



# Ultrasound Based Analysis- A Non-invasive Method to Predict Respiratory Morbidity in the Newborn

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# ABSTRACT

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**Title:**

Ultrasound Based Analysis – a Non-invasive Method to Predict Respiratory Morbidity in the Newborn.

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**Background:**

Approximately 11% of all live births globally are premature, meaning birth before 37 weeks of gestation. Preterms are affected by higher rates of mortality and morbidity, especially respiratory complications due to the late development of the fetal lungs. One of the most common disorders is RDS, Respiratory Distress Syndrome, which is a potentially fatal condition and associated with surfactant deficiency. Today the only way of assessing fetal lung maturity is through amniocentesis and the amount of surfactant in the amniotic fluid. It is an invasive procedure entailing risks such as infection and miscarriage. The possibilities to assess fetal lung maturity through ultrasound based quantitative texture analysis have been greatly explored during the last years. A Spanish research team has developed an algorithm, QuantUS-FLM with the intention to be able to predict respiratory morbidity by quantitative texture analysis of ultrasound images of fetal lungs.

A similar master thesis project evaluating the predecessor to the current algorithm, AQUA, was recently conducted at Sahlgrenska Hospital Östra by medical student Lars Cedergren. During this study difficulties for an inexperienced student to obtain ultrasound images of sufficient quality to be analysed in the algorithm were discovered. Because of this a quality assurance model to raise the number of approved images was developed.

**Aims:**

To explore if ultrasound based quantitative texture analysis is a practicable way of predicting neonatal respiratory morbidity. A secondary aim was to evaluate the previous developed quality assurance model of ultrasound image acquisition.

**Method:**

41 ultrasound images of fetal lungs from pregnant women admitted to the delivery wards at Sahlgrenska Hospital Östra were obtained during October 2013 to Mars 2014. Inclusion criteria were gestational weeks 25+0 to 40+0, maternal BMI below 35, no antenatal steroids administrated between

the image acquisition and the parturition, delivery occurring within 48 hours from the image acquisition and no malformations that could potentially affect the respiratory function. An additional 37 images obtained by medical student Lars Cedergren were added to the study. The images were then analysed with the QuantUS-FLM software online application developed by the Spanish research team. A review of both the mother's and the child's medical records was done in regards to maternal age, maternal conditions and neonatal data. Statistical descriptive analysis was performed using SPSS.

### **Results:**

QuantUS-FLM analysed a total of 71 images and predicted 16 as high risk of developing a neonatal respiratory morbidity. Among these 16, five neonates developed RDS and two developed another respiratory morbidity. None of the 55 neonates that were classified as low risk of developing a respiratory morbidity eventually did so.

Using the quality assurance model developed by a previous master thesis student raised the initial approval rate of the first collection phase from 50% in the previously carried out study, to 87% in this study.

### **Discussion:**

This study might indicate some optimism of the ability of QuantUS-FLM algorithm to predict respiratory morbidity through quantitative texture analysis. Due to low incidence of RDS, the formation of the study and the student's pre-requisites for this study a sufficient amount of included patients to achieve a moderately strong indication was not achieved. As this is only a local study within a larger multicentre study such a number of included patients was, on the other hand, never the essential goal. A test that could predict neonatal respiratory morbidity is of special clinical importance in the group of neonates born in gestational week 28 to 36 where the obstetrician is facing the dilemmas of delivery planning and antenatal steroid administration.

### **Conclusions:**

Any conclusions regarding QuantUS-FLM and its ability to predict respiratory morbidity in the newborn cannot be made due to lack of power in this study.

The quality assurance model of image acquisition will likely have contributed to increase the number of ultrasound images approved for analysis with the algorithm by the Spanish research team. More education and practical training of the student and clearer guidelines concerning optimal image features would possibly increase it even more.

## **BACKGROUND**

The purpose of the background text is to create a greater understanding of the strong connection between preterm birth and respiratory morbidity. I means of doing so this introduction begins with a short summary of the human embryology leading on to the critical steps in fetal lung development and neonatal respiratory disorders. It also covers the routines of antenatal steroids and fetal lung maturity assessment procedures known today. Finally it will provide an update of the current knowledge and on-going research of ultrasound-based methods for fetal lung maturity assessment.

### **Basic Embryology**

During a fetus' third week of development, the trilaminar germ disc is present in the blastocyst. It consists of a layer of ectodermal cells, a newly formed layer of mesodermal cells, and a layer of endoderm cells. On one side of the trilaminar germ disc is the amniotic cavity and on the other side is the yolk sac. It is generally believed that cells of the ectodermal layer migrate in the direction of the primitive streak and slip underneath it to form the mesodermal layer. This process is called invagination. The mesodermal layer thereafter comes in contact with the extra-embryonic mesoderm covering the yolk sac and amnion. The mesoderm will give rise to muscles, dermis, subcutaneous tissue of the skin, serous membranes, excretory units of the urinary system.

Cells invaginating the primitive streak will also move straight forward and eventually form a solid cord, known as the notochord. The notochord forms the midline axis and will serve as the basis of the axial skeleton. The ectoderm covering notochord forms the central nervous system. It begins with thickening of the ectoderm, thereafter folding of the lateral edges of the neural plate towards the depressed midregion, forming a groove and later the neural tube. This tube will give rise to the spinal chord and the brain. The parietal mesoderm and overlying ectoderm form the outer body wall.

The visceral mesoderm and the endodermal layer form the wall of the gut by a folding process mainly caused by the rapid longitudinal growth of the central nervous system and the transverse folding of the somites derived by mesoderm. This folding process develops an endoderm-lined cavity. In the anterior part the foregut is formed, in the caudal region the hindgut is formed and in between these two the midgut is formed. The midgut temporarily remains in contact with the yolk sac via the omphalomesenteric duct [1].

## **Fetal lung development**

Normal human lung development is a series of events that has traditionally been divided into five phases [2], even though there is a gradually occurred transition of these phases and a considerable overlap. The International Congress of Anatomists officially established the nomenclature of the first four stages in Leningrad 1970 [3]. There is a great variation between phases within a lung's different areas and the process of lung development is highly individual [4].

### **Embryonic stage (Conception-6 weeks of gestation)**

The lung develops as an outgrowth of the ventral wall of the primitive foregut by invasion of epithelial cells from the foregut endoderm into the surrounding mesenchyme [4]. Initially the outgrowth, also known as the respiratory diverticulum, is in open communication with the foregut. The foregut and the respiratory diverticulum, or primitive trachea, become separated as the diverticulum branches caudally [1] into the main right and left bronchi and thereafter continues to branch into lobar and segmental bronchi during week 4-5 [5]. The lobar branches correspond to a mature lung in means of three lobar bronchia distally of the right main bronchi and two distally of the left [4]. During this phase the pulmonary arteries and veins are developed from the sixth aortic arches and accompany the pre-acinar airways and grow around the respiratory tubules by vasculogenesis. This process overlaps into the next stage [3, 5].

### **Pseudoglandular stage (week 6-16)**

In this stage further branching of the airway and vascular network occurs [4] and a total of 20 generations of branching have occurred by the end of week 16 [3]. The branching pattern is regulated by epithelio-mesenchymal interactions [4]. All pre-acinar structures and its vascular system are formed during this phase, from the trachea to the terminal bronchioles. Further growth and increase in size occurs later on, but there will be no new branches formed after this stage [4, 5]. A differentiation of mesenchymal cells takes place to form adult structures of cartilage in the trachea, bronchial smooth muscle and the presence of mucous glands. Columnar cells proximally and cuboidal cells distally, replace the early pseudo-stratified epithelium that lined the pre-acinar airways. The cuboidal cells are rich in glycogen and represent the immature type II cells [3-5]. Glycogen is an essential part of surfactant that later on will be secreted by the pneumocytes type II cells that derive from immature type II cells [3].

### **Canalicular stage (week 16-24)**

The primitive acinar (gas-exchanging) structures are formed in this stage of development and consist of respiratory bronchioles, alveolar ducts, sacs and alveoli [5]. There are two important steps made in this stage. One is the differentiation of pneumocytes type I and II, which is initiated by flattening of acinar epithelium in week 22-24. Pneumocytes type II produces surfactant, which is detectable in utero by week 24 but not in sufficient amounts until approximately week 30 [2, 4]. The other important step in this phase is the beginning of the formation of the alveolar-capillary barrier [5]. The lumina increases and vascular networks continue to form. At first the vascular networks and the alveolar epithelium are separated by mesenchyme, but a gradual decrease in mesenchymal tissue results in capillaries becoming more adjacent to the airway epithelium, thus enabling the future gas-exchange [3, 4].

### **Saccular stage (week 24-36)**

A dilation of the acinar tubules takes place to form alveolar saccules - premature alveoli. The purpose of the saccular phase is to increase the gas-exchanging surface [4] and during this phase the primitive alveoli increase in number and become more and more efficient gas-exchangers. The alveolar walls are thicker in the saccules than in the final alveoli but the wall gradually becomes thinner [2]. A characteristic for this stage is an explicit vascular expansion. The blood vessels grow in length and diameter and also form new vessels. An important step in the vascular development is the formation of the double capillary layer surrounding the saccules [3]. Even though the respiratory structures are not fully developed, they may be able to sustain life in a preterm infant [2]. Surfactant is produced in a larger amount during this stage and provides the alveoli with stability. It prevents the alveoli from collapsing during expiration by reducing the membrane tension of the air-water line in the alveoli [1, 3].

