TITLE PAGE

PREDICTION OF NEONATAL RESPIRATORY MORBIDITY BY QUANTITATIVE ULTRASOUND LUNG TEXTURE ANALYSIS: A MULTICENTER STUDY.

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CONFLICTS OF INTEREST

MP, TC, JD and EG have served as scientific advisors to Transmural Biotech.

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Quantitative analysis of fetal lung texture predicted neonatal respiratory morbidity with an accuracy comparable to invasive tests assessing fetal lung maturity.

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ABSTRACT

BACKGROUND Prediction of neonatal respiratory morbidity may be useful to plan delivery in complicated pregnancies. The limited predictive performance of the current diagnostic tests together with the risks of an invasive procedure, limit the use of fetal lung maturity assessment.

OBJECTIVE To evaluate the performance of quantitative ultrasound texture analysis (quantusFLM®) to predict neonatal respiratory morbidity in preterm and early-term (<39.0 weeks) deliveries .

STUDY DESIGN A prospective multicenter study in 20 centers worldwide. Fetal lung ultrasound images were obtained at 25.0-38.6 weeks' gestation within 48 hours of delivery, stored in DICOM format and analyzed with quantusFLM®. Physicians were blinded to the analysis. At delivery, perinatal outcomes and the occurrence of neonatal respiratory morbidity, defined as either respiratory distress syndrome or transient tachypnea of the newborn, were registered. The performance of the ultrasound texture analysis test to predict neonatal respiratory morbidity was evaluated.

RESULTS A total of 883 images were collected but 17.2% were discarded due to poor image quality or exclusion criteria, leaving 730 observations for the final analysis. The prevalence of neonatal respiratory morbidity was 13.8% (101/730). quantusFLM® predicted neonatal respiratory morbidity with a sensitivity, specificity, and positive and negative predictive value of 74.3% (75/101), 88.6% (557/629), 51.0% (75/147), and 95.5% (557/583), respectively. Accuracy was of a 86.5% (632/730) and positive and negative likelihood ratios were 6.5 and 0.3, respectively.

CONCLUSIONS quantusFLM® predicted neonatal respiratory morbidity with an accuracy similar to that previously reported for other tests with the advantage of being a non-invasive technique.

Key words

Fetal lung maturity, ultrasound, sonography, computational methods, quantitative texture analysis, amniotic fluid analysis, amniocentesis, respiratory distress syndrome, transient tachypnea, neonatal respiratory morbidity, biomarker, diagnostic indices, predictive values.

INTRODUCTION

Neonatal respiratory morbidity (NRM) due to either respiratory distress syndrome or transient tachypnea of the newborn is the most common complication in infants born preterm and early term.[1-3] Assessment of fetal lung maturity for the prediction of NRM may be relevant, particularly after 34 weeks, when the risk of NRM ranges 5-20%, in order to better assess the risk/benefit ratio of elective delivery in late pregnancy complications[4-6] and/or the use of corticoisteroids.[7, 8] In current clinical practice, evaluation of the risk of NRM relies on the study of different components of the amniotic fluid and requires an amniocentesis.[9, 10]

Prediction of fetal lung maturity using fetal ultrasound has long been proposed as a non-invasive alternative to amniocentesis.[11, 12] Several approaches using computer analysis of fetal lung ultrasound images have been attempted over the last 25 years including gray scale measurements,[13, 14] lung tissue motion[15, 16] or the relation between image features of fetal lung versus placental or liver tissue.[17] These studies generally showed a good correlation with NRM but the diagnostic accuracy was insufficient for clinical use. However, over recent years image resolution of fetal ultrasound and computer image processing have evolved immensely. Quantitative texture analysis is a powerful technique to extract information from medical images and quantify tissue changes non-visible to the human eye, allowing training of computer programs that may predict clinical events.[18, 19] Earlier studies reported that texture analysis can be applied to fetal lung ultrasound images and correlates with gestational age[20] and with the results of fetal lung maturity tests in amniotic fluid.[21] In a recent single-center study, we tested a software based on quantitative texture analysis of fetal lung (quantusFLM®) trained to predict NRM. The software achieved a

predictive accuracy similar to that commonly reported for fetal lung maturity tests in amniotic fluid.[22]

Here we report the results of a large multicenter study designed to evaluate the performance of quantusFLM® to predict NRM. Fetal lung ultrasound images were obtained for analysis within 48 hours of delivery in a large cohort of pregnancies at 25.0-38.6 weeks' gestation. Neonatal respiratory outcomes were prospectively recorded and the ability of the software to predict NRM was analyzed.

