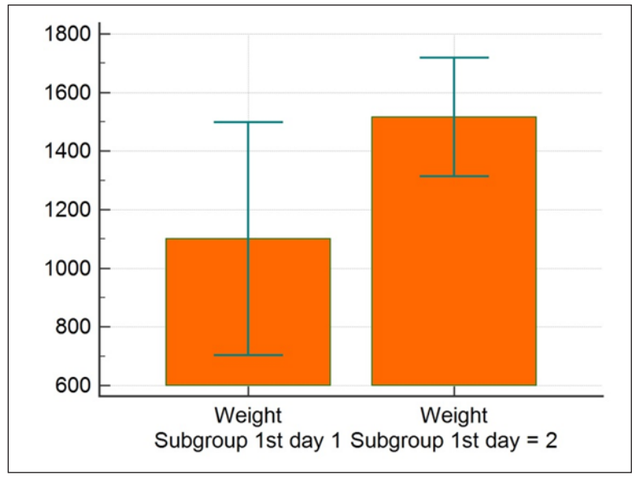


Figure 39. Comparison of birth weight within ultrasound day 1 subgroups-1 and 2

We did not find any statistical significant difference in gestational age between Ultrasound day 2 subgroup-1 and subgroup-2 (t=1,609, P = 0,1106, 95% CI of difference = -0,1608 to 1,5455) where arithmetic mean for subgroup-1 was 31,0000 (SD=2,4191; SE=0,4572) and for subgroup-2 was 31,6923 (SD=1,7604; SE=0,1993) (Figure 40).

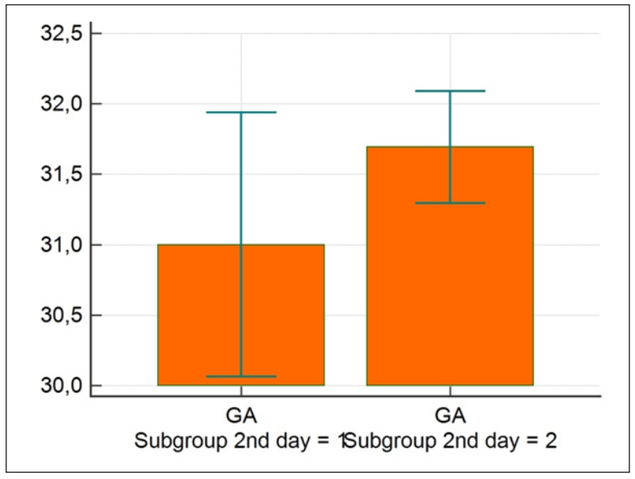


Figure 40. Comparison of gestational age within ultrasound day 2 subgroup-1 and 2

There is no statistical significant relationship between Ultrasound profile 2 subgroups and outcome (Fisher's exact test P = 0,113805588) (Figure 41).

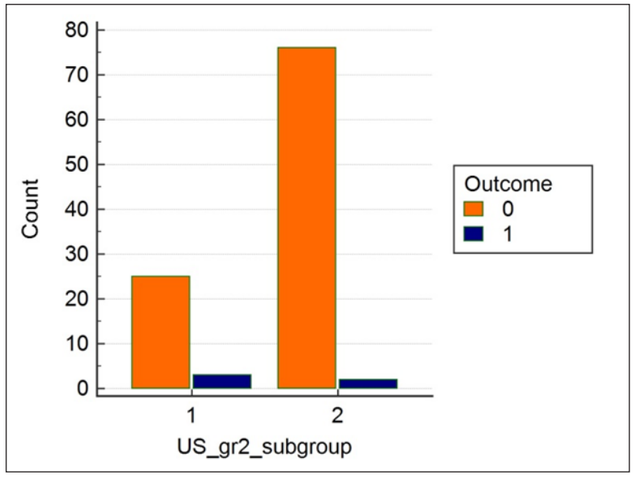


Figure 41. Absolute frequency within ultrasound day 2 subgroups and outcome (0-live;1-dead)

There is no statistical significant difference in birth weight between Ultrasound day 2 subgroup-1 and subgroup-2 (t=0,438, P = 0,6625, 95% CI of difference = -154,1280 to 241,4357) where arithmetic mean for subgroup-1 was 1725,0000 (SD=509,5877; SE=96,3030) and for subgroup-2 1768,6538 (SD=431,0051; SE=48,8017) (Figure 42).

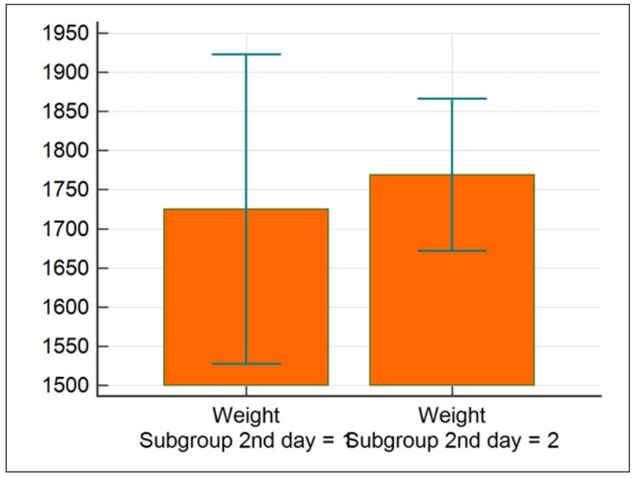


Figure 42. Comparison of weight within ultrasound day 2 subgroups

There is no significant difference in CPAP according to Ultrasound day 1 subgroup-1 and subgroup-2 (Mann-Whitney U= 48,50; P = 0,3949, Figure 43).

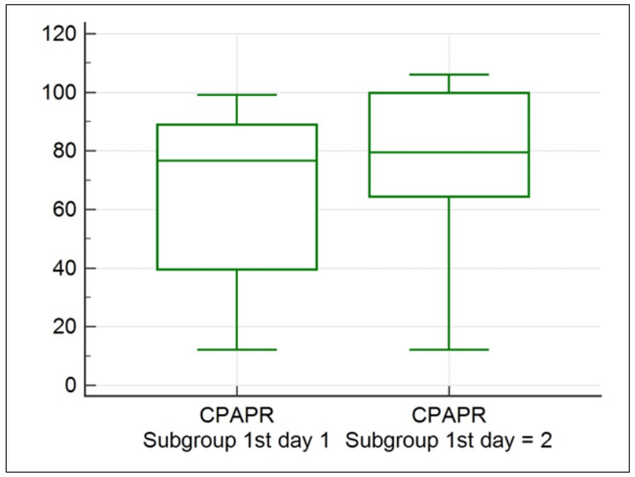


Figure 43. Comparison of cpap within ultrasound day 1 subgroups

Also, there is no difference in mechanical ventilation (MV) either between Ultrasound day 1 subgroup-1 and subgroup-2 (Mann-Whitney U= 33,50; P = 0,1339, Figure 44).

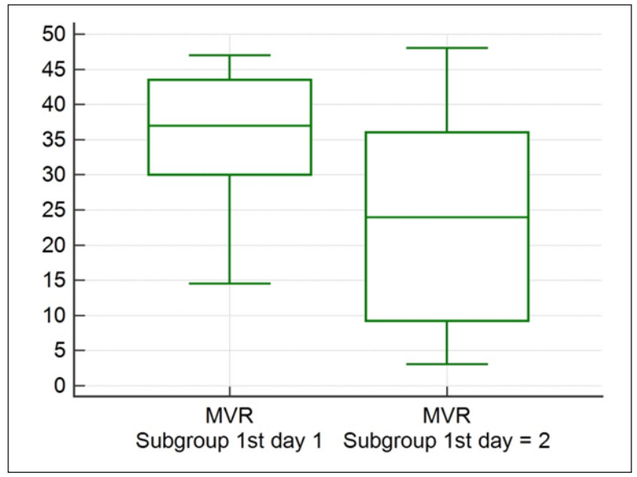


Figure 44. Comparison between ultrasound day 1 subgroups and mechanical ventilation

Mann-Whitney test for independent samples showed no difference in Oxygen therapy (O2) between Ultrasound day 1 subgroup-1 and subgroup-2 (Mann-Whitney U= 71,50; P = 0,5686, Figure 45).

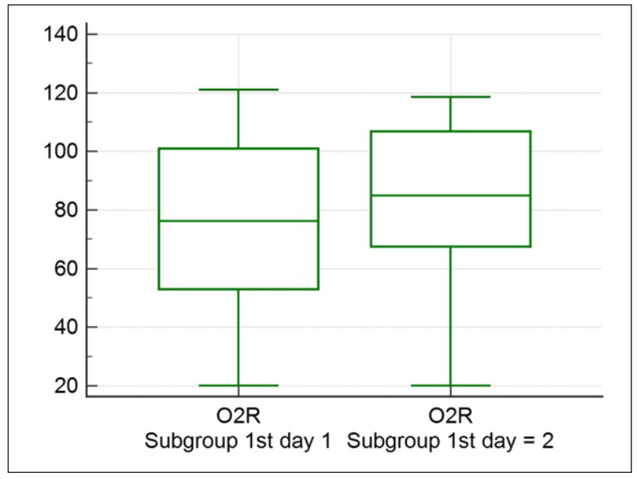


Figure 45. Comparison of O2 within ultrasound day 1 subgroups

There is no significant difference in CPAP between Ultrasound day 2 subgroup-1 and subgroup-2 (Mann-Whitney U= 485,00; P = 0,4805, Figure 46).

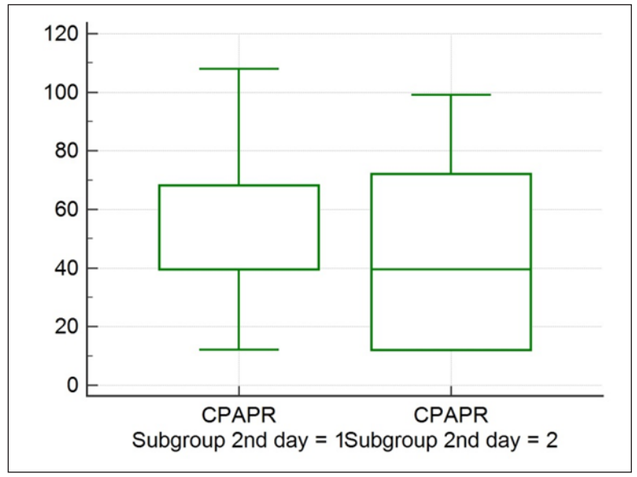


Figure 46. Comparison of cpap within ultrasound day 2 subgroups

We did not find difference in mechanical ventilation (MV) either between Ultrasound day 2 subgroup-1 and subgroup-2 (Mann-Whitney U= 54,00; P = 0,4527, Figure 47).