### **Alveolar stage (week 36-8 years of childhood)**

During this phase secondary septa forms, the interstitium and epithelium decrease in thickness and the final alveoli develop [2, 6]. These developments also contribute to the lungs physiological attributes and thereby the respiratory system in whole. The general comprehension is that the first mature alveoli are present at 36 weeks of gestation, marking the start of the alveolar stage. This final phase continues into childhood, and even up to the age of 8 years old [2, 4] but with the highest increase of alveoli occurring during the child's first two years [4]. In a fully developed lung there is approximately 300-600 millions alveoli, which is about 1000 per acinus [6]. At birth there is approximately 20-50 millions of alveoli present [5].



### **The infant's first breath**

During the fetal period, the lungs are filled with a fluid secreted by the pulmonary epithelium combined with amniotic fluid that the fetus swallows. The amount of fluid existing in a fetal lung is not yet completely established but the general opinion is that the volume of fluid is larger than the functional residual capacity [7]. The fluid thereby exerts distention to the lungs and contributes to maintain a certain lung volume. These factors combined with the breathing movements seen in fetuses as early as week 10 are major contributors to lung growth and development[5]. The characteristic that marks the infant's first breath is the clearance of lung liquid from the lungs. This process begins with the onset of labor. Changes in intrauterine space during labor changes fetal posture and with that the fetal chest wall configuration. The transpulmonary pressure increases and the amount of liquid in the lungs decreases. The fetus experiences a large release of adrenaline during labor that promotes the pulmonary epithelium to stop secretion and start reabsorption of fluid. Another important process is the fetus' journey through the distal birth canal that exerts compression and stretching of the fetus' thorax. It has been shown that infants born with caesarian section to a lower extent retains air at the end of their first breath. Liquid fills the airways until the infant draws its first breath and the inspiratory efforts play a critical role in the liquid clearance[7].

### **Antenatal glucocorticoids**

The first positive effects of glucocorticoids in fetal lung maturity were recorded in 1969. A study showed that if parturition was induced in sheep at approximately 80% of the normal gestation length, the premature lambs were viable if the fetal lambs had been infused with dexamethasone [8, 9]. Since then many studies have shown the beneficial effects of antenatal glucocorticoids [9]. Antenatal administration of glucocorticoids has proven to reduce both the incidence of RDS with 35-45% and the overall neonatal mortality, as well as a reduction in the need of neonatal respiratory support [9, 10]. Today glucocorticoids are routinely given to women at risk of preterm delivery before 34 weeks of gestation [11, 12]. Betamethasone is more potent than dexamethasone and is therefore the drug of choice [10].

However, it has been shown that there are no measurable quantities of glucocorticoids in cord serum in babies born later than 40 hours since the last betamethasone dose was given. Also the effectiveness of antenatal steroids for promoting lung maturation declines after seven days[10, 11]. This has resulted in clinical praxis of repetitive administration of antenatal glucocorticoids [11] even though there is a lack of scientific evidence that the benefits of this outweigh the risks of adverse outcome in the short and long term [12]. Concerns about the

risks that antenatal steroids would produce permanent neuromotor and cognitive deficits in the newborn have been raised during the recent years. It has been shown that prolonged increased levels of glucocorticoids can lead to degeneration of the hippocampus [13]. Antenatal steroids have also been shown to elevate fetal blood pressure and also to give rise to significant higher blood pressures in children who were treated with antenatal steroids as fetuses. Increased cardiac output through direct effects on the myocardium and a decrease of fetal heart rate variability have also been reported [14, 15]. Further antenatal steroids have been associated with febrile responses, reduction of lymphocyte proliferation and cytokine production and thereby risk of infections[16].

### **Endogenous glucocorticoids**

Many studies have been conducted to understand the effects of glucocorticoids on fetal lung development and maturity. The fetus is constantly exposed to cortisol but in a highly controlled manner through active transporters in the placenta, liver and brain but also via the level of activity of the 11-beta hydroxysteroid dehydrogenase enzyme-family ( $11\beta$ -HSD) in the placenta. There are two isoforms of this enzyme:  $11\beta$  HSD-1, which convert cortisone to the biological active form cortisol and  $11\beta$  HSD-2, which converts cortisol back to cortisone. In this way, the fetus is protected towards excess levels of endogenous maternal glucocorticoids that potentially could cause hyperglycemia and cardiovascular abnormalities. The levels of  $11\beta$ -HSD-1 increases with gestational length and the levels of  $11\beta$ -HSD-2 decreases which leads to an increase of cortisol exposure to the fetus at the end of the pregnancy [10]. A human fetal lung binds glucocorticoids with high affinity as early as the second month of gestation [9]. Circulating levels of cortisol in the fetus are low in comparison to the maternal levels but start to increase at about 30 weeks of gestation and rising from approximately 14 nmol/L to 125 nmol/L at term. During labor the levels of cortisol double and a few hours after parturition levels of 500 nmol/L have been detected [14].

### **Exogenous glucocorticoids**

Synthetic corticoids such as Betamethasone cannot work as a substrate for the 11-beta hydroxysteroid dehydrogenase enzyme-family and is therefore able to cross the placenta and act on the fetus' organs, imitating the action of endogenous cortisol [10]. An immature lung responds in a complex way to cortisol and it involves acceleration of surfactant synthesis and secretion [3, 17] as well as lung tissue remodeling, cell differentiation and reabsorption of lung liquid [3]. Glucocorticoids in its active biological form cortisol, bind to specific intracellular receptors located in the pulmonary epithelial type II cells' cytoplasm, so called

glucocorticoid receptors (GR) [8, 9]. The receptor-ligand-complex thereafter translocate into the cells' nucleus and binds to specific DNA-sequences altering its expression. It is believed that the altered expression of co-factors accelerates the surfactant synthesis in the immature lung after steroid administration [10]. Antenatal glucocorticoids have proven to affect the immature lung structure resulting in thinner alveolar walls [9] but produce larger, and fewer alveoli in total. It has been indicated that the positive effects by antenatal glucocorticoids may be due to the structural implications exclusively [18].

### **Surfactant**

The alveolar walls are made up by a monolayer of type I and type II pneumocytes. The pneumocytes type I line 95% per cent of the alveolar walls and form the blood-air-barrier together with the basal lamina and endothelial cells. The pneumocytes type II are in contact with the alveolar surface only with their apical portion. Pneumocytes type II produce and secrete surfactant. A specific characteristic of the pneumocytes type II is the lamellar bodies in their cytoplasm. Lamellar bodies are storage units of surfactant that can be secreted into the alveolar space by exocytosis [19]. Surfactant is a lipoprotein complex[20], which is a mix of lipids (90% of total weight) and proteins (10% of total weight). There is also a very small amount of carbohydrates, less than 0,1% of total weight. 90% of the lipids consist of Lecithin and Phospholipids and the role of lipids in surfactant is to lower the surface tension. The proteins are of great importance in the adsorption process of the lipids to the air-water interface [19]. There are four types of surfactant protein; SF-A, SF-B, SF-C and SF-D. The surfactant proteins are divided into two groups depending on their attributes. SF-A and SF-D are hydrophilic surfactant proteins that regulate the surfactant secretion and recycling and also play a role in the lung's immune response system [17]. SF-B and SF-C are hydrophobic and are the hydrophobic proteins that play a role in the biophysical surfactant functions [21]. The biophysical functions of surfactant are to lower the surface tension of the alveoli, support the alveolar stability during the respiratory cycle, allow alveoli of different sizes to function with equal efficiency, increase lung compliance, help maintain the gas exchange area of the lung, counteract pulmonary edema through balancing of hydrostatic forces and to reduce the work of breathing [17, 21]. In summary the pulmonary surfactant prevents the lung from collapsing at low lung volumes [20].

### **Preterm birth and respiratory morbidity**

Approximately 11% of all live births globally are preterm and prematurely born infants constitute a relatively large compound of the newborn deaths. Preterm birth is defined as birth

before 37 completed weeks of gestation and is subdivided into very preterm birth (birth before 34 weeks of gestation) and extremely preterm birth (birth before 27 completed weeks of gestation) [16, 18]. Preterm infants are affected by all forms of respiratory morbidity at a higher rate than infants born in a more advanced gestational week [2]. Many preterm infants do not have an adequate spontaneous respiration at birth and require assisted ventilation [22]. Respiratory illness is the single most common cause of neonatal mortality and morbidity [18]. The most common causes of respiratory distress are respiratory distress syndrome, transient tachypnea of the newborn and meconium aspiration syndrome.

### **Respiratory distress syndrome, RDS**

RDS is the most common cause of respiratory distress in premature infants, and is also known as hyaline membrane disease. It has been established to correlate with structural and functional lung immaturity [23]. It is described as respiratory distress in infants that sets in within a few hours of parturition. The incidence of RDS is inversely proportional to gestational length and has been estimated to range from 30% below gestational week 28 to 5% in neonates born in a gestational week above 34 [17, 23, 24]. Surfactant deficiency was proven to be the cause of RDS as early as 1959 [9, 25]. Insufficient amounts of surfactant cause small alveoli to deflate and larger alveoli to overinflate, which creates a mixed situation of atelectasis and overdistention [26]. In turn, atelectasis causes pulmonary vascular constriction, hypoperfusion and lung tissue ischemia [23]. The name, hyaline membrane disease, is originated by the findings that insufficient amount of surfactant causes fibroblasts to invade the alveoli and alter the membranes' thickness [3]. There are also components of pulmonary interstitial edema and cellular debris in these hyaline membranes [26]. Thicker membranes compromise the alveolar exchange of oxygen and carbon dioxide and cause respiratory distress [3].