MATERIAL AND METHODS

This was a prospective multicenter study involving 20 centers. Patients were recruited from June 2011 to December 2014. Eligible cases included pregnancies between 25+0 and 38+6 weeks gestation and in whom an ultrasound was obtained within 48h of delivery. Cases were considered non-eligible if corticosteroids were used for lung maturity between the ultrasound and delivery, when maternal BMI was ≥ 35 and when fetuses had known congenital malformations. Furthermore, neonates with the following conditions were excluded: neonatal sepsis, umbilical artery pH <7, hemodynamic failure, symptomatic anemia (hemoglobin <12mg/dl), postnatal diagnosis of structural or chromosomal abnormalities and meconium aspiration, since these conditions could directly predispose or lead to NRM irrespective of lung maturity.

Ultrasound images were obtained following a detailed acquisition protocol. Briefly, an axial section of the fetal thorax at the level of the four-chamber cardiac view was magnified by adjusting only depth, but not the zoom option, until the thorax occupied about two thirds of the screen, avoiding obvious acoustic shadows from the fetal ribs (Figure 1 A). Images were acquired without any type of post-processing manipulation such as smoothing, color Doppler, or any calipers or pointers. The use of tissue harmonic imaging and adjustment of image settings such as gain, frequency and time-gain compensation were left to the discretion of the physician performing the ultrasound scan.

Before starting recruitment, each center submitted a minimum of five ultrasound images of the fetal lung according to this acquisition protocol, which were reviewed by

imaging engineers' (EB and AP) to ensure that quality criteria were fulfilled and if not further images were requested as appropriate. All study images were collected and stored in the original Digital Imaging and Communication in Medicine (DICOM) format and sent to the coordinator via a file transfer protocol. DICOM scans were anonymized removing all the information related with the patient. To track the scan a new random number was generated for each new image. Lung images for the study were then inspected for image quality control by the engineer's team, and discarded if one or more of the requirements mentioned above were not fulfilled. Images passing the quality criteria were then loaded via internet through a restricted access to the commercial software website and delineated using the quantusFLM® web interface (quantusFLM®, Transmural Biotech, Barcelona, Spain). Delineations were performed either by the same clinicians acquiring the images at each participating center, or by research clinicians at the coordinating center. Delineation of the region of interest (ROI) included the largest possible area of the fetal lung proximal to the transducer, avoiding the heart and great vessels (Figure 1 B). The web software contained an automatic filter to accept the delineation only when it contained at least 400 pixels. Delineated ultrasound images were then analyzed automatically with quantusFLM®. Features of the software used by quantusFLM® have been described in detail elsewhere.[22] The software contains algorithms that analyze the textural patterns of the area delineated in the ultrasound image. These algorithms have been "trained" by means of a machine learning approach to estimate the probability of NRM, using hundreds of cases of fetal lung ultrasound images in which the occurrence of NRM was known. The software used in this study utilizes different sequences of texture features adapted to gestational age ranges.[16] Therefore, gestational age in weeks was not used

to calculate any *a priori* risk of NRM, but to decide the specific algorithm used to calculate the probability of NRM. The software used in this study provided categorical results, i.e. either “high” or “low” risk for NRM.

For each case recruited, the centers prospectively recorded the maternal baseline characteristics and the neonatal outcomes in a database purposely designed for this study. Anonymized clinical information from each case was submitted to the coordinator through a customized file transfer protocol and stored in a database available only to the clinical researchers of this project (MP and TC) who confirmed eligibility criteria and the absence of exclusion criteria for each case. Analysis of neonatal clinical information was supervised by a neonatologist (FB). The study protocol was approved by the coordinator’s Institutional Review Board (2011/6291, 2013/8892). Patients included in the study were receiving care in the participating institutions and enrolled either in a specific protocol for the evaluation of fetal lung maturity, in studies involving the use of fetal ultrasound or in studies where ultrasound was used as part of the clinical management approved by the local review boards. All patients included in the study gave written informed consent for the use of ultrasound images and perinatal data. None of the observations here reported has been previously used in another study.