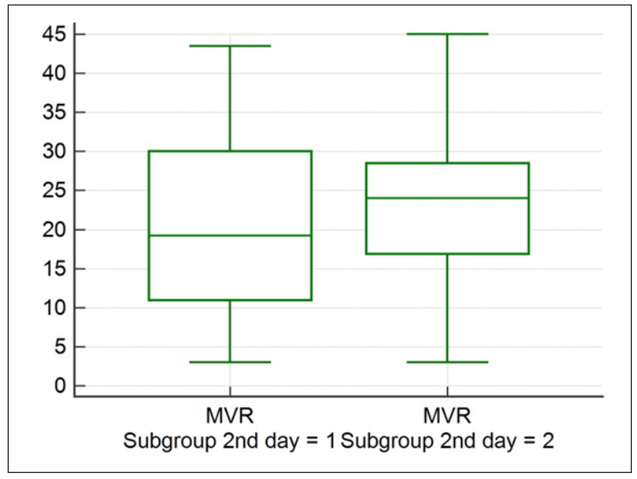


Figure 47. Comparison of mv between ultrasound day 2 subgroups

Statistical significant difference has been detected in Oxygen therapy (O2) between Ultrasound day 2 subgroup-1 and subgroup-2 (Mann-Whitney U= 497,00; P = 0,0493, Figure 48). Higher value was recorded in subgroup-1.

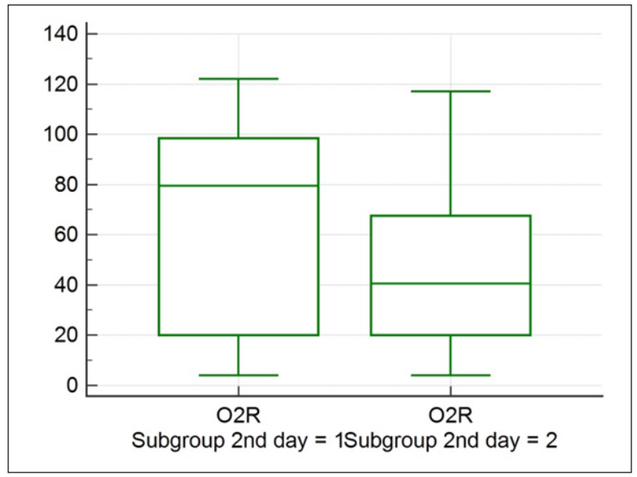


Figure 48. Comparison of o2 within ultrasound day 2 subroups

After the implementation of the Wilcoxon paired samples test, no statistically significant difference in grades has been found between Ultrasound Day 1 and X-ray (large sample test statistics Z=-0,786959; P = 0,4313, Figure 49). For additional confirmation, Monte Carlo significance value test was applied, which confirmed the results of the previous analysis (n permutations=99999, P = 0,36356).

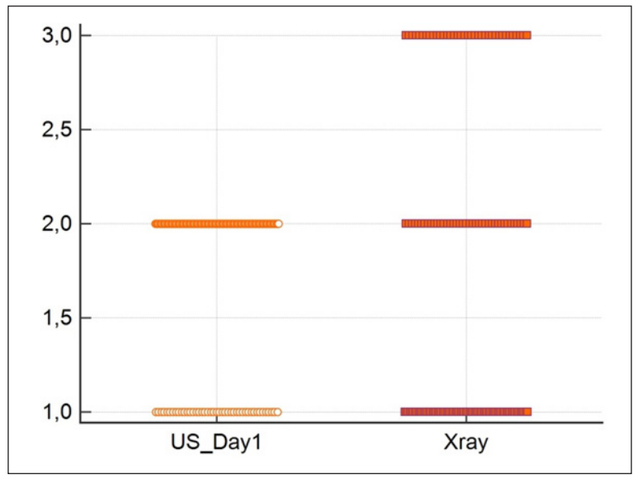


Figure 49. Comparison of grades between ultrasound day 1 and x-ray

When we compare Ultrasound Day 1 and Ultrasound Day 2 this result shows a statistically significant difference (large sample test statistics Z=-5,442449; P < 0,0001, Figure 50) in way that 39 patients showed positive difference (Ultrasound Day 2 in compared to Ultrasound Day 1) since none showed negative. The Monte Carlo significance value test has confirmed the results of the previous analyze (n permutations=99999, P = 0,00001).

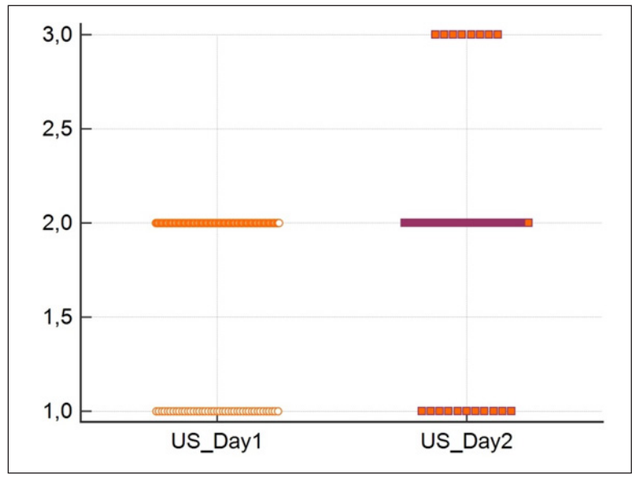


Figure 50. Comparison of grades between ultrasound day 1 and day 2

The same test showed a statistically significant difference (large sample test statistics Z=-5,346671; P< 0,0001, Figure 51) between Ultrasound Day 2 and Ultrasound Day 3 in way that 46 patients showed the positive difference (Ultrasound Day 3 in compared to Ultrasound Day 2) since three showed negative. The Monte Carlo significance value test has confirmed the results of the previous analysis (n permutations=99999, P = 0,00001).

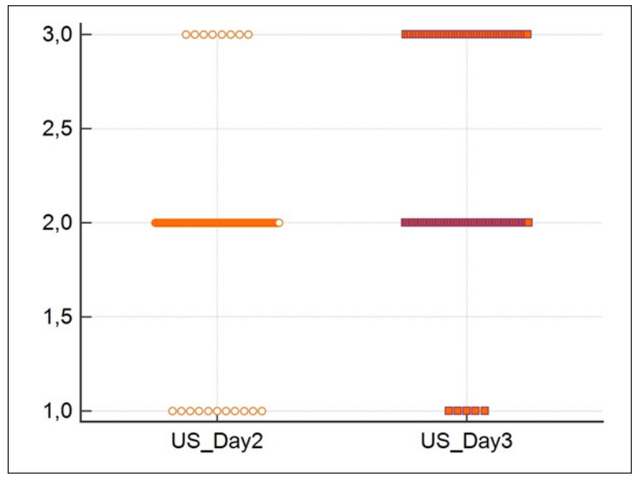


Figure 51. Comparison of grades between ultrasound day 2 and day 3

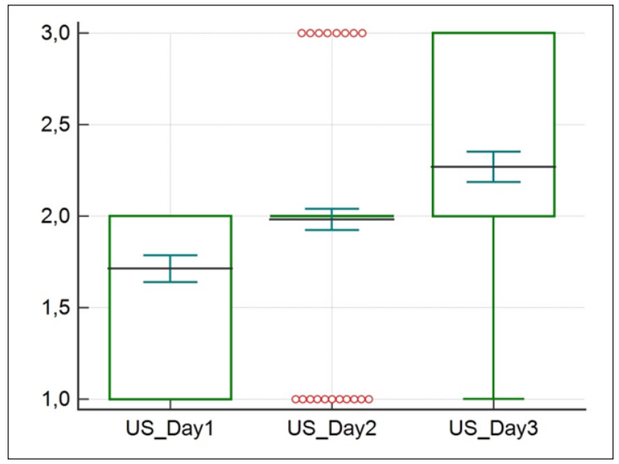


Figure 52. Comparison of grades between ultrasound day 1, 2 and 3

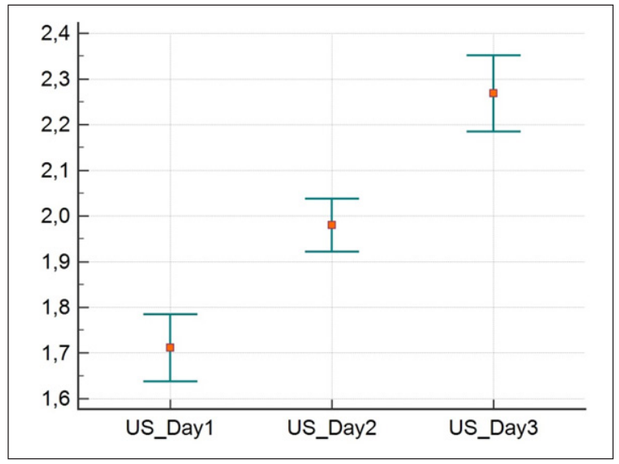


Figure 53. Comparison of grades between ultrasound day 1, 2 and 3

Logistic regression showed that the APGAR score in 1st minute contributes significantly to the prediction of the outcome (regression coefficient =-0,48674, P=0,0002). It means that a lower value of APGAR score in 1st minute gives a worse prognosis of survival. The same results were detected for female group (regression coefficient =-0,48246, P=0,0073), as well as for group of males (regression coefficient =-0,49253, P=0,0077). In case of APGAR score in 5th minute, these contributes are even stronger (for all patient - regression coefficient =-0,96613, P=0,0003; group of females - regression coefficient = -0,82770, P=0,0153; group of males - regression coefficient =-1,13 P=0,0082).

Gestational age (GA) has a significant role in the outcome in the sense that lower values give worse prognosis for survival. This was observed in the total sample (regression coefficient =-0,78357, P<0,0001), but also within group of women (regression coefficient =-0,61681, P=0,0031) and men (regression coefficient =-1,19, P=0,0031). Similar situation, but not with the same intensity, we found for weight parameter (for all patient - regression coefficient =-0,0066890, P<0,0001; group of females - regression coefficient = -0,0070373, P=0,0016; group of males - regression coefficient =-0,0063574, P=0,0029).