Clinical symptoms of RDS are grunting, chest wall retractions, tachypnea, hypoxia and cyanosis in a premature infant immediately after birth. Typical signs in a chest-x-ray are homogenous infiltrates [23] that are commonly called "ground glass opacity" and signs of atelectasis [18, 23]. The introduction of antenatal steroids and surfactant therapy has markedly increased the survival rate of infants suffering from RDS [26]. Antenatal steroids decrease the incidence of RDS with approximately 50% and surfactant treatment reduces the severity of RDS, the progression of the disease and the incidence of death associated with RDS [18]. According to the European Consensus Guidelines on management of neonatal respiratory distress syndrome 2013 mechanical ventilation should be avoided, and when possible a non-

invasive ventilation such as the CPAP should be used instead due to the risk of lung injury [12]. Research indicates that early surfactant treatment, at or within minutes of birth, combined with initiation of nasal CPAP significantly reduces the need for mechanical ventilation and chronic lung disease [25-28].

### **Transient tachypnea of the newborn, TTN**

This condition is the second most common respiratory disease in premature infants [29]. It is a benign condition that occurs when the timing of lung fluid clearance has failed and lung fluid remains in the lungs after parturition [23]. The risks of TTN are high in infants who are born precipitously, without active labour or with caesarean section [29]. It is a condition that usually requires minimal intervention and resolves over a 24 to 72h period but may however cause severe morbidity such as hypoxia and respiratory distress and give rise to unnecessary use of antibiotics [30]. The most typically occurring symptom is tachypnea at or within two hours of birth and chest x-ray can show signs of excess lung fluid such as diffuse infiltrates, a “wet silhouette” surrounding the heart or intralobar fluid accumulations [23].

### **Persistent pulmonary hypertension, PPHN**

Pulmonary hypertension in the newborn is generally believed to be caused by failure of the normal cardiopulmonary transition at or shortly after birth [31]. During the fetal life, most of the blood returning to the right atrium directly proceeds into the left atrium through the foramen ovale. Blood entering the right ventricle shunts through the ductus arteriosus and flows directly in to the aorta [32] resulting in that less than 15% of the combined ventricular cardiac output perfuses the lungs [33]. Before birth, the resistance in the pulmonary arteries are very high due to low oxygen tension in the alveoli. As the infant starts to breath air at the parturition, the resistance in the pulmonary arteries rapidly decreases as the oxygen tension in the alveoli increases. This reaction is mediated by mitochondrial production of ATP that in turn stimulates eNOS to produce nitride oxide causing pulmonary vasorelaxation [32]. Other mediators such as prostacyclin (PGI<sub>2</sub>) have also been detected in this reaction. In addition to this there is another mechanism of decreased levels of active vasoconstrictors [31]. When this transition fails, the pulmonary artery resistance remains high and as a result the blood is continuously being shunted into the systemic circulation due to persistent foramen ovale and ducuts arteriosus [34]. This bypassing of the pulmonary circulation leads to severe hypoxemia, which in turn may be therapy resistant against oxygen treatment [31, 35]. Risk factors of developing PPHN are intra uterine growth restriction, maternal SSRI exposure and NSAID exposure in utero during third trimester. Even though inhalation of NO is regarded as

the golden standard, PPHN still has a considerable high mortality rate. Therefore ECMO treatment is recommended when pharmacological treatment fails [32].

### **Lung maturity tests by amniocentesis**

In 1996 and then again in 2008 [36, 37] The American College of Obstetricians and Gynaecologists recommended the assessment of fetal lung maturity before elective deliveries at less than 39 weeks of gestation because of the risk of neonatal RDS [38, 39]. Predictive tests of RDS through amniocentesis have been available since 1971 [40]. The main indications for lung maturity testing have been to discriminate foetuses in high risk of developing RDS from those who will not have a significant respiratory disease [41]. It is currently performed for the purpose of delivery planning concerning patients with complicated pregnancies [42, 43]. In many conditions preterm delivery is not possible to avoid and fetal lung maturity testing is of less value in these cases. Because of this it has been suggested that lung maturity testing preferably should be considered more important when the time of delivery is elective, such as in cases with premature rupture of membranes or placenta praevia and accrete, when advanced gestation may cause increased risk for both mother and foetus [42].

However, there have been studies reporting that there has been a decrease in the amount of lung maturity tests performed in the US the last years. The studies have shown that this is due to physicians' opinions that lung maturity testing is not medically necessary for patient care, concerns about the risks of amniocentesis and patients' refusal of amniocentesis. Still, only a small number of the physicians participating in the survey agreed to the statement that they could provide the same level of care without the benefaction of lung maturity test results available today [40, 41]. The American College of Obstetricians and Gynaecologists released new recommendations this year and do no longer recommend fetal lung maturity testing for the reason that if preterm delivery is necessary it should not be delayed because of the lung maturity test results. On the other hand if it is possible to safely delay the delivery, there is no actual indication for preterm delivery [44]. These guidelines are in accordance with the Swedish tradition concerning lung maturity testing and preterm deliveries.

Due to the fact that fetal lung liquid is directly communicating with the amniotic fluid the assessments available today are based on indirect estimation of the amount of surfactant through amniocentesis [17]. To be clinically useful fetal lung maturity tests should have a high diagnostic sensitivity and high negative predictive value. Unfortunately, the tests

available today continue to be relatively poor predictors of lung maturity. One study showed that 10% of children born to hospitalized women with mild preeclampsia after a positive lung maturity test still developed RDS. This indicates that there needs to be improvements in fetal lung maturity tests available today [17, 42].

### **Lecithin/Sphingomyelin ratio**

This was the first produced biochemical assessment for fetal lung maturity. Lecithin is a surfactant lipid and increases in concentration during the third trimester. Sphingomyelin is a surfactant phospholipid but remains relatively consistent throughout the pregnancy and can therefore be used as an internal standard against which lecithin concentrations may be compared. The concentrations are determined by thin layer chromatography. A minimum of 3-4 ml of amniotic fluid is required and the current cut-off point for lung maturity is a ratio of 2.0. The L/S ratio have been proven to have a relatively good sensitivity but lack of specificity [17]. The method is difficult to perform and interpret and it also requires a few hours for analysis [39]. Another disadvantage is the fact that contamination of the amniotic fluid of either blood or meconium can influence the ratio to either a positive or negative outcome [17].

### **Phosphatidylglycerol**

The existence of phosphatidylglycerol (PG) occurs several weeks later than Lecithin and is considered a late marker of lung maturity. When detectable, it is a very strong indicator that RDS will not occur. Phosphatidylglycerol is not present in either blood or meconium, which is a major advantage. In 1983 an immunochemical approach to detection of PG was reported and it was based on the agglutination of antibodies with PG containing lamellar bodies. This biochemical assessment was named Amniostat FLM. It is rapid and not affected by meconium or blood but because of the late appearance of phosphatidylglycerol, many infants that test immature never develop RDS. Even so the test does demonstrate a high negative predictive value [17].

### **Surfactant/Albumin ratio**

The surfactant/albumin ratio is the most commonly used test today and is based on the competitive binding of a fluorescent probe to pulmonary surfactant and albumin in the amniotic fluid. It has been improved since the development in 1976. The last modification was made in 1995 and the method was renamed to TDx FLM II Assay. Recommendations by the manufacturer have set the cut-off levels to over 50 for mature lung and beneath 39 for immature lung. Several studies have shown the TDx FLM II assay to have excellent

sensitivity but to its disadvantage not quite as good specificity. The national Academy of Clinical Biochemistry recommended the surfactant/albumin ratio to become the standard routine analysis of fetal lung maturity [17]. A study has shown the correlation between antenatal administered corticosteroids and increase in the TDx FML II value the following week. This is interpreted to strengthen the fact that antenatal steroids enhance lung maturity and that the TDx FML II value is a relevant predictor of the fetal lung maturity [43].

### **Lamellar body count**

Surfactant is stored as lamellar bodies in the cytoplasm of the pneumocytes type II cells. The lamellar bodies are secreted into the alveolar space and release surfactant. This assessment method is based on a standard haematological cell counter to quantify the number of lamellar bodies in the amniotic fluid [17]. It is possible to use the haematological cell counter because of the fact that lamellar bodies are similar in size to platelets [39, 45]. Counts  $>50\,000/\mu\text{L}$  estimates maturity and counts  $<15\,000/\mu\text{L}$  suggests immaturity [17]. A disadvantage of the method is that results may differ depending of the brand of the blood cell counter but advantages are that it is inexpensive and a relatively simple test [42, 45, 46]. Studies have shown LBC to be equal the L/S ratio in sensitivity and specificity and LCB is also suggested to be equal surfactant/albumin ratio, even though no studies directly have compared the two [17, 45]. It is recommended that either lamellar body count or TDx FML II Assay should be performed as an initial test for prediction of lung maturity since both these methods are rather rapid [17, 47].

### **Risks associated with amniocentesis**

The general risks associated with amniocentesis are leakage of amniotic fluid, pregnancy loss, infection, failure to obtain a sample and thereby multiple needle punctures, contamination of amniotic fluid with blood and possibly, but rarely, direct fetal injury [48-50]. Most of the publications on this matter are studies made on amniocentesis for chromosomal analysis indication. A miscarriage risk of 1 in 200 is widely quoted and was published by the American College of Obstetricians and Gynaecologists in 2001. These numbers are approximately the same for women that have not gone through amniocentesis [51], making it difficult to know whether the miscarriage is due to the amniocentesis or to unrelated events [48]. Another conclusion that can be made is that amniocentesis is a relatively safe method performed by experienced practitioners [51]. Some studies have shown an additional risk of pregnancy loss at 0,6% compared to control groups that do not go through amniocentesis. They also prove that ultrasound guidance during the amniocentesis lower the incidence of



pregnancy loss, multiple needle punctures and the contamination of the amniotic fluid with blood [48]. The risk of pregnancy loss is naturally a great concern in the women about to undergo amniocentesis and the risk is clearly small but nevertheless existing [50].