The primary clinical outcome of the study was NRM including respiratory distress syndrome (RDS) or transient tachypnea of the newborn (TTN). Respiratory distress syndrome was defined based on clinical criteria, including grunting, nasal flaring, tachypnea and chest wall retraction, or the need for supplemental oxygen, together with typical chest radiography findings and admission to the neonatal intensive care unit for respiratory support.[2] Transient tachypnea of the newborn was diagnosed

based on early respiratory distress (isolated tachypnea, rarely grunt, minimal retraction) and a chest X-ray showing hyperaeration of the lungs and prominent pulmonary vascular pattern.[23]

The performance of quantusFLM® to predict NRM was analyzed by the clinical researchers of this project (MP, TC) by matching quantitative ultrasound analysis and clinical outcome. Descriptive statistical methods were used to summarize the distribution of all the variables: for continuous variables mean and standard deviation were obtained, and for categorical variables frequencies and percentages were reported. Descriptive statistics were performed with R language (R Foundation for Statistical Computing, Vienna, Austria, 2015; https://www.R-project.org).

RESULTS

A total of 883 cases were recruited. Of these, 135 (15.2%) were discarded after image quality control and 18 (2.0%) were excluded due to one or more clinical exclusion criteria (42/164, 20.4% in the 25-33.6 weeks' group and 111/566, 16.4% in the 34.0-38.6 weeks' group), leaving a total of 730 images for analysis (Figure 2). The final number of cases included per center and the ultrasound equipment locally used are described in the supplementary material (Tables 1S and 2S). The clinical characteristics of the pregnant women enrolled in the study and relevant conditions for which ultrasound was indicated are detailed in Table 1. The study included: 17 (2.5 %) women at<28 weeks; 128 (18.7 %) women at 28.0-<34.0 weeks; 176 (25.7%) women at 34.0-<37.0 weeks; and 364 (53.1%) women of ≥ 37.0 weeks of gestation. Perinatal and neonatal outcomes and the characteristics of the respiratory support are shown in Tables 2 and 3, respectively.

The prevalence of NRM was 13.8% (101/730), of which 66.3% (67/101) were diagnosed as RDS and 33.7% (34/101) as TTN. All newborns diagnosed with RDS were treated with at least one of the following: oxygen higher than 40%, continuous positive airway pressure, or non-invasive ventilation, or high frequency ventilation and an endotracheal tube for invasive ventilation, or surfactant use. quantusFLM® analysis predicted the occurrence of NRM with a sensitivity, specificity, positive predictive value, and negative predictive value of 75/101 (74.3%), 557/629 (88.6%), 75/147 (51.0%), and 557/583 (95.5%), respectively. Accuracy was of 632/730 (86.5%) and positive and negative likelihood ratios were 6.5 and 0.3, respectively. The predictive performance stratified by gestational age is shown in Table 4.

COMMENT

Principal findings of the study

The main finding of this large multicenter study is that quantitative texture analysis of fetal lung ultrasound images predicted NRM with a similar accuracy to that of laboratory tests using amniotic fluid, which have reported sensitivities and specificities ranging from 74 to 89% and from 54 to 89% respectively, [9, 24, 25] although a wide range of figures has been reported (Table 5 and 3S). Furthermore, the observed risk of respiratory neonatal morbidity by gestational age described in a large cohort of late preterm and early term infants published recently (Table 4S)[2] is similar than the predicted one here.

Results of the study in the context of other observations

Several attempts have been made to predict fetal lung maturity using ultrasound images. Serizawa[13] and Maeda[14] compared the ultrasonic gray level histogram width of the fetal lung and liver while Bhanu Prakash et al.[17] compared the values of fetal lung to those of liver. La Torre et al.[16] correlated several patterns of fetal breathing movements with fetal lung maturity tests, and Tekesin et al.[26] evaluated the mean gray value of fetal lungs. The accuracy to identify NRM in all these studies has ranged from 73% to 96% (Table 3S). However, no prospective studies have been conducted to validate the associations observed above. The approach used in this study was different from previous attempts to non-invasively assess fetal lung maturity. The method used here is based on the combination of texture extraction with machine learning methods, allowing the identification of texture patterns in the ultrasound image that correlate with the clinical outcome. This approach has been shown to be

reliable and robust to small variations in the conditions of the image acquisition, including depth and changes in the gain of the image and does not need other tissues to be compared to (placenta, fetal liver…).[20] Besides, a previous pilot study reported on the ability of this non-invasive technology to predict NRM.[22]

Clinical implications

Liggins and Howie[27] stated that the use of antenatal corticosteroids could enhance fetal lung maturity in preterm pregnancies and as a result, corticosteroids use is common practice in pregnancies up to 34 weeks' gestation. [28-30] Now, the question whether late preterm fetuses may benefit of such an intervention is on the rise.