There is no statistically significant relationship between the proportion of females and males in total samples and the outcome (χ^2 =0,089, P = 0,7652). Neither female nor male has more frequency of survival.

The proportion of surviving patients (89,9%) is statistically significantly higher than patients who did not survive (10,1%)(χ^2 =95,040, P = < 0,0001).

An ROC analysis was performed where the criterion was the gestational age (GA) value, with the aim to estimated whether the GA value has a differentiation character in terms of outcome. ROC analysis showed statistically significant differentiation reliability (AUC = 0,886, SE = 0,0545, 95% CI 0,824 to 0,932, P <0,0001). A cut off value of ≤28 was observed for maximum sensitivity 80,00 and specificity 89,55 (Figure 54). This result indicates that if gestational age is equal to or less than 28 weeks, the negative outcome of a newborn in terms of survival can be predicted with great specificity and sensitivity.

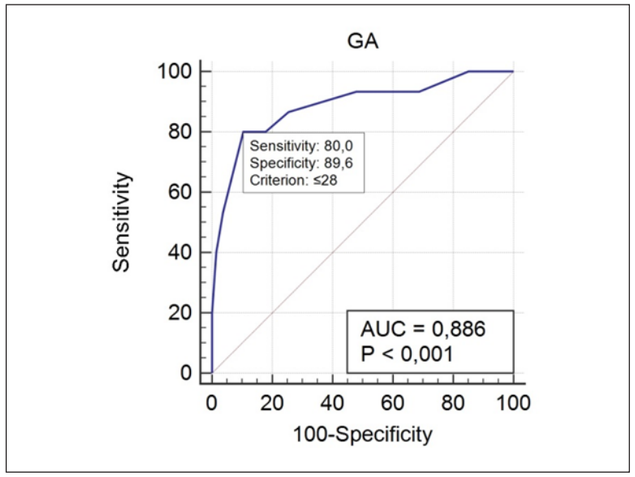


Figure 54. Receiver operating characteristic (ROC) curve for gestational age

In a case of weight, also statistically significant differentiation reliability was noticed (AUC = 0,961, SE = 0,0149, 95% CI 0,916 to 0,986, P<0,0001). A cut off value of ≤1210 was observed for maximum sensitivity 100 and specificity 90,30 (Figure 55). This result indicates that if weight is equal to or less than 1210 g, the negative outcome of a newborn in terms of survival can be predicted with great specificity and sensitivity.

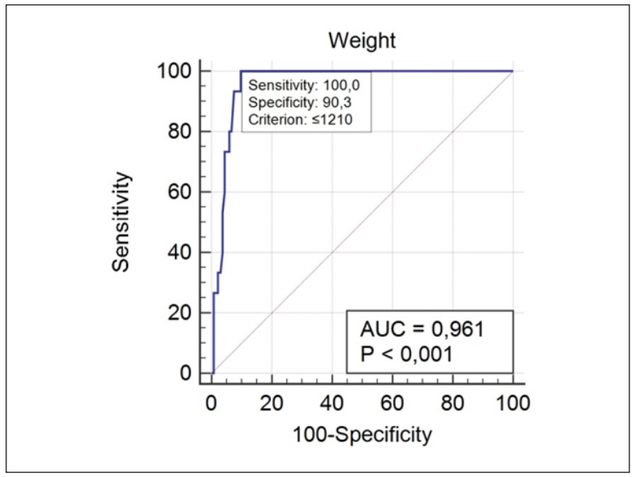


Figure 55. Receiver operating characteristic (ROC) curve for weight

Inter-rater agreement statistic (weighted Kappa) test showed moderate strength of agreement between x-ray and Ultrasound Day 1 (weighted Kappa = 0,41, SE = 0,045, 95% CI 0,315 to 0,490; Figure 62). Result of Spearman's coefficient of rank correlation has confirmed such relationship (rho=0,481; P<0,0001).

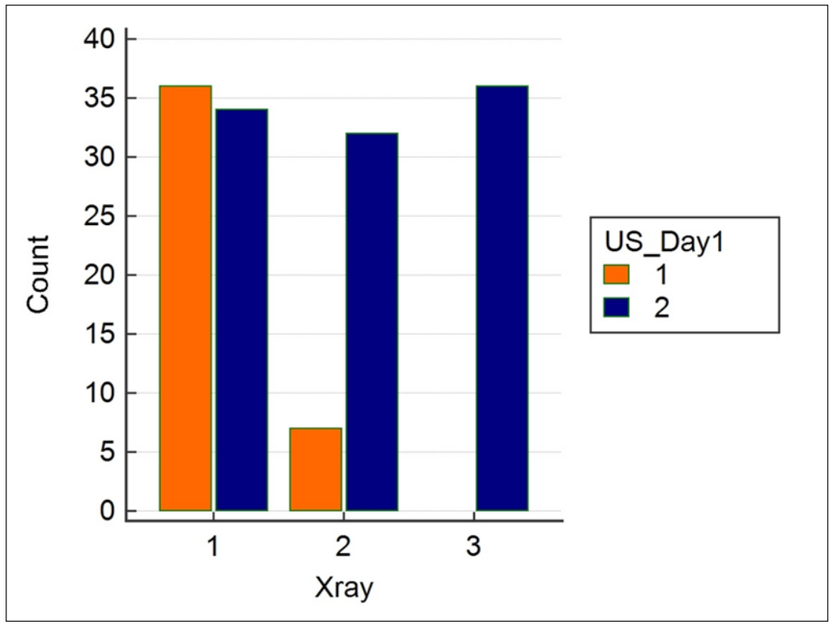


Figure 62. Inter-rater agreement test between x-ray and ultrasound day 1

9.5. Twins subgroup

Statistical analysis were made between clinical parameters such as Apgar score, birth weight, gestational age and outcome within pairs of twins.

There is no statistically significant difference between pairs of twins when we have observed APGAR score in 1st minute (P = 0,3750), as well as APGAR score in 5th minute (P = 0,1953). Correlation test showed clear relationship between these parameters within group of twins (for APGAR-1, rho=0,645, P=0,0016, 95% CI for rho 0,295 to 0,842; for APGAR-5, rho=0.722, P=0,0005, 95% CI for rho 0,399 to 0,886).

Also, there is no difference in weight between pairs of twins (Paired samples t-test = 1,379, P = 0,1831, 95% CI of difference -43,3119 to 212,3596).

There is clear strong correlation between twins when we observe their mutual weight (r=0,8581, P<0,0001, 95% CI for r 0,6773 to 0,9412) (Figure 54).

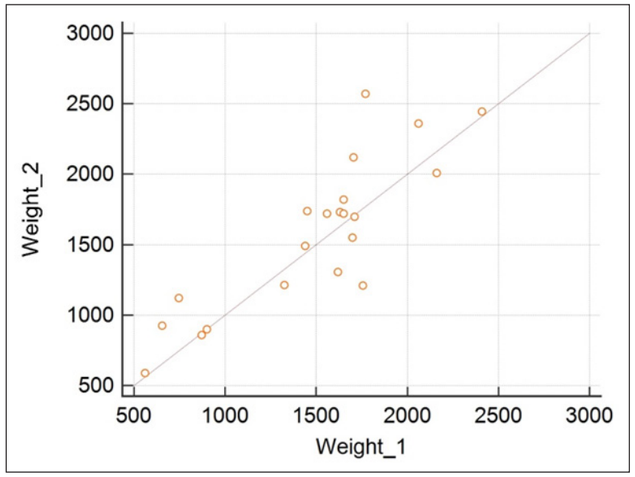


Figure 56. Scatter diagram of weight correlation between pairs of twins

It is important to emphasize that there is no statistically significant difference between the pair of twins when we observe RDS (Small rank test P = 0,2402). We did not find and the relationship between first-born and second-born twins and survival outcome (Fisher's exact test P>0.9).

It is quite clear that there is no correlation between the observed clinical parameters and the fact that the patient is first or second-born twins due to non-significant results of Apgar score, weight, and outcome.

9.6. Multivariate analysis

When we include RDS grade, Apgar1, Apgar5, GA and weight all together, non-parametric MANOVA test show statistical significant difference regarding outcome (permutation N = 9999, total sum of squares= 4,184E07, within-group sum of squares= 3,185E07, F= 44,23, P= 0,0001).

When we have observed groups as RDS grades, after Apgar-1, Apgar-5, gestational age, and weight parameters were included, non-parametric MANOVA test show statistical significant difference in RDS grades (permutation N= 9999, total sum of squares = 4,333E07, within-group sum of squares= 4,025E07, F= 3,539, P= 0,0187). Table 12. shows differences among pairs of groups (RDS grades).

RDS	Grade 1	Grade 2	Grade 3	Grade 4
Grade 1				
Grade 2	0,7708			
Grade 3	0,9931	0,6586		
Grade 4	0,0019*	0,0054*	0,0108*	

Table 12. Results of non-parametric manova test (p value) among pairs of groups (permutation N= 9999, total sum of squares= 4,333E07, within-group sum of squares= 4,025E07, F= 3,539, P= 0,0187)

9.7. Complications

In a total cohort of 150 patients, 19 patients had some complications during the treatment and hospital stay. Table 13. shows the total number and types of complications. The majority of patients had a pneumothorax, following with pulmonary hemorrhage and BPD. One of these patients had two complications, initially started with pulmonary hemorrhage and later following with BPD due to prolonged time on mechanical ventilation. permutation N= 9999, total sum of squares= 4,333E07, within-group sum of squares= 4,025E07, F= 3,539, P= 0,0187

Type of complication	Number of patients	%
Pneumothorax	7	4,6%
Pulmonary hemorrhage	5	3,3%
BPD	5	3,3%
Sepsis	2	1,3%
Total	19	12,6%

Table 13. Complications of rds

9.8. Doses for x-ray examinations

Results for cumulative doses shown in Table 14. and calculation were done for Kerma in air-Kae, Kerma air product- KAP and Effective dose-E.

		K_{ae} (μGy)			KAP (Gycm²)			E (mSv)		
		Mean	Median	IQR	Mean	Median	IQR	Mean	Median	IQR
Gestational age (weeks)	≤29	226.87	172.11	172.11	0.0686	0.052	0.052	0.158	0.12	0.12
	30–32	184.21	86.05	86.06	0.0557	0.026	0.026	0.128	0.06	0.06
	>32	163.28	86.05	86.06	0.0493	0.026	0.026	0.114	0.06	0.06
	Total	188.56	86.05	86.06	0.057	0.026	0.026	0.131	0.06	0.06

Table 14. VaLues of mean, cumulative entrance surface air kerma (Kae), Kerma air product (KAP) and estimated effective dose (E) for neonates with different gestational age; mean ($\bar{x}$), median ($\tilde{x}$), third quartile (Q3), and interquartile range (IQR).