### **Previous literature on ultrasound based assessment of fetal lung maturity**

The basic idea of ultrasonography is to send a fine beam of ultrasound waves through the human tissues to receive characteristic echo reflections and form an ultrasound image. The different grey levels in the image represent the acoustic properties of the tissue such as attenuation of acoustic waves, speed of sound and acoustic impedance [52]. Ultrasound can not measure biochemical changes in the growing lung nor histological changes but it is reasonable to assume that both morphological and biochemical changes alter the propagation properties in the fetal lung [53]. The possibilities of using ultrasound as a non-invasive method for assessment of fetal lung maturity, have been greatly explored over the last 30 years [54]. A lot of earlier studies have shown that the echogenicity of the fetal lung changes as the pregnancy progresses and also in relation to other fetal organs such as the liver, placenta and fetal gut [53, 55].

A drawback in ultrasonography diagnosing is that it is in general very subjective and dependent on the sonographer's ability to observe certain textural characteristics from the image and compare them with those developed for different pathologies in order to determine if a disease or abnormality exists or not. In order to solve this problem a computer based quantitative texture analysis model has been developed and it has proven favourable results when diagnosing liver-, breast- and thyroid diseases [52]. Due to the previous results the method was found interesting to use in fetal lung maturity assessment. A few studies have shown encouraging results in this field with evident changes in fetal lung texture correlating with increasing gestational age and also when compared to fetal liver textural characteristics [52, 53, 56]. Unfortunately because of controversial results, limitations in sample size and difficulties in recording the parameters in standardized ways, development of the results into clinically applicable solutions has so far been prevented [54].

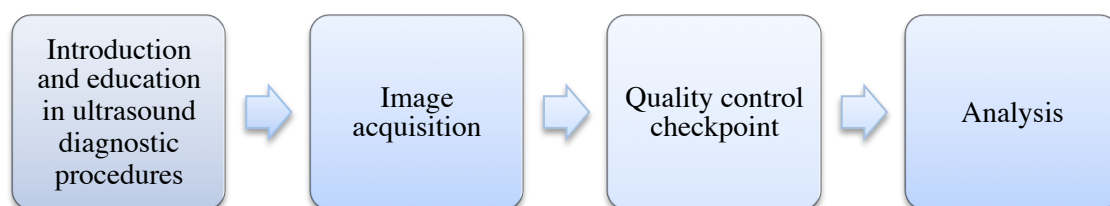
Recently a Spanish research team led by M.D Ph.D Montse Palacio has developed a quantitative ultrasound analysis algorithm, AQUA, that has been proven to be able to extract features from fetal lung that strongly correlate with gestational age [57]. The same algorithm has also been proven to correlate with TDx-FLM results with a sensitivity of 95,1%, a specificity of 85,7% and an accuracy rate of 90,3% in predicting mature and immature fetal

lungs [54]. Those results are the basis of a current multicentre study, originating from Barcelona, Spain, now examining the clinical usefulness of the quantitative texture analysis algorithm [58].

### **Procedures of ultrasound image acquisition**

During the summers of 2012 and 2013, medical student Lars Cedergren executed his master thesis project in medicine at Sahlgrenska University Hospital Östra, examining the clinical usefulness of quantitative texture analysis of fetal lungs to predict respiratory morbidity using the AQUA algorithm. In the summer of 2012, the Spanish research team approved only 21 out of 40 obtained images for analysis, which gave an approval rate of only 52,5%.

To ensure that more images met the distributed guidelines and would be approved in the future, Lars Cedergren launched a new model for quality assurance. This involved proper introduction to the ultrasound machine for future new students and the institution of a quality control checkpoint before images analysis. The checkpoint was constituted of an experienced physician in fetal ultrasound diagnostics that would double-check the images and select the preferable image to be sent for analysis. The student would give his or her suggestions of which image to be selected and the physician would either agree or disagree and give feedback in return to the student. This new quality assurance model resulted in a 100% approval rate in the summer of 2013 [59].



**Fig.1. Flow chart visualizing the proceedings of the quality assurance method developed by medical student Lars Cedergren in 2013 regarding ultrasound image acquisition.**

## **AIMS**

The primary aim of this project is to determine if ultrasound based quantitative texture analysis of fetal lungs is a practicable method to predict the risk of neonatal respiratory morbidity.

The secondary aim is to evaluate the quality assurance method developed by Lars Cedergren in image acquisition.

## **METHOD**

### **Literature review**

To collect background information and to become conversant with the current knowledge about the subject, a non-structured literature review was undertaken. PubMed was the solitary used database and the search terms used were different terms concerning fetal lung maturity and development, premature births and respiratory complications, assessment of fetal lung maturity and former research concerning ultrasonography and fetal lung maturity testing. Some articles were found through suggested related searches. The student alone without supervision or verification has conducted the literature review. As this is not supposed to be a structured literature review, the journals impact factor has not been taken into account.

### **Collection of ultrasonography images**

This project is part of a large multicentre prospective observational study originating from the Department and Research Centre of Maternal-Fetal Medicine, Hospital Clínic-IDIBAPS in Barcelona, Spain. There are a total of 17 institutes from 10 different countries participating in this study, among them Sahlgrenska University Hospital, Gothenburg. The goal of the multicenter study is to gather at least 1000 images divided in a minimum of 200 between 25 to 30 weeks of gestation, 300 between 31-35 weeks of gestation and 500 at 36-40 weeks of gestation to achieve a statistical difference. This was measured using the prevalence of RDS, approximated to 20% overall, and the estimations that 200 cases would be enough to make an

adequate difference. The Spanish research team has not conducted any power calculations. Each participating clinical center should be providing at least 50 images and preferably 100-300.

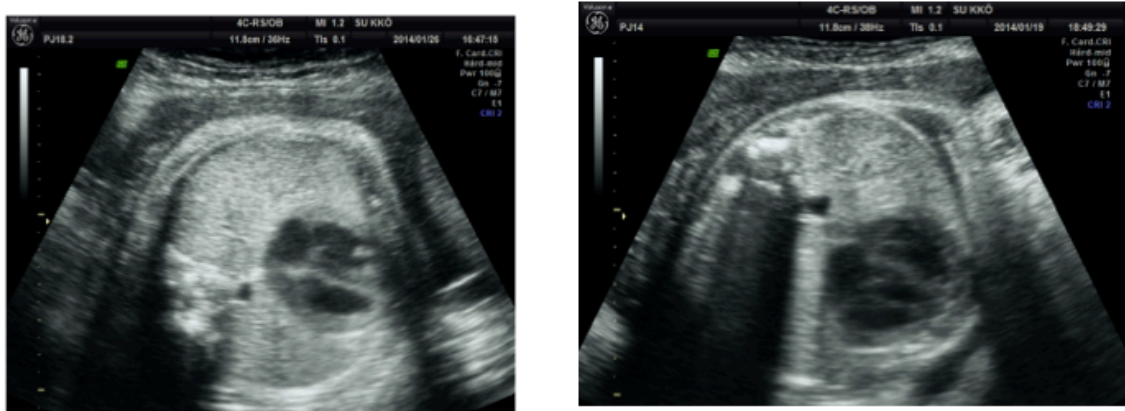
Inclusion criteria were women delivering between 25+0 and 40+0 weeks of gestation and in which an ultrasound image of the fetal lungs is acquired within 48 hours prior to delivery. Exclusion criteria were maternal steroids administered after the ultrasound image acquisition and before delivery, fetal malformations that could potentially affect lung maturity and maternal body mass index greater than 35. Images collected of fetuses that were delivered after more than 48 hours after the ultrasound image acquisition were deleted [58].

Images were collected during a period of 25 weeks, from October 2013 to March 2014. The images were primarily collected at the special delivery ward with ultrasound machine Voluson model E. The student was introduced to the ultrasound machine and to the proceedings of fetal diagnostics at two occasions in October 2013. The first included patient was informed and the first image acquisition was done with supervision. Thereafter the student obtained all the ultrasound images without further supervision. When images had been obtained and delivery had occurred within 48 hours, up to five suggested images were uploaded to drop box for evaluation of quality and selection for analysis according to Lars Cedergren's previous developed process [59]. The images were evaluated and selected by M.D Panagiotis Tsiartas. The ultrasound images had to be close-up cross-sections of the fetal thorax with a four-chamber view of the heart. To achieve as high quality as possible for the developed analysis method, guidelines were distributed from the Spanish research team (see figure 2).

**Fig.2. Guidelines on how to obtain an optimal image for analysis with QuantUS-FLM**

**Optimal image attributes**

- Spine located at 3 or 9h (horizontally)
- Cardiac pre-sets are preferred
- The thorax section must occupy almost the totality of the screen and entire thorax structure must be shown
- Avoid shadows from ribs, spine or other limb bones
- No pointers or markers
- No Doppler, colours or measurements
- Avoid post-processing



Example of an optimal image

Example of shadowing of one lung due to the location of the spine

### **Data collection procedures**

If the delivery occurred within 48 hours, a review of both the mother's and the newborn's medical records were performed concerning certain medical aspects (see table 1). These data were filled into an excel-document and double-checked by the Spanish research team before the analysis to exclude any type of incongruences. The excel-document originated from Spain and all the clinical centers involved in this study filled in an equal document. The medical record reviews were mostly conducted 48-72h after the delivery. If it was a full-term delivery without neonatal complications, the review was only conducted at this one time. If the neonate was admitted to the NICU, a review of its medical records was conducted repeatedly until discharge.