The practice of testing for fetal lung maturity is extremely variable worldwide, being widely used in some areas and completely ignored in others. Estimation of fetal lung maturity might reduce the use of corticosteroids in late preterm deliveries (34 to 36 weeks' gestation), where the risk of NRM is relevant but relatively low, ranging 10% to 20% . As recently shown, steroids decrease by one third the occurrence of NRM in late preterm deliveries,[8, 31-34] and the number needed to treat to reduce one case of NRM in the circumstances described is 25[8]. These findings have resulted in the publication of a SMFM Statement on the use of antenatal corticosteroids in the late preterm period[35] which recommends treatment under the strict inclusion criteria of the ALPS study, although warning against overtreatment in those cases not meeting the inclusion criteria. Even if mid and long term follow-up of babies exposed to corticosteroids has shown no adverse effects or no benefits,[36-39] antenatal corticosteroids might be associated with potential side effects related to overexposure later in life[40-42] particularly in term-born babies.[43, 44] A substantial proportion of fetuses

treated with corticosteroids are delivered long after one week of the initial dose or even at term.[45-50] Rescue doses are debatable[51, 52] and benefits and risks have to be evaluated when repeated doses are considered long after an initial course was given early in pregnancy[53-55] or if an early term elective cesarean section is planned.[56] Thus, strategies to define the target population, are urged.

On the other hand, the fear of overtreatment has to be counterbalanced against the fact that restrictive messages may limit the use of corticosteroids in those cases which the intervention has been proven of benefit and in which additional information from quantusFLM® is of limited value (i.e. preterm delivery at 25 weeks). For instance, some data showed that among cases with potential benefit, only 80% of cases receive one dose and 70% received two doses.[57] On the contrary, there are studies that show that a wide use of corticosteroids might not be of benefit in all contexts.[58]

All these aspects have been discussed in recent reviews and therefore, the issue remains controversial.[59, 60] It is in this context that the selection of a low risk group for respiratory morbidity by a non-invasive tool might reduce exposure in a large fraction of pregnancies, avoiding the risks of overexposure in an unselected population and optimizing intervention in those cases in which it is needed.

Additionally, a common argument against testing for fetal lung maturity is that there is either a clear indication for elective preterm delivery or there is not, in which case the results of fetal lung maturity would not be of help.[4, 61] This view might be challenged by studies reporting that about a 23% of deliveries in late-preterm deliveries had no clear indication for delivery,[62] or that they were delivered after a “non-evidence based” indication.[63] Therefore, a fraction of complicated pregnancies may fall within a

grey zone, in which elective delivery may be considered as an option, but there is not a strict indication according to clinical protocols or guidelines.[64] Likewise, access to advanced neonatal care is not readily available in all settings even in high-resource countries. In these circumstances, knowing the risks of respiratory morbidity with an acceptable accuracy might help clinicians and parents to make more balanced decisions and/or determine the most appropriate place of delivery.[65] Finally, among the reasons for avoiding fetal lung maturity testing may be the fear for complications of amniocentesis, reported to occur in around a 0.7% of cases,[66, 67] medical costs and/or maternal discomfort. This perception, and consequently, the attitude of physicians and parents seeking information about fetal lung maturity might be reconsidered if this information can be obtained with a non-invasive test.

Strengths and limitations

The results of this multicenter study are in line with those obtained in a previous smaller study in which the technology was prospectively and blindly evaluated in a single center in 144 patients.[22] These findings and the multicenter nature of the study support the fact that, provided the quality criteria in the acquisition of the images are respected, the test is robust and yields similar performances in different clinical settings enhancing the likelihood that results are generalizable.