In the analysis of x-ray examinations, we found that the average number of x-ray was 2,1 ranging from none to 16 per patient, with the highest number of x-ray performed in the most severe disease with fatal outcome (Figure 57).

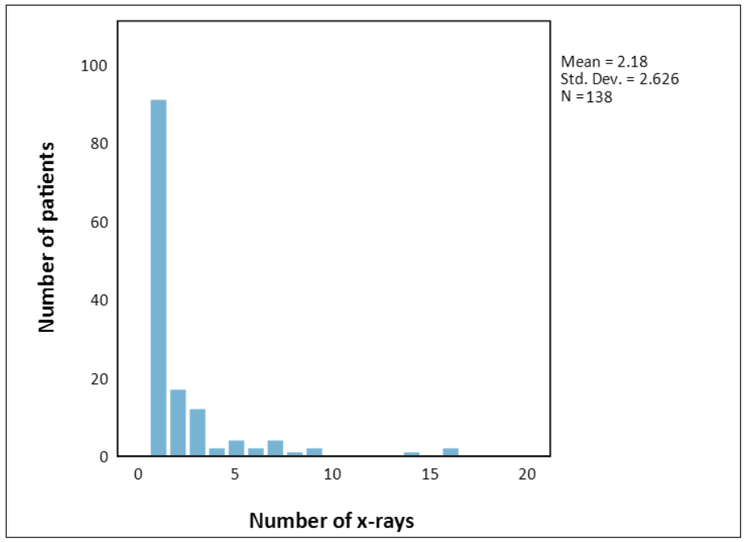


Figure 57. Total number of x-ray examinations

There is a statistically significant correlation between gestational age and body mass with equal distribution between genders. Results shown in Figure 58 (p< 0,001)

Differences in average cumulative doses expressed as Air Kerma, KAP, and effective dose in different body mass groups are shown in Figures 59, 60, and 61. with a significant correlation between parameters.

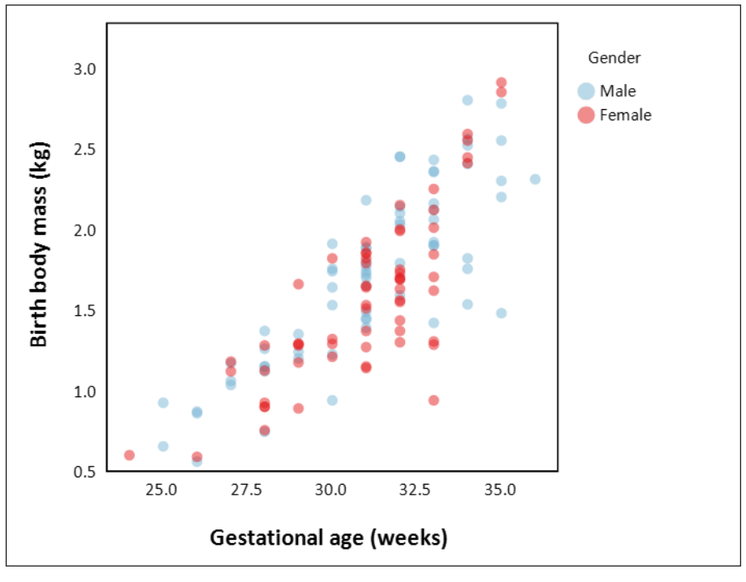


Figure 58. Correlation between gestational age and birth body mass for male and female neonates.

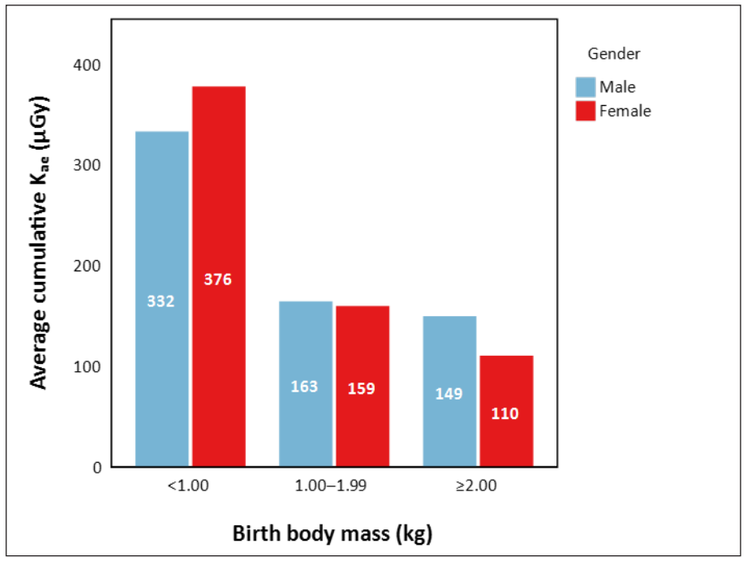


Figure 59. Average cumulative entrance surface air kerma in different body mass group.

We have a statistically significant result between parameters (Pearson correlation coefficient p = 0.008) (Figure 59).

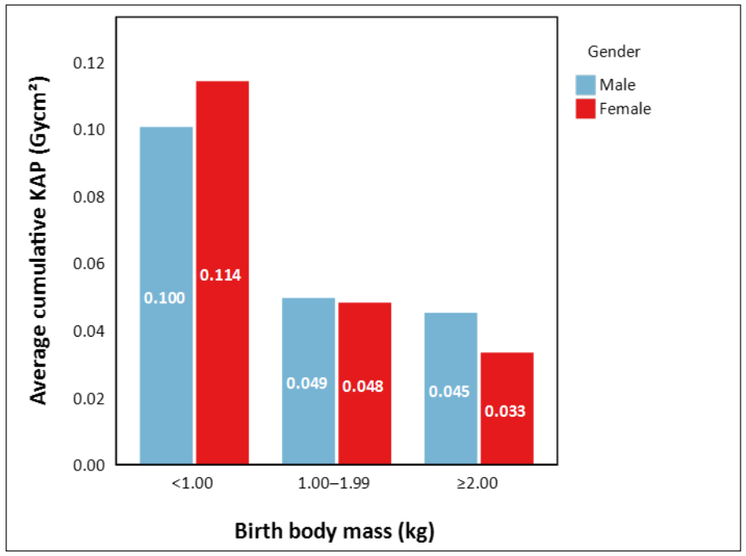


Figure 60. Average cumulative KAP in different body mass group.

The correlation between the two parameters is significant. Pearson correlation (p = 0.004)(Figure 60).

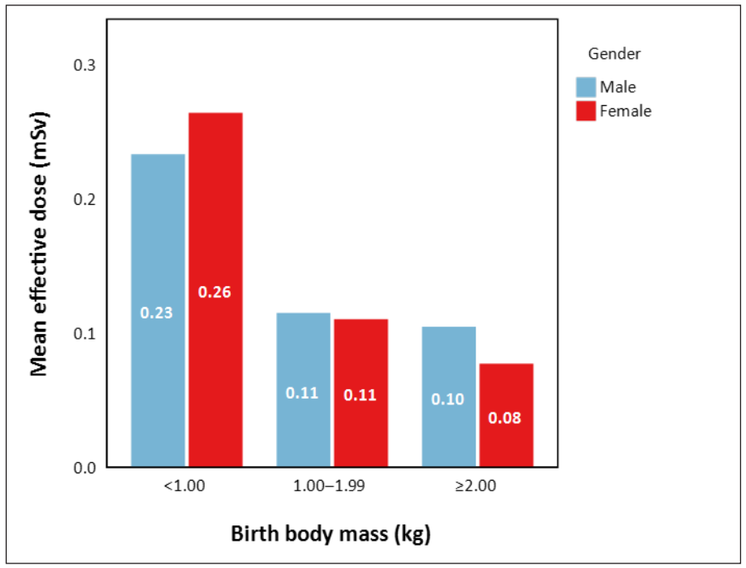


Figure 61.The average cumulative effective dose in different body mass groups

The correlation between the two parameters is significant. Pearson correlation (p = 0.003) (Figure 61).

The entrance surface air kerma for a single chest radiography exposure is 86,05 µGy, which corresponds to 0.060 mSv effective dose. On average, patients have exposed 2.1 times, so the average cumulative effective dose was 0.12 mSv.

The estimated effective dose from public exposure was 1.25×10−4 mSv, which could be considered negligible.

10. Discussion

The initial idea for starting this study was to evaluate the diagnostic possibilities and accuracy of lung ultrasound in the evaluation of respiratory distress syndrome in neonates. The standard way of diagnosing patients with respiratory distress syndrome is done by x-ray examination in correlation with the clinical status of a patient. Presumption for our study was the clue that lung ultrasound will be equally good or potentially even superb in diagnosing this clinical problem, which has very high morbidity among premature patients. With the introduction of ultrasound as a non-harmful method, it would be possible to reduce the number of performed x-ray examinations by replacing some of them with ultrasound and by that reducing the ionizing radiation delivered to the neonates.

Also, it is essential to emphasize that lung ultrasound, despite its extensive use in clinical settings, still is not a part of official recommendations or algorithms in the patient management of various respiratory and thoracic pathological conditions. Clinical diagnosis is the preferred method for diagnosing RDS in comparison to radiological findings, although it could be used in the differentiation of extrapulmonary and intrapulmonary causes.[47,94]

The relative limitation of a method is related to the fact that ultrasound examination is highly operator dependent and prone to subjectivity. Although there are exiting profiles and ultrasound artifacts for the classification of different respiratory pathology, there is always a question regarding variability in the technique of the examination. In our opinion, this obstacle could be avoided with continuous training and practice work, and also with the use of defined protocol of the examination (published in the form of recommendations by ultrasound consensus groups and societies).

Children are more vulnerable to potentially harmful effects of ionizing radiation, especially premature with immature tissues and cells which have high growth potential.

One of the study goals was to evaluate the exact number of performed x rays and also to measure the total radiation dose that patients received as a result of ionizing radiation.

During this process, scatter radiation was calculated because x-ray examinations were performed in the room with two or three patients.

The parameter that we used for the evaluation of the final patient outcome was survival or eventually, the death of a patient.