**Tab.1. Medical aspects registered for each included patient.**

- |   |   |
|---|---|
| 1. ID patient (individual code)   | 19. NICU admission (yes/no)   |
| 2. US Scan Machine (Brand)  | 20. Date of NICU admission (if any)   |
| 3. US Scan Machine (Model)  | 21. Date of NICU discharge (if any)   |
| 4. Last menstrual period (by 1 <sup>st</sup> trimester scan)  | 22. Use of oxygen therapy >40% (yes/no)                                     |
| 5. Date of scan   | 23. Respiratory support (yes/no)  |
| 6. Date of amniocentesis (if any)   | 24. Nasal CPAP (yes/no)   |
| 7. Test of lung maturity used (if any)  | 25. Nasal NIV/BIPAP (yes/no)  |
| 8. Value of test of lung maturity (if any)  | 26. Mechanical ventilation or endotracheal intubation (intubation) (yes/no) |
| 9. Result of test of lung maturity (if any)   | 27. Days of intubation (if any)   |
| 10. Date of 1 <sup>st</sup> steroid dose  | 28. HFV – high frequency ventilation (yes/no)                               |
| 11. Date of 2 <sup>nd</sup> steroid dose  | 29. Surfactant (yes/no)   |
| 12. Date of delivery  | 30. Courses of surfactant (if any)  |
| 13. Gestational age at delivery   | 31. Respiratory distress (yes/no) *   |
| 14. Mode of delivery (spontaneous vaginal delivery, operative vaginal delivery, elective cesarian section, non-elective cesarian section) |   |
| 15. Birthweight   | 32. Transient tachypnea of the newborn (yes/no)                             |
| 16. Apgar 1'  | 33. Other respiratory disorders (yes/no) **                                 |
| 17. Apgar 5'  | 34. Describe other respiratory disorder **                                  |
| 18. Umbilical artery pH   | 35. Describe other relevant conditions ***                                  |

\*Definition of RDS:

Early onset of the following signs: tachypnea, tachycardia, chest wall retractions (recession), expiratory grunting, nasal flaring and cyanosis during breathing efforts associated at least a reticulogranular pattern on chest film or oxygenation response to surfactant. As the disease progresses, the newborn may develop ventilatory failure (decreasing oxygen levels and rising carbon dioxide concentrations in the blood) and prolonged apneas

\*\* Include: PPH (persistent pulmonary hypertension), apnea, pneumothorax

\*\*\* Include: Pneumonia, meningitis, sepsis workup, sepsis (culture proven) or NEC (necrotizing enterocolitis), seizures or convulsions, IVH (intraventricular hemorrhage) grade I-II or IVH grade III-IV, hypoglycemia, feeding problems, TPN (total parenteral nutrition), hypothermia, hyperbilirubinemia, or jaundice requiring phototherapy

## **Quantitative texture analysis**

Quantitative imaging techniques of medical images are based on the development of an algorithm to improve the information obtained from the images. The purpose is to extract quantitative information from an image to detect non-visible changes and to be more of an objective path for prediction and diagnosis [60]. In other words, to turn subtle non-visible

image information into a numeric score and by those means compare it with other digital images [61]. The Spanish research team initially developed a custom made quantitative texture analysis algorithm, AQUA (automatic quantitative ultrasound analysis) that was developed to be invariant under illumination changes because it did not use the direct grey level in the image [62]. It estimates texture features based on conditional random fields (CRF) in a manner in which the texture features robustly converges to different tissue characteristics, independently to the overall acquisition context, such as scanning settings and operator skills [54, 62]. A conditional random field is a form of graphic statistical model often used for image segmentation and to combine features from different sources [62].

### **QuantUS-FLM- algorithm**

The AQUA-algorithm was however found not to be robust enough and was further developed into a new algorithm, the QuantUS-FLM (QuantusFLM®, Transmural Biotech, SL, Barcelona, Spain). QuantUS-FLM is a mathematical classification algorithm especially designed to predict neonatal respiratory morbidity through the analysis of textural image features from fetal lung images. The software is composed of two modules:

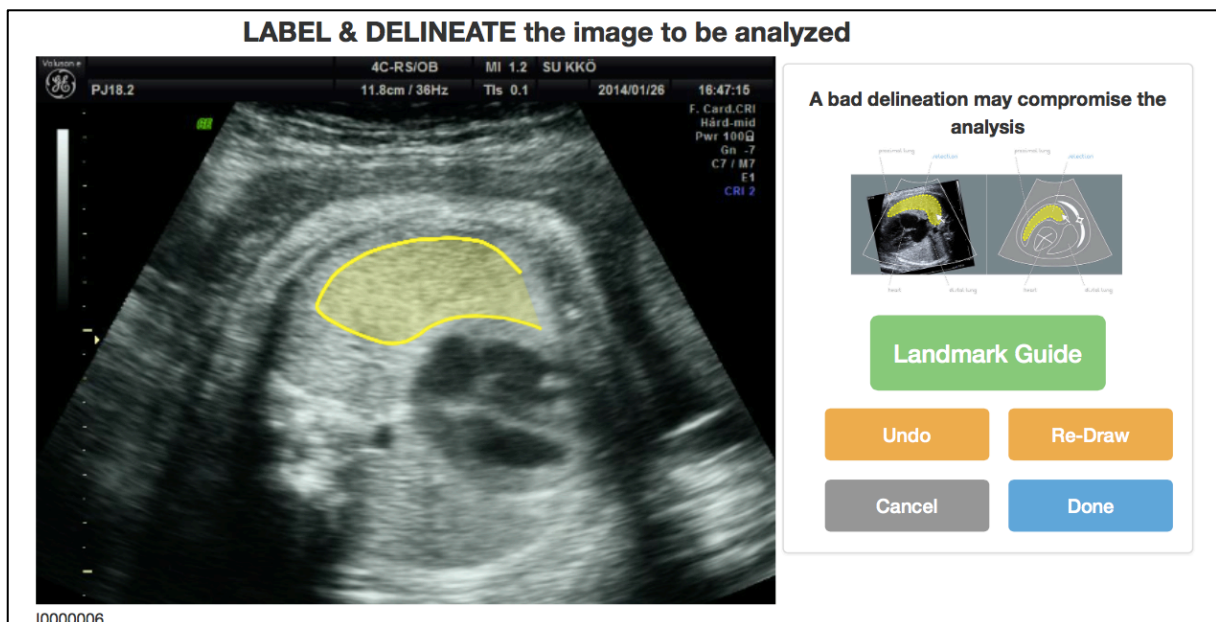
1. Texture feature extractor module
2. Classification module

The texture feature extractor module has already previously been optimized to select ultrasound image textural features that are more characteristically identifying fetal lung and that are more robust to variations occurring in image acquisition in clinical practice. The module was developed by combining several existing mathematical methods for texture extraction, in different iterations, using theoretical samples and real ultrasound images. In total the development used more than 10 million image samples and over 7 billion computerized experiments.

The second module, the classification module, is based on an algorithm that combines gestational age and the texture features obtained with the first module to predict the occurrence of neonatal respiratory morbidity. The algorithm used in this module is the result of computer training process using machine-learning methods that combined different mathematical approaches with lung ultrasound images from more than 1000 real clinical cases[63].

### Analysis with QuantUS-FLM

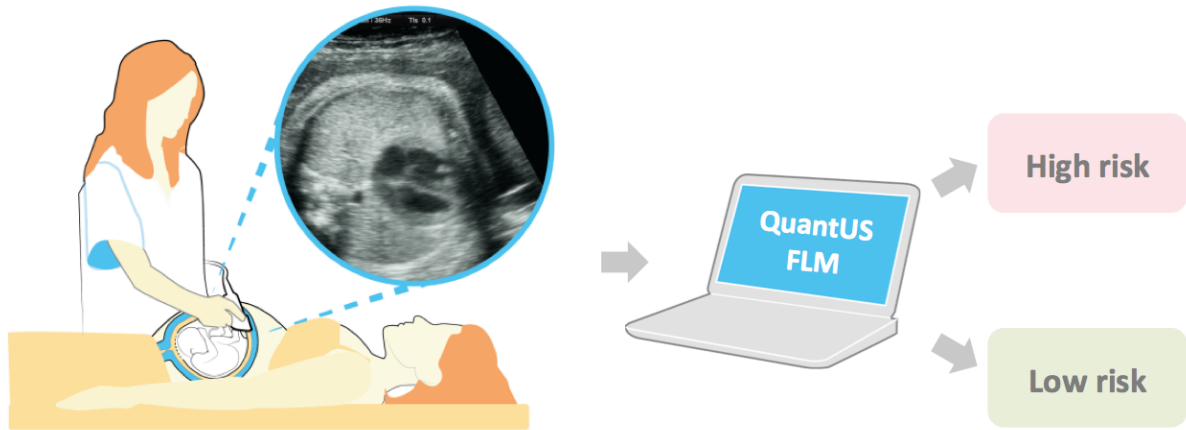
The images were digitally collected in the DICOM (Digital Imaging and Communication in Medicine) format. All images were uploaded to Spain where the research team approved them and uploaded them anonymously to their newly developed software application for on-line analysis. The student manually processed each image with a manual delineation of each lung so that no tissue except lung-tissue would be analysed by the software. The application does not check the user's delineation, and a poorly performed delineation may compromise the results. It is important that the delineated area is as wide as possible, of the proximal lung (in relation to the ultrasound probe) and not containing any blood vessels or other structures such as the heart, diaphragm or ribs (see figure 3).