However, this study has some limitations. The method tested in this study uses an indirect approach to estimate lung maturity. By definition, prenatal prediction of NRM is hampered by the fact that the outcome is largely, but not exclusively, determined by the fetal lung maturity status. Thus, in circumstances such as neonatal sepsis, malformations potentially affecting lung function or intrapartum hypoxic-ischemic

events, newborns with normal lung maturity in utero may present respiratory impairment. Also, specific conditions such as fetal growth restriction, multiple pregnancy, diabetes or premature rupture of membranes were not analyzed separately. Differences in the performance of quantusFLM® in these subgroups cannot be excluded and requires further research. On the other hand, the performance of the software for each specific gestational age was not assessed in this study because the algorithms used were not designed to predict NRM for each specific gestational age. Future algorithms with 1 or 2-week gestational age intervals would be more precise, although whether this could improve the accuracy reported here remains to be assessed. Regarding the mode of delivery, cesarean section rate is high around 50%. This is due to the fact that delivery had to occur within 48h of the image acquisition to meet inclusion criteria. This may overestimate elective cesarean section rate in our study population although could be comparable to some settings. Accordingly to clinical practice also, elective and non-elective cesarean section are more frequent in preterm deliveries. Finally, despite the ultrasound image required to perform the test was an axial section of the thorax, which is considered as a standard section, a relatively high number of images were eventually discarded due to the lack of compliance with the quality criteria required to perform the test. This stresses the fact that obtaining a valid ultrasound axial section of the fetal thorax at late gestation might not always be straightforward, and in particular cases the test might require special care or training to ensure an optimal acquisition of the image.

Conclusions

In summary, the results of this large multicentre study are consistent with the findings of a pilot study on the ability of a non-invasive technology to predict NRM from fetal

lung ultrasound images.[22] The technology also showed an accuracy that is similar to the previously reported performance of tests in amniotic fluid. Therefore, quantusFLM® provides a non-invasive tool which might help clinicians in the decision-making process.

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FIGURES

Figure 1. A) Lateral axial transverse section of the fetal thorax at the level of the 4-chamber section of the fetal heart. B) Region of interest delineated.

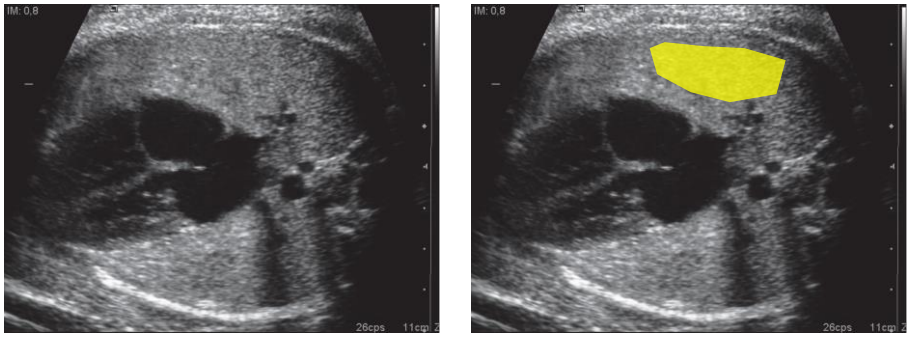
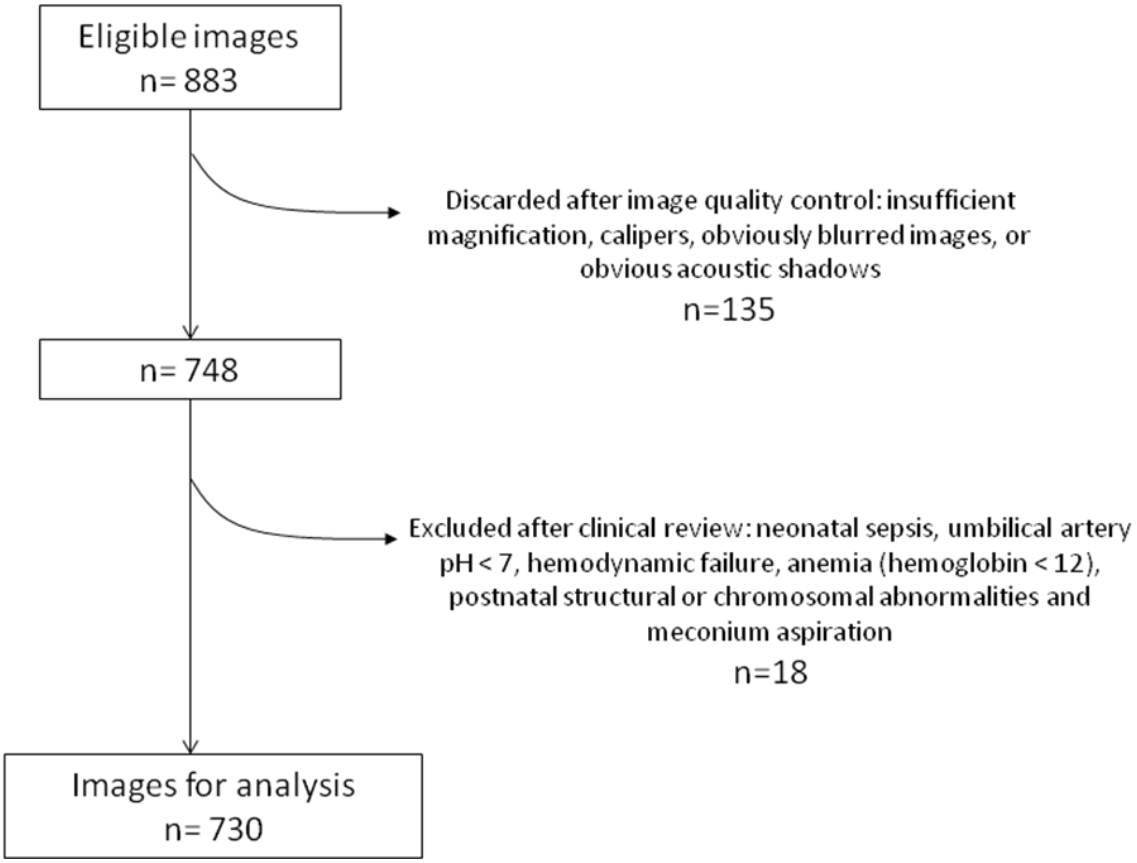