When performing lung ultrasound, it is necessary to examine whole lungs, considering that there is limited time for the patient examination; in most cases, patients are in the incubators and a decrease of the inside temperature has to be minimal.

Preterm babies are especially sensitive to temperature changes and predisposed to hypothermia, due to low birth weight and low gestational age, presenting with acidosis and hypoglycemia, but also concerning that hypothermia at birth increase the risk for

respiratory distress in those infants.[95] According to WHO recommendations, preventing the hypothermia is one of the main concerns in newborn infants, particularly preterm infants and those born outside of the hospitals.[96]

In our study, incubators were with automated temperature control (temperature range depending on gestational age and weight between 32ºC and 35º+/-0,5ºC).97 During the examination, we opened only side doors, so in average decrease of inside (incubator) temperature was 0,4º C. Examination time varied between 1min 26 sec and 3 min 48 sec. There is a reason for this wide variation in examination time, because, during the time, examiner/radiologist become more skillful, and also in all cases when a nurse or resident helped with manipulation of the patient, the exam performed more rapidly. Very small babies have to be examined quickly with a complete evaluation of both lungs, especially if they request surfactant therapy which needs to be administered as soon as possible, mostly upon their arrival to the NICU department. In severe cases when surfactant therapy applies in the delivery room, ultrasound examination can be done right after patient transport to NICU, so the control studies can be compared to the initial one, to establish a complete clinical and radiological picture of the patient condition. Because ultrasound is not harmful to the patient, it can be repeated as many times as it is necessary, contrary to the chest x-ray examination which using ionizing radiation. It is indisputable that the x-ray exam has its advantages in the diagnosing of respiratory distress syndrome and it can be done as an initial exam together with tube insertion checking examination (umbilical catheters or nasogastric tubes positioning checking).

In our study results showed an almost equal number of male and female patients (75/74). The average gestational age was 31 weeks, and the average birth weight was 1660 gr.

Delivery with cesarean section was in 68% of patients (32% of patients- spontaneous delivery).

In the total cohort, 89,9% of patients survive, and 10,1 % died, which assumed as a high survival rate (gestational age of included patients ranged between 24-35 weeks). This result is similar to the results of other studies with an overall survival rate of 80,4 %, and 81,3% respectively, but they included neonates from 22-32 weeks.[98,99]

We found statistically significant results with ROC analysis for gestational age and birth weight with cut-off values, meaning that a baby with a gestational age of ≤ 28 weeks and birth weight of ≤ 1210 gr will have a higher probability of developing the more severe grade of respiratory distress syndrome and worse outcome. Figures 54, 55. These results show high sensitivity and specificity, especially for birth weight (sensitivity 100%; specificity 90,3%) and respectively for gestational age (sensitivity 80%; specificity 89,5%).

Kohn et al. described birth weight cut off value of 1600 gr for the differentiation between high and low-risk infants in the prediction of neonatal morbidity. Also, they concluded that gestational age is a better predictor for patient's morbidity with a cut off value of

32 weeks. For gestational age values, less than 37 weeks were significant, but this is a more wide parameter, accounting that premature are divided into low, moderate and late preterm, and all these are accounted as less than 37 weeks.[100]

In our study birth weight was shown to be a better predictor of patient morbidity and outcome. This result is contrary to studies that concluded that gestational age as a single parameter is more appropriate than birth weight for the evaluation of premature outcome and assessment of potentially severe postnatal morbidities.[99,101] Although, the recently published paper described that there is no significant difference between gestational age and birth weight to morbidity and outcome in premature patients.[102]

Statistically significant results were found for Apgar scores in the 1st and 5th minute, and these scores contribute significantly to outcome with the results even stronger for Apgar scores in the 5th minute (P=0,0003), Apgar 1st minute (P=0,0002) respectively (Tables 5,6). These results for the Apgar score are similar to the results of a study published by Cnattingius et al.[103]

It is assuming that scores below 7 consider as "low" values.[104]

Patients with lower Apgar scores have more severe forms of RDS and worse outcomes. In a study published by Razaz et al., researchers compared the outcome for the Apgar score in the 1st, 5th, and 10th minute. The study showed significant results for scores in 5th minutes compared with scores at 10th minutes, but authors also analyzed results for scores between 7 and 10 in each measurement time (1st, 5th and 10th minute).[105]

According to a study published as a part of Euro-Peristat report, there is considerable variation between Apgar score values in neonates in different countries, so this variety is not reliable mark for prediction of patient morbidity and mortality. Conclusion of this study refers to the evaluation of the distribution of Apgar score during the time, rather than to value of Apgar score itself.[106]

Our results show that 65,2 % of pregnant women received antenatal corticosteroid therapy, which should be considered as moderately satisfactory coverage. Kumar et al. published less coverage of 44% in a similar patient cohort of 163 patients. [107]

Although this percentage of 65,2% in our study group seems acceptable, it is essential to point out that the antenatal corticosteroid therapy course has not been completed in all patients, meaning that some women received only a single dose of corticosteroids. Recommendations published by ACOG clearly stated that antenatal corticosteroids should be given in period up to 7-14 days before delivery with possible one "rescue" dose, in pregnancies 32 6/7 weeks and if delivery happens between second and third week, after corticosteroids.

ACOG (American College of Gynecologists) does not recommend antenatal corticosteroids before 24 weeks, contrary to the Royal College of Obstetricians who support this recommendation.[36,39,108]

Also, there is a recommendation for antenatal corticosteroids for women at risk for premature rupture of membranes (PROM), published in an article by Roberts et al.[109]

In our study, the youngest premature have been delivered at 24 weeks, without administered antenatal corticosteroids, consequently with the most severe form of RDS and fatal outcome, despite the treatment.

Nonparametric statistical analysis of our results shows that there was no significant correlation between premature rupture of membranes (PROM) and grade of respiratory distress syndrome, showed in Figures 31,32., but in sub-analysis of patients with PROM, we found that among of these 32 patients (36,4%), 46,8 % of them have not received antenatal corticosteroids.

There is a higher risk for developing of RDS in a case when PROM occurs before 32 weeks of gestation.[110]

Surfactant is used as a standard of care, as substitution therapy in the management of premature patients with respiratory distress syndrome.

Less than half of our patients, 41,3% have received surfactant, and as expected those were in majority patients with low gestational age and most severe grades of RDS. There was no significant relationship between RDS grade and applied surfactant therapy, Figures 33,34. These results can also be connected to results regarding applied CPAP therapy and administered O2, where we have found weak, but statistically significant correlations between these parameters and grade of respiratory distress syndrome (P=0,0075). Patients with higher grades of RDS spent more days on CPAP therapy. For administred Oxygen therapy significant results were found between RDS grades 3 and 4 with higher values in grade 4 (Figure 28), although there were no significant differences between O2 therapy and RDS grades 1 and 2 (Figure 27).

Surfactant deficiency can be a result of inherited or acquired disorders during fetal development. Even though the detection of inherited causes of surfactant deficiency has not been the scope of our work, undoubtedly is clear that revealing possible gene mutations in these patients will give a more profound understanding of respiratory distress syndrome in different patients, and give an explanation for unfavorable patients outcome. These scopes should be subject to further investigations in a larger patient cohort.

In contrary to the results for CPAP and O2, there was no statistically significant correlation between mechanical ventilation and grade of RDS (Figures 25,26), probably to the fact that we had a limited sample of 150 patients and fewer numbers patients on mechanical ventilation.

Analysis by logistic regression of different parameters shows that patients with higher gestational age have better prognosis and outcome, contrary to the grade of RDS, where our results show that higher grades of RDS on x-ray have poorer prognosis and outcome (P<0,0001).

These results are expected, and they are in the concordance with basic postulates of respiratory distress pathophysiology. The prevalence of RDS is in inverse correlation with gestational age and birth weight of patients. The incidence is the highest, and disease has a more severe form in low gestational age and low birth weight. [111]

10.1. Subgroup analysis (x-ray and ultrasound grades)

Analysis of RDS grades has been based on the evaluation of different scores on x-ray and ultrasound examination, as explained in the Material and methods section.

The x-ray scale has four grades, and the ultrasound findings classify according to 3 profile gradations. These gradation scales have inverse relation, meaning that Ultrasound profile 1 corresponds to X-ray grades 3 and 4, which are the most severe forms of RDS.

Comparison between a chest x-ray and lung ultrasound in the diagnosing of neonatal respiratory disease was investigated by different authors, which published significant results in favor of lung ultrasound as a highly sensitive method in the evaluation of neonatal lungs.[26,82,112]

Although our results showed a considerable difference between x-ray and ultrasound examination in favor of ultrasound as presented in Table 10, Figure 13, when we used a 4-grade x-ray scale, it statistically was challenging to compare uneven gradation scales. Initially, when we compare subgroups of x-ray, grades 3 and 4 we did not find any statistical difference between them, which enables the modification of the radiographic (x-ray) scale to inverse a 3-grade scale. In this way, the comparison between ultrasound and x-ray was balanced with scales that have even gradation scoring with mutual correspondence in numbers.

The initial x-ray exam was comparing to Ultrasound–day 1. The reason for not including other Ultrasound examinations on day-2 and 3 in comparison to x-ray, is because repeated x-ray exams have not performed at regular intervals and they were dependent on the clinical status of patients, based on the judgment of neonatologist.

The majority of patients, in summary, on ultrasound examination, had RDS grade 2, and on x-ray, it was RDS grade 1 in 70 patients (Figures 9,10,11,12). It is important to point out that this correlation was used based on inverse modifies x-ray scale where grades 3 and 4 were fused, and it correlates to ultrasound profile 1. On ultrasound, when we analyzed each day separately, profile 2 dominated. The comparison between x-ray ultrasound on day 1 is crucial when more than 40 patients had profile 1, and the rest of the patients had profile 2. On this way results between ultrasound and x-ray show mutual correlation.

In the analysis of each clinical parameter and patient characteristics, including the type of delivery, presence of premature rupture of membranes, antenatal corticosteroids and a grade of respiratory distress we have not found a significant difference between each x-ray groups.

We did not find a statistically significant difference between x-ray grades 1 and 2 for the patient weight (Figure 35). Although, there was a significant difference between x-ray grades 3 and 4 for patient weight, meaning that grade 4 has a lower median weight of 1251 gr, comparing to 1697 gr in grade 3 (Figure 36). In other parameters, there was no significant difference between groups 3 and 4.