**Fig.3. Visualization of the manual delineation process in the QuantUS-FLM online application.**

After the delineation process the QuantUS-FLM software was applied to extract the texture features of the delineated parts of the lungs. For each image submitted, a result of either low risk or high risk (for developing respiratory morbidity) is received within minutes of the analysis, which is an on-line procedure done through the QuantUS-FLM custom made application. A visualization of the process of this study is showed in figure 4. When all images had been run through analysis the Spanish research team sent a file in which the IDs of the images were revealed.





**Fig.4. Flow chart visualizing the image collecting process and analysis with QuantUS-FLM.**

### **Statistical analysis**

The statistical analyses have been done using SPSS and have been performed with supervision of Dr Tsiartas. All statistical analyses are based on the data retrieved from the medical record review. No comparative analyses have been used, solely descriptive analyses. The calculation of birth weight deviation score (Table 3) has been made using the Marsal method [64] taking gender, birth weight and gestational age at delivery under account to calculate the deviation from estimated expected birth weight. This calculation was done by PhD student Jonas Bacelis.

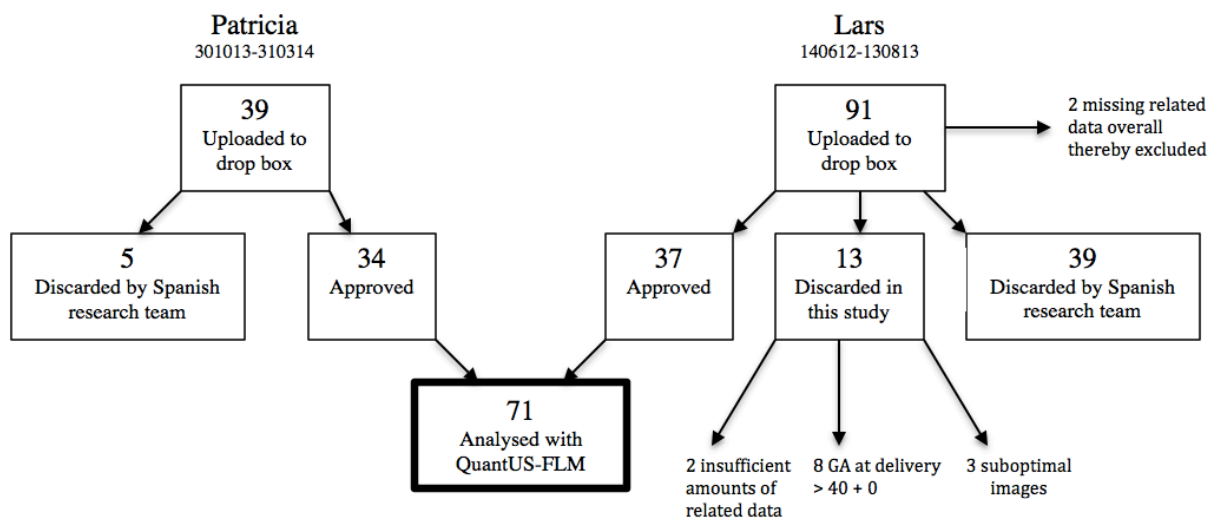
### **ETHICS**

Women admitted to the two delivery wards at the Sahlgrenska University Hospital/Östra were invited to participate in the study. They were given an oral presentation about the study as well as a written brochure and thereafter asked to sign a consent form stating that they had received the information given to them and agreed to participate out of free will. The ultrasonography-images collected were solely containing clinical information. Each participant was given an individual code only known to the person collecting the images. Any requests from the parents, regarding other ultrasonography diagnostic procedures such as malformation- or gender evaluation during the image collection, were rejected due to the student's limited diagnostic competence. This local study received an ethical approval by Regional Ethical Review Board in Gothenburg on March 19, 2012 (application approval number 184-12).

## RESULTS

A total of 59 images of individual foetuses were collected during this study. Of these 15 were excluded due to delivery beyond 48 hours from the image acquisition, two were excluded due to maternal BMI above 35 and one was excluded because of delivery before gestational week 25+0. The remaining 41 images that fulfilled the inclusion criteria were uploaded to drop box was Dr Tsiartas double checked the quality and select one of the suggested images to be analysed. Due to the low number of included patients a third classification of images were installed during the double check procedure, in addition to approved and unapproved, which was named uncertain of sufficient quality. These seven uncertain images were also sent for analysis but with the knowledge that they would most likely be disapproved. Two images were discarded during the double check procedure and 39 images were thereby sent to Spain to be anonymized and thereafter analysed in the online application.

In the end, five images were unapproved due to poor image quality by the Spanish research team, although only two of these had been classified as uncertain and the remaining three had been classified as approved during the double check procedure. In summary, a total of 34 images was eventually analysed of the images collected in the time period 30-10-13 to 31-03-14, which provides an approval rate of 87%. 37 images of neonates with no respiratory outcome, collected by medical student Lars Cedergren, were added to the 34 images collected by Patricia Johansson. The results below are based on the merged total of 71 images analysed by QuantUS-FLM, collected during 14-06-12 to 31-03-14.



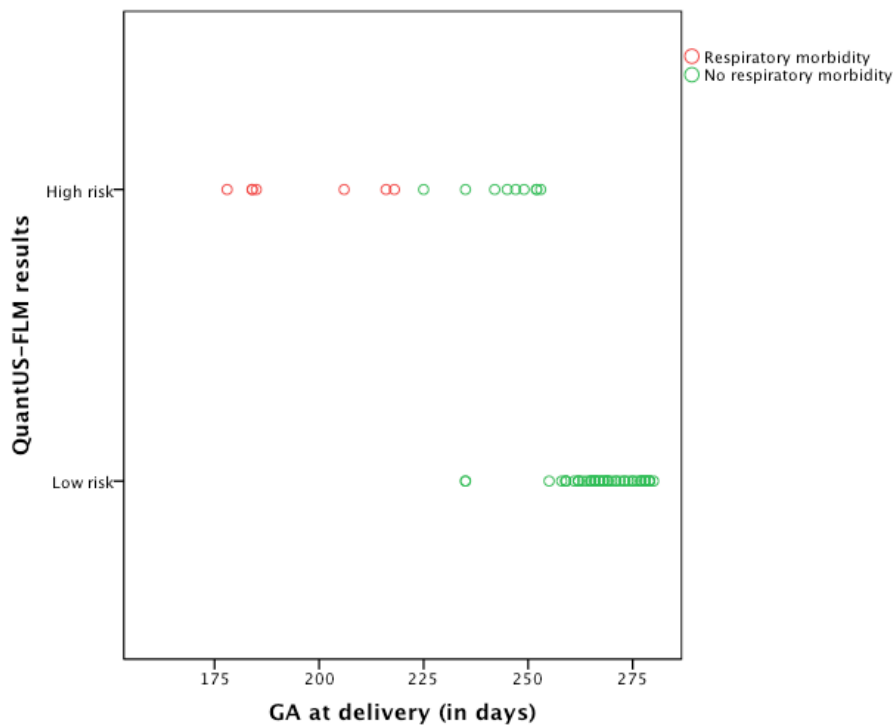
**Fig. 5. Flow chart visualizing the number of images existing in this study and the distribution of approved and discarded images.**

**Tab. 2. Maternal Demographics and Neonatal Data**

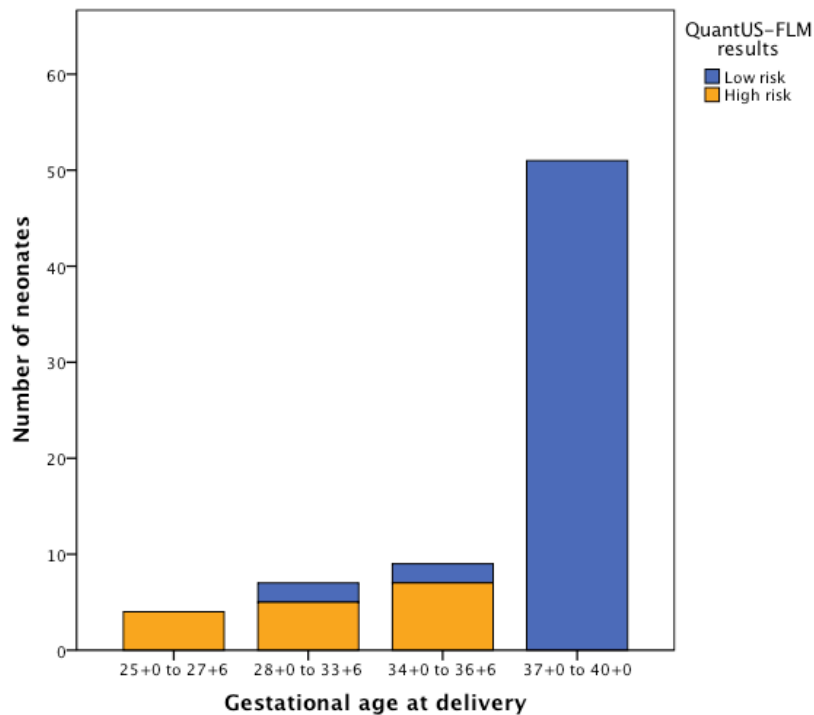
	Neonates with respiratory morbidity n=7 (9.9%)	Neonates without respiratory morbidity n=64 (90.1%)
<b>Maternal age (years, median, IQR*)</b>	<b>31 (8)</b>	<b>31 (7)</b>
<b>Nulliparous (n=, %)</b>	<b>4 (57.1%)</b>	<b>36 (56.3%)</b>
<b>Preeclampsia</b>	2 (28.6%)	12 (18.8%)
<b>PTL</b>	2 (28.6%)	5 (7.8%)
<b>PPROM</b>	2 (28.6%)	2 (3.1%)
<b>Twin pregnancy</b>	2 (28.6%)	8 (12.5%)
<b>Antenatal steroids (n=, %)</b>	<b>7 (100%)</b>	<b>5 (7.8%)</b>
<b>Vaginal delivery (n=, %)</b>	<b>3 (42.9%)</b>	<b>49 (76.6%)</b>
<b>Caesarean section (n=, %)</b>	<b>4 (57.1%)</b>	<b>15 (23.4%)</b>
<b>Gestational age at delivery (weeks+days, median, IQR*)</b>	<b>26+3 (32 days)</b>	<b>38+1 (16 days)</b>
25+0 to 27+6	4 (57.1%)	0
28+0 to 33+6	3 (42.9%)	4 (6.2%)
34+0 to 36+6	0	9 (14.1%)
37+0 to 40+0	0	51 (79.7%)
<b>Birth weight (gr, median, IQR*)</b>	<b>850 (1390-670)</b>	<b>3200 (3485-2750)</b>
0 to 1000g	5 (71.4%)	0
1001 to 2000g	2 (28.6)	4 (6.2%)
2001 to 3000g	0	20 (31.3%)
3001 to 4000g	0	35 (54.7%)
4001 to 5000g	0	5 (7.8%)
<b>NICU* admission (n=, %)</b>	<b>7 (100%)</b>	<b>11 (17.2%)</b>
<b>APGAR* score &lt; 7 (n=, %)</b>		
at 1 min	6 (85.7%)	8 (12.5%)
at 5 min	1 (14.3%)	1 (1.6%)