Figure 2. Flow chart of the eligible samples



TABLES

Table 1. Clinical characteristics of the women included in the study

	Total n= 685	GA range at scan [25.0-33.6] n= 145	[34.0-38.6] n= 540
Maternal Age	32.3 (5.8)	31.4 (5. 8)	31.3 (5.8)
Nulliparity	340 (49.6%)	70 (48.3%)	270 (50%)
Ethnicity			
Caucasian	400 (58.4%)	93 (64.1%)	307 (56.7%)
Black	40 (5.8%)	9 (6.2%)	31 (5.7%)
Asian	44 (6.4%)	0	44 (8.1%
Hispanic	121 (17.7%)	24 (16.6%)	97 (18.0%)
Other	53 (7.7%)	18 (12.4 %)	35 (6.5%)
Multiple pregnancy	65 (9.5%)	21 (14.5%)	44 (8.1%)
Maternal or fetal relevant conditions			
Preterm labor	48 (7%)	26 (17.9%)	22 (4.1%)
PPROM	158 (23.7%)	70 (48.3%)	88 (16.3%)
Preeclampsia	116 (16.9%)	40 (27.6%)	76 (14.1%)
IUGR	148 (21.6%)	32 (22%)	116 (21.5%)
Pre-gestational diabetes	15 (2.2%)	3 (2.1%)	12 (2.2%)
Antepartum hemorrhage	10 (1.5%)	3 (2.1%)	7 (1.3%)
Other*	160 (23.4%)	31 (21.4%)	129 (23.9%)

Mean (SD) or n(%) when appropriate. *Hypothyrodism, hypertensive disorders, Placenta previa, Lupus, Human immunodeficiency virus positive, assessment of fetal wellbeing, fetal presentation. PPROM: preterm premature rupture of membranes. IUGR: intrauterine growth restriction. GA, gestational age;

Table 2. Perinatal and neonatal outcomes of the newborns included in the study

	Total n=730	Gestational Age at scan [25.0-33.6] n=164	[34.0-38.6] n=566
Gestational age at delivery (weeks)	36.0 (2.6)	31.4 (2.2)	37.2 (1.2)
Ultrasound-to-delivery lapse of time (days)	0.6 (0.7)	0.7 (0.7)	0.6 (0.6)
Mode of delivery			
Spontaneous vaginal delivery	294 (40.3%)	50 (30.5%)	244 (43.1%)
Operative vaginal delivery	48 (6.6%)	4 (2.4%)	44 (7.8%)
Non-elective cesarean section	125 (17.1%)	36 (22.0%)	89 (15.7%)
Elective casarean section	263 (36.0%)	74 (45.1%)	189 (33.4%)
Birthweight (g)	2517 (760)	1554 (486)	2796(575)
Female gender	365 (50.0%)	70 (42.7%)	295 (52.1%)
Apgar at 5min < 7	10/729 (1.4%)	7/163 (4.3%)	3/566 (0.5%)
pH UA 7.00-< 7.10	18/479 (3.8%)	5/124(0.04%)	13/355(3.7%)
Hyperbilirrubinemia (phototherapy)	152 (20.8%)	86 (52.4%)	66 (11.7%)
Other relevant conditions:			
Apnea	20 (2.7%)	20 (12.2%)	0
Bronchopulmonary displasia	8 (1.1%)	8 (4.9%)	0
Persistent Pulmonary hypertension	3 (0.4%)	2 (1.2%)	1 (0.2%)
Intraventricular hemorrhage (III or IV)	3 (0.4%)	3 (1.8%)	0
Necrotizing enterocolitis	3 (0.4%)	3 (1.8%)	0
Neonatal death < 28 days	3 (0.4%)	3 (1.8%)	0
NICU admission	242 (33.2%)	148 (90.2%)	94 (16.6%)
Length of stay at NICU	18.7 (19.5)	25.5 (21.4)	8.2 (9.0)
Discharged alive from NICU	239/242(98.8%)	145/148(98.0%)	94/94(100%)