In further analysis, we made a subgroup analysis of ultrasound findings. Evaluation of ultrasound profiles was made according to the three profile scale, explained previously in the material and methods and introduction section. Apart from the classification in one of three profiles, different ultrasound findings were observed. One of the main pathological/ ultrasound sign is the presence of B-lines, which represents the accumulation of fluid in interstitial space and alveoli. All of our patients had this positive sign, meaning that one of the conditions for the diagnosis of respiratory distress syndrome is the presence of B lines. Thick pleura was also very common in our cohort, although it is not specially characteristic sign for the diagnosis of RDS. Partial atelectasis also was described as one of the pathological findings in these patients.

In our study group, we found, especially on day 1, the presence of subpleural consolidations, together with confluent B lines and other signs of RDS. In the analysis of these patients, we found a statistically significant correlation for this parameter, and these patients that have an initial ultrasound exam presence of subpleural consolidations had severe forms of RDS and worse outcomes (Figures 37,38,39,48). We consider these results as one of the most significant findings in this study, even though initially we did not consider to evaluate this parameter in detail, and these findings come from the analysis of whole material with a statistical significance which deserved further evaluation.

Based on these findings, we made an additional classification of Ultrasound findings into subgroup 1 and subgroup 2, depending on the presence or absence of subpleural consolidations, regardless of the ultrasound profile. Subgroup 1 were patients with consolidations.

We had statistically significant differences for gestational age values where US-Subgroup 1-(patients with consolidations), have a lower gestational age of 27,7 weeks in comparison to US subgroup 2 with a mean gestational age of 30,5 weeks (Figure 37).

Also, a significant difference was founded in the US subgroup 1 for outcome and median weight values, wherein subgroup 1, mean weight was 1101 gr, and for subgroup 2 it was 1516 gr (Figures 38,39).

On the second day, we found a statistically significant difference in subgroup 1 for applied therapy with O2 that shows higher values (P=0,0493) (Figure 48) which can be explained with a higher need for oxygen in a severe grade of RDS.

There were no significant differences between ultrasound subgroups 1 and 2 with CPAP, MV and O2 (Figures 43,46; 44,47 and 45)

These findings also are connected to the grade of RDS, so the combination of subpleural consolidation with completely “white lungs” or grade 1 RDS will have a worse prognosis. In different papers, subpleural consolidations were mentioned as one of pathological ultrasound findings, but without its correlation to prognosis, treatment, and outcome. [75,86]

In the book, published by Liu and Sorantin, on the contrary, subpleural consolidations were mentioned as essential findings with the claim that without the presence of consolidations, diagnosis of respiratory distress syndrome can not be established. [26]

We can not agree absolutely with that claim, because some of our patients develop consolidations on the second day, but some of them did not have any consolidations at all. Explanations for these findings can be in the fact that severe grades of RDS, in the majority had consolidations, but mild forms (grades) of disease were without it.

Based on our results, we concluded that the current classification of ultrasound findings into three profiles is insufficient and too “ wide” for the classification of patients with RDS, especially for the patients classified into Profile 2. Considering that subpleural consolidations represent significant findings, we proposed a modified ultrasound classification, which, in our opinion, will serve better in the evaluation of the pathological conditions in respiratory distress syndrome (Table 15).

US profile	Findings		
Profile 1	„White“ lungs- confluent B lines without spared area of lungs	1a	With subpleural consolidations
Profile 2	Prevalence of confluent B lines	2a	With subpleural consolidations
Profile 3	Prevalence of A-lines		

Table 15. Modified ultrasound classification

Since the ultrasound examination was performed three days in a row, we analyzed findings between each day. Results show a significant difference between day 1 and day 2, but also between day 2 and day 3, with a positive trend, which means that the progress of the disease has been gradual (Figures 50,51,52,53).

Ultrasound scores ranged from 1 to 3, where profile 1 represents a most severe form of respiratory distress, and during the treatment, it is expected that grading in successful treatment will pass from 1 or 2 to profile 3, which represents the resolution of distress with normal or almost regular aeration of the lungs.

The initial examination had the lowest scores, so with the treatment resolution of pathological findings progress to higher values of ultrasound scores, which explains the significant difference between each day, as a result of successful disease resolution for most of the patients.

We also made a sub-analysis of the twin patients, where we compared different clinical data with RDS grade and outcome. Even though in the literature, there is evidence that second-born twin has more severe disease and worse outcome, in gestation after 28 weeks, but without the difference in gender.[113,114]

In our results, we did not find the relationship between first-born and second-born twins and survival outcome (Fisher’s exact test P>0.9). Also, we did not find statistically significant correlations between other parameters that support these clues, most probably because our sample was too small for comparison. The important fact in the

evaluation of respiratory distress syndrome morbidity and mortality in twin patients is the knowledge regarding their zygosity, but this specific data were beyond the scope of our study, nevertheless, it could be an interesting topic for further investigations.[29]

Non-parametric MANOVA test evaluates in multiparametric analysis correlations between RDS grade, Apgar score in 1st minute, Apgar score in 5th minute, gestational age, and weight. Results presented in Table 12 show, among all these parameters, a significant correlation between the grade of respiratory distress syndrome and outcome, which is in concordance with previously confirmed results. Patients with severe grades of RDS have the worst outcomes with significant differences between grades 1,2, and 3 in comparison to grade 4 (P= 0,0187). These results should be interpreted simultaneously with all other parameters.

Our results show specific cut off values for the evaluation of patients prognosis and outcome as following: Patients with gestational age ≤ 28 weeks, less than 1210 gr and Apgar score in 5th minute values ≤7 with RDS grade 3 or 4 (on x-ray) or grade 1 (on ultrasound) and ultrasound subgroup 1 will have worse outcome.

Inter-rater agreement was calculated with the Kappa test, which measures agreement over and beyond agreement that happens by chance alone.[115] Kappa values showed moderate agreement between x-ray and ultrasound on the first day, results displayed in Figure 62. The reason for the moderate score is probably because we compared these scores in 1st day (at disease onset), when on ultrasound examination score 3 was completely missing because this score represents normal finding, which some our patients had only in 3rd day.

In Table 13 are data for the percentage of different complications during the treatment of RDS. Most often, in 4,6% of patients, the complication was pneumothorax, following with 3,3% of patients who had pulmonary hemorrhage and 3,3% with BPD, and only 1,3 % had sepsis.

Although, pneumothorax can be diagnosed with ultrasound, in the cohort of our patients, a chest x-ray was the radiological method of choice for the confirmation of this diagnosis. Our results show the incidence of 4,6% which is similar to other studies, where incidence was 5% or 8,1% in a study where authors prove a decrease in pneumothorax incidence with a reduction of ventilator parameters in neonates with respiratory distress syndrome. [116,117]

For our data collection, after a performed chest x-ray and confirmed diagnosis of pneumothorax, respectively in the same patient, we perform the ultrasound, in 5 patients out of 7 we found positive cases of pneumothorax. We did not include these data into results because the sample was too small and in 2 patients when pneumothorax occurs, the radiologist was not at disposal at that specific moment. Ultrasound signs of pneumothorax were the same as described in the literature: absent lung sliding, thick pleural line, lung point, and "barcode" sign in M mode (motion mode).[118,119]

Lung sliding represents contact between parietal and visceral pleura, but in the case of pneumothorax, air fills this space and thus enables contact between two pleurae, and this

sign is missing. Sometimes it is challenging to identify the absence of lung sliding. In our opinion, M mode is more important and more specific than examination in B mode alone and comparing to other signs of pneumothorax, so the examiner can more confidently set the diagnosis of pathological finding. Similar results also were observed in published articles.[120,121]

Our results show a 3,3% of pulmonary hemorrhage, which has proven with pleurocentesis (hemorrhagic fluid) and even 3,3% of bronchopulmonary dysplasia. These diagnoses have set according to clinical and laboratory signs together with a chest x-ray. Literature shows excellent results for the ultrasound examination and evaluation of the mentioned pathological conditions.[122,123] In our patients, these complications developed later during the treatment course and design of our study concentrated on three ultrasound examination in a row (respectively first three days of disease). Despite this limit in our study, further investigations can focus on ultrasound examination of these complications in patients with RDS.

Higher risk for the developing BPD was proven in a meta analysis for the intubated patient on mechanical ventilation, with the recommendation to avoid or decrease the use of this kind of supportive therapy where it is possible.[124]

In published articles, the authors investigated the connection between bronchopulmonary dysplasia and other factors, including gestational age and birth weight, Apgar score and other complications such as pneumothorax and concluded that among others, the onset of pneumothorax in first 48 hours of life is one of the predisposing factors for developing BPD.[125]

10.2. Doses of x-ray examination

One of the goals in our study was a measurement of doses delivered to patients after a chest x-ray examination. One of the most often performed radiological procedures in Intensive care units is chest or chest-abdomen x-ray exam. Besides the number of x-ray exams, we wanted to objectivize the amount of radiation delivered to patients with the diagnosis of respiratory distress syndrome. Based on the recommendation of ICRU (International Commission on Radiation Units) doses can be expressed as KAP (Kerma air product-Gycm^2), which is the preference in Europe, or as RAK (Reference Air Kerma- Gy) which has preferred in the USA.[126]

KAP-dose area product is more appropriate for diagnostic x-ray exams, and it is a surrogate measure of the amount of energy delivered to the patient.[63]

Delivered dose to premature patients depends on their body weight, so the patients with less body mass will be more susceptible to potentially harmful effects of ionizing radiation. Cumulative entrance surface air Kerma and effective dose are in direct connection with a number of x rays and patient weight. In Table 14. are shown summarized data for Ka,e, KAP, and effective dose in our patients. Doses were calculated based on the number of chest x-ray examinations.

In our cohort, we found a significant correlation between different body mass and cumulative entrance surface air kerma, KAP, and effective dose (Figures 59,60,61).

These correlations were specially expressed in extremely low birth weight premature (< 1000 gr.) The mean Ka,e for these patients was 376 μGy, KAP value was 0,014 Gycm^2 and Effective dose 0,26 mSv.

The similar results have been observed in neonates with low gestational age. The female patients received a higher dose than males in the weight group < 1000 gr.