\* IQR = Interquartile range. PTL= Preterm labour, PPROM= Preterm premature rupture of membranes, NICU= Neonatal intensive care unit. APGAR= Test of neonatal viability in regards to appearance, pulse, grimace, activity and respiration assessed at 1 minutes and 5 minutes after birth.

As showed in table 2 the maternal age and parity are equally distributed between the group of neonates with and the group without respiratory morbidity. Maternal conditions seem to be more commonly occurring in the group with respiratory morbidity and the neonates are born with caesarean section to a greater extent when observing these numbers. Neonates in the group with respiratory morbidity were of much lower gestational age at delivery, following a much lower birth weight and constitute a higher percentage of neonates with APGAR score below 7 at 1 minute. All of the neonates with respiratory morbidity were admitted to the neonatal intensive care unit compared to 17,2% of the group without respiratory morbidity.



**Fig.6. Distribution of QuantUS-FLM results in relation to gestational age at delivery expressed in days.**



**Fig.7. Distribution of QuantUS-FLM results in relation to gestational age at delivery expressed in weeks+days.**

Seven of the neonates developed a respiratory morbidity and all of them were correctly classified as high risk with QuantUS-FLM. Another nine neonates were also classified as high risk of developing respiratory morbidity without actually developing respiratory morbidity. Within the group of low-risk none developed a respiratory morbidity. As visualized in figure 6 the low risk classified neonates were existent in gestational ages at delivery above 28 weeks of gestation and high-risk classified neonates existed in gestational ages below 36 weeks of gestation which formed a small overlap. The main quantity of low risk classified neonates was above 35+5 weeks of gestation at delivery (visualized in figure 7).

The Spanish research team were requested to confirm the accuracy of the received results. M.D. PhDc Teresa Cobo re-delineated the 71 images and received a result that was 94,4% consistent to the results above. Two of the neonates that did not develop a respiratory morbidity but nevertheless were classified as high risk turned out as low risk in the second analysis. Further on two of the neonates classified as low risk but never developed a respiratory morbidity were in opposite classified as high risk in the second analysis.

**Tab.3. Maternal and neonatal characteristics in the group of neonates with high risk QuantUS-FLM results.**

	Respiratory outcome	GA at delivery (weeks+days)	Maternal morbidity	Antenatal steroids	Type of delivery	Admission to NICU >1 week	Weight-deviation score
1	RDS	26 +3	Preeclampsia	Yes	NCS	Yes	-37,8%
2	TTN	31+1	Preeclampsia	Yes	NCS	Yes	-24,1%
3	Other	30+6	PPROM	Yes	NCS	Yes	-19,4%
4	RDS	26+2	PTL	Yes	IVD	Yes	-14,7%
5	RDS	26+2	PTL	Yes	IVD	Yes	-12,7%
6	RDS	25+3	PPROM	Yes	SVD	Yes	+3,1%
7	RDS	29+3	GH, IUGR	Yes	NCS	Yes	-55,7%
8	None	35+2	PTL	No	SVD	Yes	-19,9%
9	None	36+0	PTL	No	SVD	No	+4,4%
10	None	35+0	Vaginal bleeding	No	IVD	No	-2,0%
11	None	34+4	Preeclampsia	No	IVD	No	-11,2%
12	None	36+1	No	No	IVD	No	-15,2%
13	None	36+0	Preeclampsia	No	IVD	Yes	-36,8%
14	None	33+4	GH	Yes	NCS (failed induction)	Yes	-31,1%
15	None	32+1	PTL	Yes	ECS	No	-6,6%
16	None	35+4	PTL	No	SVD	No	+32,1%

RDS= Respiratory distress syndrome, TTN= Transient tachypnea in the newborn, Other = see previous specification, in this case apnea, PPROM= Preterm premature rupture of membranes, PTL= preterm labour, IUGR= Intrauterine growth restriction, GH= Gestational hypertension, NCS= Non-elective caesarean section, IVD= Induced vaginal delivery, SVD = Spontaneous vaginal delivery, ECS= Elective caesarean section, NICU= Neonatal intensive care unit

Nine individuals were classified as high risk and did not develop a respiratory morbidity. They are described in more detail in table 2. All were born between 32 weeks of gestation to 36 weeks of gestation and had a close to equal amount of maternal conditions as the true positives. Only two of them received antenatal steroids and the weight-deviation score varied from -36,8% to +32,1%. Vaginal delivery, induced or spontaneous, was overly represented in this group of neonates.

## **DISCUSSION**

The results in this master thesis project might indicate some optimism concerning QuantUS-FLM's ability to predict neonatal respiratory morbidity.

### **§1 Clinical Relevance**

The most interesting results are the overlap of high risk and low risk test results in the gestational ages 28+0 to 33+6 and 34+0 to 36+0. These are the groups of pregnancies with the most clinical relevance. In these groups the obstetrician is facing decisions concerning delivery planning and has to balance the benefits of a preterm birth on both mother and child against the obvious risks of preterm births for the neonate. A reliable test that could predict the risks of respiratory complications if delivered within 48 hours would strengthen the comprehensive assessment of each case. Also antenatal steroids are not routinely given in the group of neonates born in gestational week 34-36 due to the uncertain long-term adverse effects (see background). A theoretical possible way of using a high-risk result in this group would be to use as an indication for antenatal steroids. Also, as stated in some published articles on the subject, a non-invasive test that could predict fetal lung immaturity could be used repeatedly to estimate the effects of the antenatal steroids given[65].

Unfortunately there are not enough patients included in this study to be able to see any real indications. QuantUS-FLM did detect all of the neonates that developed respiratory morbidity but only three of these were born in the overlapping groups discussed above and none of these were born in week 34+0 to 36+0.

Research in a larger scale on the subject is currently on going at the Clinical Hospital, Barcelona, Spain, led by M.D Ph.D. Montse Palacio and M.D Ph.D. Teresa Cobo. As described earlier their study is formed as a multicentre study in which several clinical centres and countries are involved to gain more power to their results. The data collection phase of this study was recently closed and any results have yet not been released. The intentions are to publish an article, which already has been sent to the Journal Of Obstetrics and Gynaecologists for approval. Previous studies of the predecessor to QuantUS-FLM, AQUA, have shown that the algorithms results strongly correlate to gestational age and also to fetal lung maturity testing with TDx-FLM [54, 57]. It will be interesting to see the results and to compare them to other non-invasive ultrasound based methods to predict fetal lung maturity such as fetal lung to liver ratio. That method has recently proven in one study to predict fetal

lung immaturity with a prediction rate of 96%[65]. The future will have to state which method really is the most practicable in clinical praxis.

### **§ High risk neonates**

Nine neonates were predicted to develop RDS, TTN or other respiratory morbidity but did not. Three of these were admitted to NICU but none were in need of any respiratory support. The drawback of receiving results such as this in a clinical situation is the risk of prolonging a complicated pregnancy when is not really necessary. A prolonged pregnancy can in certain situations entail risks for both mother and child. No neonates were predicted to not develop a respiratory morbidity but eventually did anyway. That is promising results since it would possibly be devastating in a clinical situation.

No general conclusions could be made of these nine individuals that would explain why they received a high-risk result without developing respiratory complications. Vaginal delivery is more commonly occurring in this group, which may have contributed to the fact that none of these nine neonates developed a respiratory morbidity. That point of view would be cohesive with an epidemiological study conducted in Gothenburg 1986, which stated that RDS is more commonly occurring in neonates that were born through caesarean section compared to vaginal delivery[66].