Mean (SD) or n(%) when appropriate. NICU: neonatal intensive care unit.

Table 3. Characteristics of the respiratory support and respiratory morbidity

		Gestational Age at scan	
	Total n= 730	[25.0-33.6] n=164	[34.0-38.6] n=566
Need for respiratory support (any)	115 (15.6%)	89 (54.3%)	26 (4.6%)
Oxygen therapy ≥ 40%	55 (7.5%)	37 (22.6%)	18 (3.2%)
CPAP	117 (16 %)	94 (57.3%)	23 (4.1%)
NIV/BPAP	23 (3.2%)	22 (13.4%)	1 (0.2%)
Intubation required	31 (4.3%)	28 (17.1%)	3 (0.5%)
Days of intubation (if any)	6 (9.4)	6.7 (9.9)	1.8 (1.5)
HFV (high frequency ventilation)	12 (1.6%)	10 (6.1%)	2 (0.4%)
Surfactant use	34 (4.7%)	32 (19.5%)	2 (0.4%)
Doses of surfactant (if any)	1.4 (0.7)	1.4 (0.7)	2 (1.4)
Neonatal Respiratory Morbidity	101 (13.8%)	72 (43.9%)	29 (5.1%)

Mean (SD) or n(%) when appropriate. CPAP: continuous positive airway pressure. NIV/BPAP: non-invasive ventilation/Bi-level positive airway pressure.

Table 4. quantusFLM® performance to predict neonatal respiratory morbidity

	Total n=730	[25.0-33.6] n=164	[34.0-38.6] n=566
Neonatal respiratory morbidity	101 (13.8%)	72 (43.9%)	29 (5.1%)
True positives	75	57	18
True negatives	557	67	490
False positives	72	25	47
False negatives	26	15	11
Accuracy	86.5% (632/730)	75.6% (124/164)	89.8%(508/566)
Sensitivity	74.3% (75/101)	79.2% (57/72)	62.1%(18/29)
Specificity	88.6% (557/629)	72.8% (67/92)	91.3%(490/537)
Positive predictive value	51% (75/147)	69.5% (57/82)	27.7%(18/65)
Negative predictive value	95.5% (557/583)	81.7% (67/82)	97.8%(490/501)
Positive likelihood ratio	6.5	2.9	7.1
Negative likelihood ratio	0.3	0.3	0.4

Table 5. Summary of performance of invasive tests in amniotic fluid used to predict neonatal respiratory morbidity (summarized from Table 3S)

	Ac	Se	Sp	PPV	NPV
quantusFLM®	86.5%	74.3%	88.6%	51%	95.5%
L/S	81.6%	74.6%	82.5%	34.1%	96.4%
PG	57.5%	82.7%	54.4%	18.0%	96.3%
LBC	75.4%	84.2%	74.4%	27.9%	97.6%
TDxII	78.7%	88.5%	77.7%	28.5%	98.5%

L/S: lecithin/sphingomyelin ratio; PG: phosphatidylglycerol; LBC: lamellar body count; TDxII:surfactant/albumin ratio.

Table 1S. Number of images included in each center.