Mean cumulative Ka,e has the highest value for neonates with gestational age ≤ 29 weeks (226,87 μGy), and lowest for neonates with gestational age > 32 weeks (163,28 μGy). Mean effective dose values were also higher for the neonates ≤ 29 weeks (0,158 mSv) and for neonates > 32 weeks (0,014 mSv). Mean KAP was higher for neonates ≤ 29 weeks (0,686 Gycm^2) and lower for neonates > 32 weeks (0,493 Gycm^2). These results are in concordance with the number of performed x-ray where the highest number has found in more severe grades of RDS in patients with lower gestational age and lower birth weight. Data from a similar study published by Aramesh et al. ranged from 0,046-0,111 mSv with a maximum three x-rays per patient.[127] Although in a study published by Narayan, values of measured doses were higher, up to 1467 μGy and maximum 31 x-rays.[128]

The entrance surface air kerma for a single chest radiography exposure was 86,05 μGy, which corresponds to 0.060 mSv effective dose. On average, patients have exposed 2.1 times, so the average cumulative effective dose was 0.12 mSv.

In a case of neonatal patients, scattered and leakage radiation from other patients x-ray examination should be considered as public exposure.[129]

Results for scattered radiation doses indicate that the effective dose should be less than 0.001mSv per patient during the hospital stay. The position of incubators in the ward potentially can influence the amount of delivered radiation. Calculation of the radiation doses has based on the simulation with the cylindric fantom and current position of incubators in the ward. The incubator in the center of the room would receive 0.000125mSv, which is approximately 1,000 times less than the dose received due to direct medical exposure.

In medical exposures, biological effects depend on the radiosensitivity of target tissue and organ that was exposed to ionizing radiation. Cells with high growth potential and tissues with fast metabolism as in embryo, fetus, or newborn are more sensitive to the effects of ionizing radiation.[130] Justification of radiological procedure and x-ray exposures in the pediatric population have to respect ALARA principle (as low as reasonably achievable), and in papers published by American College of Radiologist and IAEA (International Agency for Atomic Energy), there are defined recommendations for the use of ionizing radiation in medical purposes.[131–133]

The meta-analysis, published by Pearce et al. evaluated the estimated cancer risk in the pediatric population that undergone CT examination. The results of the study indicate that there is a small absolute risk, so the exposure to ionizing radiation should be maintained to minimum.[134]

The public exposure limit is 1 mSv, and this does not apply to medical exposure of the patient, because there is no defined limit in medical exposures in terms that benefits for the patient health overcome effects of potential biological effects of cumulative doses of ionizing radiation.[59,89]

Ultrasound and radiographic pictures with different grades of RDS were included as Appendix section

11. Limitations of the study

We did not find any major limitiation in our study.

Since we did not find significant correlation in the analysis of twins, limitation of the study could be the number of patients, so for the statistical significance is necessary to analyses larger patient sample.

Due to the high sensitivity of ultrasound, subtle changes can be noticed in a more prolonged period, which could potentially influence more extended follow-up and the duration of patient hospital stay.

12. Conclusions

Results of our study prove the primary hypothesis and show statistically significant results which enable the realization of secondary hypothesis and aims of our study as follows :

1. Lung ultrasound enables the diagnosing and follow-up of respiratory distress syndrome in premature neonates.
2. Lung ultrasound shows a significant correlation with chest x-ray, which is considered as a radiological method of choice for the diagnosis of RDS.
3. Subpleural consolidations on ultrasound examinations are a significant and important finding in patients with RDS in terms of prognosis and outcome.
4. We proposed a modification of the exiting three profile ultrasound scale for the detailed classification of patients with RDS.
5. Lung ultrasound should be included in the diagnostic algorithms for the diagnosing of RDS.
6. Calculated doses of ionizing radiation delivered to the premature patients are acceptable and far below the dose limit for public exposure.
7. Doses of scattered radiation are considered negligible.
8. Lung ultrasound can replace a considerable number of chest x-ray examinations, thus decrease delivered effective doses of ionizing radiation to the patients.

13. Further investigations

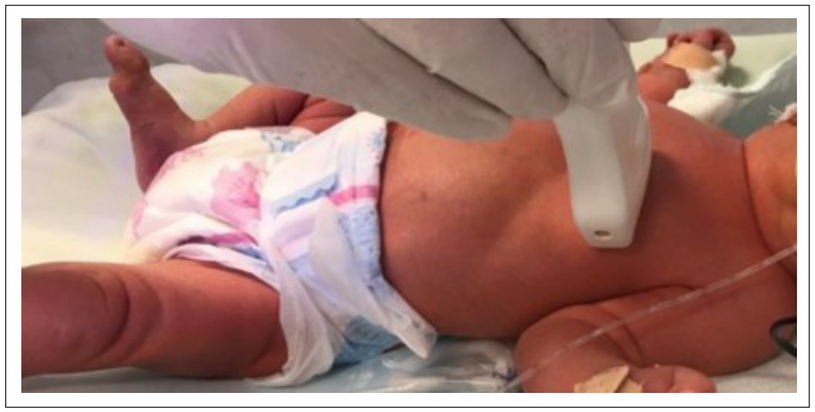
During the evaluation of results in our study, we find topics that could be a subject of potential further research and which detailed investigation was beyond the scope of this study.

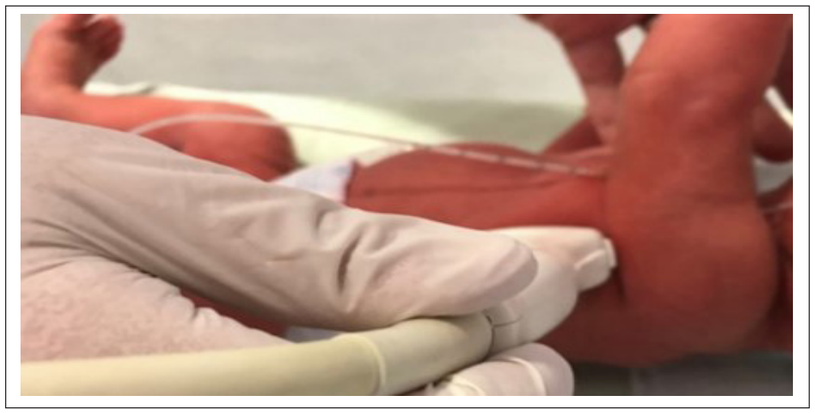
1. Study of gene mutations responsible for surfactant deficiency in neonatal respiratory distress syndrome in a larger cohort of patients.
2. Correlation of twins zygosity with RDS disease severity and the outcome.
3. Calculation of organ doses for x-ray examination.
4. Estimation of cancer risk in pediatric patients.

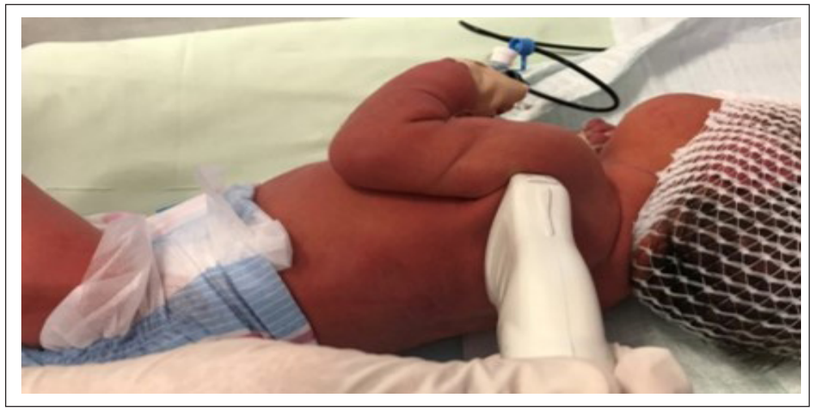
ABBREVIATIONS

RDS – Respiratory distress syndrome
ARDS – Adult respiratory distress syndrome
LUS – Lung ultrasound
ELBW - Extremely low birth weight
VLBW – Low birth weight
CDC - Center for disease control
TTN - Transient tachypnea in newborn
CRIB – Clinical risk index for babies
DPPC – Dipalmytoylphosphatidylcholine
SP-B – Surfactants protein B
SP-C - Surfactant protein C
ACOG – American College of Gynecologists
RCOG – Royal College of Obstetricians and Gynaecologists
WHO- World Health Organisation
CPAP – Continuous positive air pressure
BIPAP – nasal positive pressure ventilation
IPPV – Intermitent positive pressure ventilation
IMV – intermittent mandatory ventilation
PTV – patient triggered ventilation
VGV – Volume guaranteed ventilation
PSV – Pressure support ventilation
HFOV- High-frequency oscillatory ventilation
MV-mechanical ventilation
INSURE – Intubate-surfactant-extubate
BPD- Bronchopulmonary dysplasia
CT – Computer tomography
DRL – Dose reference levels
ICRP – International Commission for radiation protection
ICRU – International Commission on Radiation Units
IAEA – International Agency for atomic energy
Ka,e – Kerma in the air
KAP – Kerma air product
NICU – Neonatal Intensive Care Unit
PROM – Premature rupture of membranes
O2- Oxygen
ROC – Receiver operating characteristic curve
ALARA – As low as reasonably achievable