A higher amount of high-risk results were observed in neonates with low birth weight and low gestational age at delivery. A possible explanation is the fact that the algorithm takes under account the gestational age at delivery in the analysis. The gestational age of delivery has previously been proven to be a strong predictor of respiratory morbidity[67], naturally, and therefore must be included in such an algorithm. Also the texture of the fetal lungs differs along the gestational age and therefore it is a very strong factor and important to include in the algorithm. Perhaps the software needs to be custom made for different gestational ages to ensure that gestational age at delivery does not work as a confounder. There is a risk that the QuantUS-FLM is more likely determining whether a foetus is preterm or full-term than actually predicting the risk of neonatal respiratory morbidity.

### **§ Analysis process**

In this study the delineation and analysis results was made by and received directly by the student. This is an advantage in an educational point of view as the student were given the opportunity to take part in each step of the project giving a comprehensive view. In a more scientifically point of view it would have been better if a blinded and more experienced



person would have made the delineation as the student only had a few months of experience in evaluating ultrasonography-images of fetal lungs. There are primarily two risks with having the same person collecting the image and later doing the delineation in a study like this. The first is the risk that not only lung tissue got analysed because of poor delineation and the second that the person doing the analysis could try to manipulate the results because of knowledge of the real outcome. On the other hand, this is a clinical study of a diagnostic test that is intended to be used by obstetricians at delivery wards who will both be collecting the images and perform the delineation process.

The advantages of the QuantUS-FLM are that it is fairly easy for physicians and other health care professions to use the on-line application as well as the results are received very quickly, within minutes of approval of analysis. The ultrasound image is also relatively easy to collect, and especially to obstetricians and gynaecologist used to working with ultrasound diagnostic procedures. There is no need to take under account a long period of training of image acquisition and the acquisition is not time consuming. Ultrasonography is also a very gentle diagnostic method with no maternal or neonatal adverse outcomes. It does not come with any pronounced discomfort or risks associated with the invasive methods.

### **§Power**

A disadvantage of this master thesis project is the low power it holds. This could partly be explained by the pre-requisites and formation of the study combined with the low incidence of RDS and other neonatal respiratory complications. These factors led to difficulties in achieving enough included patients as would have been statistically desirable. As this is only a local project within a larger multicentre study this was never an achievable goal, although a higher number of included patients would have provided stronger indications regarding the ability of QuantUS-FLM to predict respiratory morbidity.

### **§Formation of the study**

In this master thesis project the student was not able to spend time at the delivery ward every day due to other classes in parallel. To be able to make good timings of ultrasound image acquisitions in relation to delivery, it has become evident during this project that it is of great importance that the researcher can indeed spend a lot of time at the delivery wards, being well informed by the physicians concerning the possible candidates health and delivery status. The strategy in this study was to collect ultrasound images of the possible candidates that fulfilled the inclusion criteria when possible and then delete the images if delivery did not occur within

48 hours. The disadvantage of not being able to spend time at the delivery wards every day lead to missing to include a lot of neonates who eventually developed a respiratory morbidity. This disadvantage is something to take into account when developing similar projects in the future. Important to mention is the impossibility to predict whether a foetus will or will not develop RDS and also the difficulties in estimating whether or not a foetus will be delivered within 48 hours from the image acquisition. The inclusion criteria provided a very small time window in which the image could be obtained and this combined with the student's pre-requisites made it even more difficult.

With some help from neonatologist Dr Lennart Stigsson we were able to receive some statistics of the local incidence of RDS in Gothenburg through the Perinatal Quality Register, and also the number of neonates admitted to NICU the last year. A total of 30 neonates developed RDS during almost the entire image-collecting phase of this study and these were also delivered between 25+0 to 40+0 weeks of gestation. Four of these were included in this study, which represents 13,3% of the possible RDS patients. This indicates that if more continuous time could have been spent at the delivery ward a higher percentage of included neonates that were born with respiratory complications would possibly have been achieved.

### **§Evaluation of the quality assurance model of image selection**

The quality assurance model introduced by Lars Cedergren has proven to pay off when introducing new students to the proceeding of ultrasound image acquisition. During his first image-collecting phase he received an approval rate of 52% and in this study the student received an approval rate of 87%. This indicates that proper introduction and education at the beginning and thereafter close check ups and quality control checkpoints may play a part in the increase of approval rate. Although, regarding the double-check procedure, since all the images classified as uncertain of sufficient quality was sent for approval to the Spanish research team it was found that only two of the seven uncertain images got disapproved in the end but in opposite three images that had been classified as approved eventually got disapproved by the Spanish research team. This raises question marks to whether the approval/disapproval process is a very subjective process or if even clearer guidelines and image examples should be provided for each clinical centre.

## **CONCLUSIONS AND IMPLICATIONS**

Due to lack of power this study cannot provide any conclusions except a small indication that the QuantUS-FLM algorithm could possibly be a practicable method to predict the risk of neonatal respiratory morbidity. To be able to draw any reliable conclusions the study population would have had to been a great deal larger.

Conclusions concerning the quality assurance model in image acquisition are that it may have contributed to the increase the number of approved images for a first time student. If further improvements are made in introduction and education in ultrasound diagnostic technique as well as becoming more consistent in the double check procedure it might be possible to even further increase the approval rate.

## Populärvetenskaplig sammanfattning

### Ultraljudsbaserad analys – en icke-invasiv metod för att förutspå andningsstörningar hos nyfödda

Globalt sett är ungefär var tionde födsel som sker för tidig, vilket brukar menas att barnet föds innan graviditetsvecka 37. De svårigheter som barnen kan drabbas av är framförallt andningsstörningar. Detta beror på att fosters lungor utvecklas väldigt sent, ungefär i graviditetsvecka 24 och är egentligen inte helt färdigutvecklade ens när barn föds i fullgången tid. Detta kan leda till ett tillstånd som kallas RDS, Respiratory Distress Syndrome, vilket innebär att lungorna hos barnet inte är helt färdiga och saknar ett viktigt ämne som håller lungblåsorna öppna så att barnet kan tillgodose sig med syre. Detta ämne kallas surfaktant. Andra vanliga andningsstörningar är till exempel att barnet har kvar fostervatten i lungorna längre än de ska och behöver syrgas eller att barnet drabbas av andningsuppehåll och behöver andningsstöd av respirator.

Det finns tillstånd där förlossningsläkare väljer att förlösa ett barn trots att det är för tidigt på grund av att det utgör en hälsorisk för både mamma och barn om graviditeten fortsätter. Det är i sådana situationer ett test som kan förutsäga om fostret är redo att förlösas eller inte med avseende på andningen hade varit viktigt. Detta för att förlossningsläkaren då kan vänta med förlossningen om det behövs och är möjligt.

Idag kan man mäta fosters lungmognad genom ett fostervattensprov där man försöker mäta mängden surfaktant i fostervattnet. Detta är en invasiv metod där en nål sticks in i mammans livmoder och fostervatten som omger fostrets sugts ut. Detta medför obehag och risker som infektioner efteråt, läckage av fostervatten genom hålet och en mycket liten, men inte obefintlig risk för missfall. Detta görs idag inte i Sverige på grund av att man inte tycker att det är en tillräckligt bra metod. I Spanien försöker man nu istället utveckla ett datorprogram, QuantUS-FLM, som ska kunna tolka ultraljudsbilder på fosters lungor och förutsäga om de har en risk för att drabbas av andningsstörningar efter en förlossning. Detta examensarbete är en del av Spaniens multicenterstudie där flera olika länder deltar. Ett liknande examensarbete har nyligen gjorts av en annan student där man visade att det kan vara svårt som student att kunna ta tillräckligt bra ultraljudsbilder. Den studenten utvecklade då ett system för att introducera och lära nya studenter att ta tillräckligt bra bilder och det systemet vill vi också utvärdera hur det fungerar.

**Studiens genomförande:**

Vi samlade in ultraljudsbilder på fosters lungor från gravida kvinnor som av olika anledningar låg inne på Östra sjukhusets specialförlossning- eller normalförlossningsavdelning i graviditetsvecka 25 till 40. Bilderna togs inom 48 timmar till en förlossning och efter förlossningen dokumenterades hur barnet mådde med avseende på andningen och om mamman hade några sjukdomar i botten. Dessa bilder tolkades senare i ett datorprogram där man fick ett resultat som sade antingen hög risk för att drabbas av andningsstörning eller låg risk.

**Studiens resultat:**

Datorprogrammet klassificerade alla barn som drabbades av andningsstörning som hög risk, men även nio andra barn klassificerades som hög risk utan att drabbas av andningsstörning efter förlossningen. Av alla barn som klassificerades som låg risk drabbades ingen av någon andningsstörning.

I det tidigare genomförda examensarbetet av en annan student blev nästan hälften av bilderna underkända för att de var av för dålig kvalitet. I den här studien blev bara 15 % underkända.

**Slutsatser av studien:**

På grund av att studien enbart innehåller bilder från totalt 71 foster är det inte tillräckligt för att kunna dra några vetenskapliga slutsatser kring resultatet. Mer forskning behöver göras, vilket pågår i större skala i Spanien just nu men några resultat har ännu inte fått fram. Man kan se en indikation att datorprogrammet fungerar. En nackdel av falska positiva testresultat, det vill säga de foster som felaktigt klassificeras som hög risk utan att drabbas av andningsstörning, är att det i kliniska situationer kan leda till att man låter en komplicerad graviditet fortsätta längre än man borde för att testet felaktigt säger att fostret behöver det.

Denna studie visade även att det introduktions- och kvalitetssäkringssystem som utvecklades av den föregående studenten fungerar med avseende på att öka andelen bilder som blir godkända för analysering med QuantUS-FLM.

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