Center	n= 730	%
BCNatal (Spain)	182	24.9
UZLeuven (Belgium)	77	10.5
Hradec Kralove (Czech Republic)	64	8.8
Sahlgrenska Univ. Hospit.(Sweden)	48	6.6
Clínica el Prado Medellin (Colombia)	47	6.4
Hosp. Univ. Puerta del Mar (Spain)	44	6.0
Althaia (Spain)	40	5.5
Consorci Sanitari Terrassa (Spain)	40	5.5
Hospital C U Chile (Chile)	33	4.5
Perinatology Research Branch (USA)	33	4.5
Hospital La Paz (Madrid)	28	3.8
Hospital San Cecilio (Spain)	25	3.4
KK Women's & Childr. Hosp. (Singapore)	23	3.2
Childrens' Hospital (Mexico)	14	1.9
Royal Prince Alfred Hospital (Australia)	12	1.6
Fernandez Hospital (India)	8	1.1
University of Wisconsin (USA)	4	0.6
Hospital Virgen Arrixaca (Spain)	4	0.6
UTHSC (USA)	2	0.3
Hospital Nostra Sra Meritxell (Andorra)	2	0.3

Table 2S. Ultrasound equipment used in the study in alphabetical order

Equipment	n=730	%
Aloka		
Aloka 4000	33	4.5
General Electrics		
Voluson 730	214	29.3
Voluson E6	56	7.7
Voluson S6	45	6.2
Voluson E8	123	16.8
Voluson P8	8	1.1
Samsung		
Medison	12	1.6
Siemens		
Acuson Antares	148	20.3
Toshiba		
Aplio	64	8.8
Nemio	2	0.3
Xario	25	3.4

Table 3S. Diagnostic performance of non-invasive and invasive tests in amniotic fluid used to predict neonatal respiratory morbidity

	N	Ac	Sen	Sp	PPV	NPV
Non-invasive tests						
Grey level histogram[13, 14]	22/47	-	86	72	-	-
Fetal breathing movements[15, 16]	-/43	-	92	85	92	80
Liver-to-lung texture[17]	750/1000	73-96	-	-	-	-
quantusFLM[22]	29/144	86	86	87	62	96
quantusFLM (present study)	101/730	86	74	88	51	95
Invasive tests[25, 68-72]						
Lecitin/esphingomielin ratio						
Bowie	5/52	85	80	85	36	98
Ashwood	17/187	84	82	85	35	98
Dalence	12/122	89	92	89	48	99
Fakhoury	4/28	96	75	100	100	96
Greenspoon	7/70	80	71	81	29	96
Lee	14/141	92	64	95	60	96
Karcher	13/201	88	62	89	29	97
Hagen	29/140	81	48	89	54	87
Rusell	23/294	84	96	83	32	100
Neerhof	100/833	76	81	76	32	96
Phosphatidilglicerol						
Karcher	13/204	69	92	67	16	99
Hagen	21/113	73	86	71	40	96
Rusell	16/240	80	94	79	24	99
Neerhof	100/833	47	80	42	15	94
Lamellar bodies count						
Bowie	8/56	75	88	73	35	97
Ashwood	28/247	91	71	93	57	96
Dalence	16/130	96	75	99	92	97
Fakhoury	4/28	100	100	100	100	100
Greenspoon	7/62	90	100	89	54	100
Lee	14/157	94	79	95	61	98
Karcher	13/219	76	85	75	18	99
Haymond	12/184	62	92	60	14	99
Neerhof	100/833	66	88	63	25	97
TDxII-FLM						
Karcher	13/218	78	92	78	21	99
Haymond	12/194	66	83	65	14	98
Hagen	29/140	77	90	74	47	100
Rusell	24/301	89	96	88	42	100

The outcome generally tested was RDS. N: RDS/Total. Ac: accuracy; Sen: sensitivity; Sp: specificity; PPV: positive predictive value; NPV: negative predictive value.

Table 4S. Neonatal respiratory morbidity by gestational age in late preterm and early term infants (data extracted from Hibbard et al.[2] N=81567, NRM n=1346)

GA threshold (weeks)	≥34	≥35	≥36	≥37	≥38	≥39
True positives	0	390	719	1002	1206	1346
True negatives	80221	76911	71763	61889	41624	0
False positives	0	3310	8458	18332	38597	80221
False negatives	1346	956	627	344	140	0
Accuracy	98%	95%	89%	77%	53%	2%
Sensitivity	0%	29%	53%	74%	90%	100%
Specificity	100%	96%	89%	77%	52%	0%
Positive predictive value	0%	11%	8%	5%	3%	2%
Negative predictive value	98%	99%	99%	99%	100%	0%

All figures are numbers or % where stated. GA: gestational age at delivery.

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