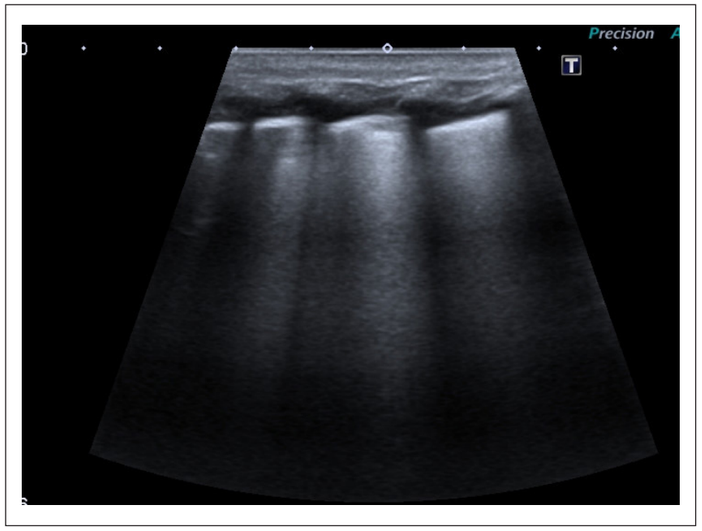
Appendix



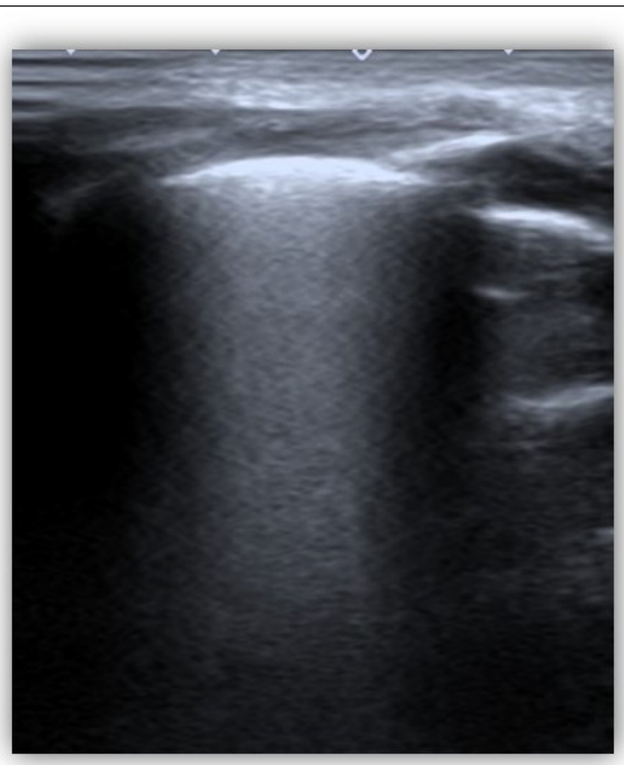




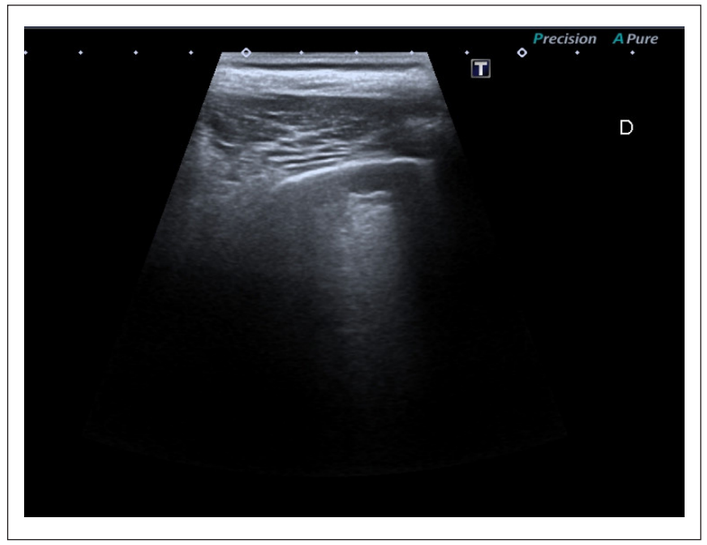
Picture 1,2,3. Examination protocol : Transverse and sagittal plane; medioclavicular, midaxillary and paraspinal line; Supine and oblique position.

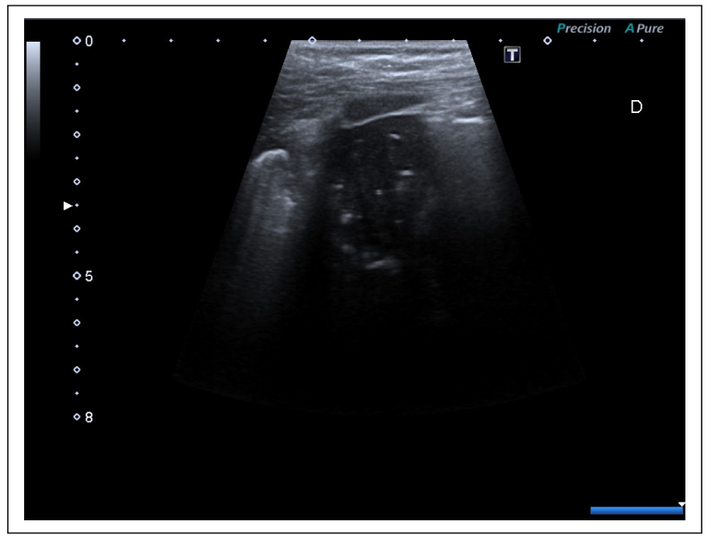


Picture 4. Ultrasound Profile 1- White lungs

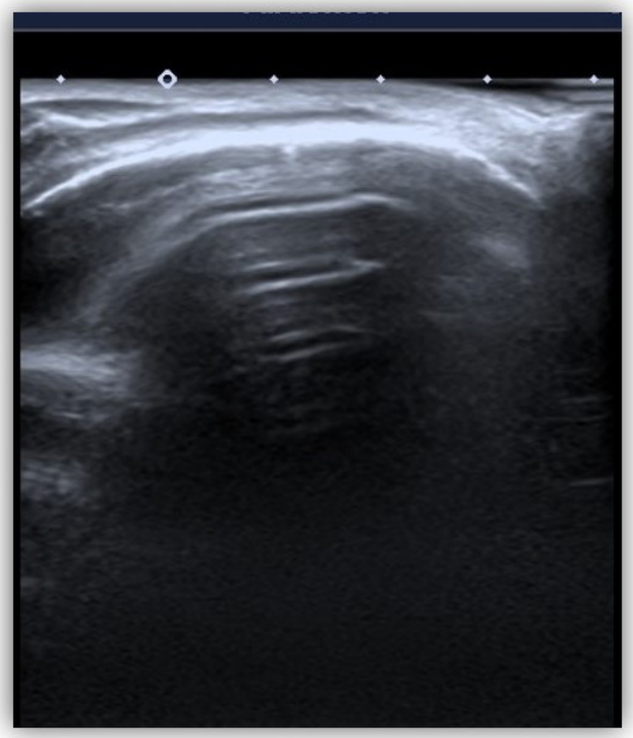


Picture 5.Ultrasound Profile 2-Prevalence of confluent B lines

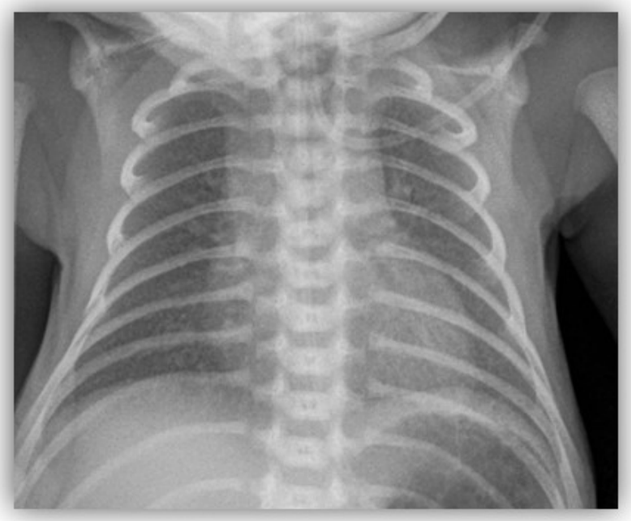




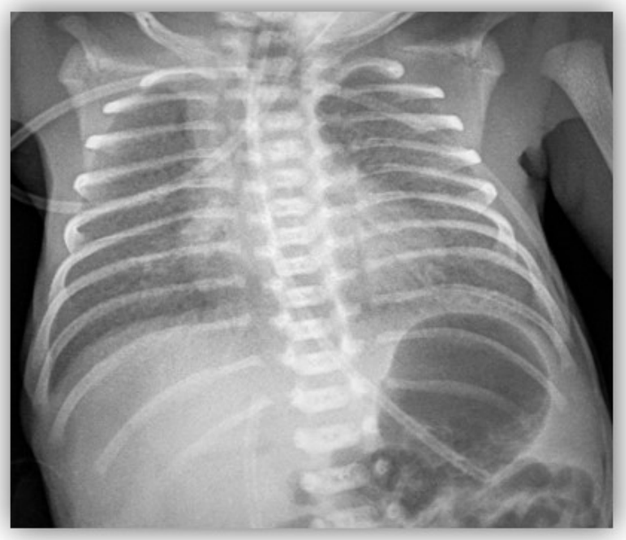
Picture 5 a,b.Subpleural consolidation-ultrasound



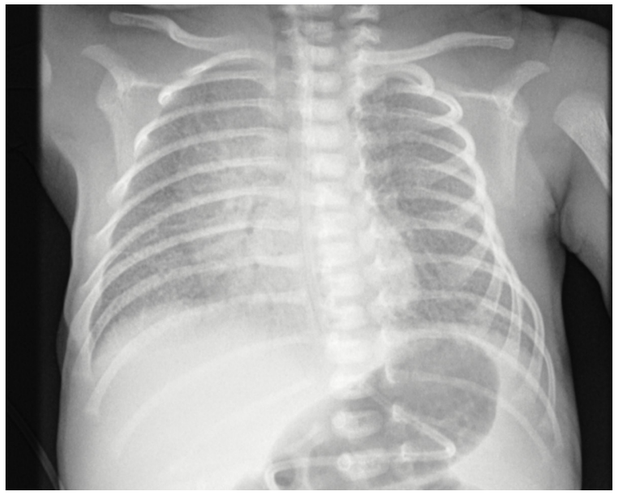
Picture 6. Ultrasound Profile 3-Prevalence of A lines



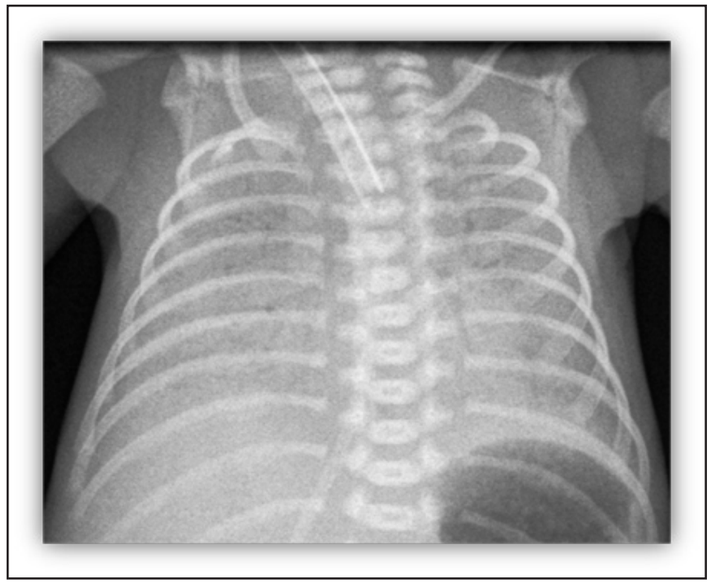
Picture 7. Grade 1-x-ray



Picture 8.Grade 2-x-ray



Picture 9.Grade 3-x-ray



Picture 10.Grade 4-x-ray

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