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A Novel Combination of Ultrasound Patterns of Fetal Breathing Movements for the Assessment of Fetal Lung Maturity

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ABSTRACT

Objective Several patterns of fetal breathing movements (FBMs) i.e. abdominal wall movements, thoracic movements (TMs), nasal fluid flow velocity waveforms (NFFVW) were investigated by ultrasound (US) technology and related to fetal pulmonary maturity/immaturity, i.e., fetal lung maturity (FLM) tests and neonatal outcome in order to validate our hypothesis that some FBMs patterns may indicate that the fetal lung is mature or immature, regardless of gender, weight and gestational age.

Design Prospective descriptive study.

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Subjects and Main outcome measures We prospectively enrolled 39 high-risk pregnancies in whom a complete US study of FBMs was performed and correlated to FLM tests. All women delivered by cesarean section within 24 hours from amniocentesis, and neonatal outcome was evaluated up to 7 days after birth. US-FLM was defined as the presence of nasal fluid flow velocity waveforms (NFFVW) detected by pulsed Doppler and spectral analysis, synchronous with thoracic movements (TMs) detected by M-mode. An US guided amniocentesis was performed in order to collect amniotic fluid (AF) and FLM was evaluated by L/S (lecithin/sphingomyelin) determination, phosphatidylglycerol (PG) analysis and lamellar bodies count.

Results Diagnostic accuracy for US evaluation of FLM, with NRDS as endpoint parameter, was as follows: sensitivity: 100%, specificity: 80%, PPV: 73% and NPV: 100%.

Conclusion FBMs are known to reflect pulmonary development and maturity and thus, are conceivably correlated with the risk of the newborn infant to develope RDS after birth. Synchronous presence of NFFVW and TMs correlate accurately with conventional FLM tests. We suggest that this non-invasive procedure may be helpful to assess FLM, using a rapid and low cost technique; its use is particurlarly important when certain situations arise, e.g., amniocentesis refusal, religious concerns, oligo-anhydramnios, laboratory logistic equipment or heavily stained AF sample.

Key words: Fetal breathing movements (FBMs), fetal lung maturity (FLM), thoracic movements (TMs), nasal fluid flow velocity waveforms (NFFVW).

Neonatal distress respiratory syndrome (NRDS) is a developmental disorder of prematurely born infants characterized by progressive atelectasis and respiratory insufficiency.

NRDS and its sequelae account for an estimated 30% of neonatal diseases.[1,2] Because of the resultant high mortality and morbidity, the assessment of fetal lung maturity (FLM) plays an important role in the management of pregnancies particurarly at risk of preterm delivery (60% incidence of RDS in newborn infants born before 29 weeks' gestation vs. 1% for those born after 37 weeks). However, gestational age per se does not predict perinatal outcome; therefore, FLM tests are required when delivery must be postponed in order to allow the fetal lung to reach maturity. For other instances, in which delivery is needed for maternal and/or fetal well-being, corticosteroid treatment should be instituted for the acceleration of FLM.[3,4]

FLM tests are divided into biochemical and biophysical and performed on amniotic fluid (AF) samples obtained by amniocentesis as suggested by Scarpelli[5] and documented by the studies of Gluck et al.[6]

Fetal breathing movements (FBMs) in utero are essential for lung development and function. Fetal pulmonary liquid (FPL) contributes up to one-third of AF volume; its osmolarity is different from that of AF; it contains surface activity compounds which originate from the alveoli, i.e., pulmonary surfactants;[7] their presence in AF is a prerequisite to assess FLM.

Traditionally, the biochemical tests consist in evaluating L/S (lecithin/sphingomyelin) both planimetric and stechiometric;[8] a value of two or more (depending on laboratory methods), is indicative of FLM;[9] phosphatidylglycerol (PG) determination is important to add diagnostic accuracy to L/S for the prediction of FLM; its concentration of 3% or more of total phospholipids predicts quite accurately FLM.[6,10] L/S and PG determination have a negative predictive value (NPV) of 100%; the positive predictive value (PPV) of a low L/S is approximately 70%. Blood and meconium in AF can reduce the accuracy of L/S.[11, 12]

The above mentioned tests suffer of some limitations. The ideal method should be: reproducible, simple and of quick performance.[12] Other attempts to evaluate FLM have been suggested, i.e., the shake test introduced by Clements et al.[13] which is based on the ability of surfactant to form a stable foam in the presence of ethanol. This technique is simple, special equipment is not required and the results are promptly available, but its accuracy decreases when AF is contaminated with blood or meconium;[14] an immature test (absence of foam at a 1 to 1 dilution) should be confirmed by L/S and PG determination in order to accurately predict FLM.[12]

Another quick test consists in lamellar bodies count; this is easily performed using a commercial cell counter but is limited by a high false immaturity rate.[15] Fluorescence polarization has also been introduced as a rapid test for FLM. This test is performed on the basis of changes of microviscosity in AF during gestational age;[16] it overestimates fetal lung immaturity.

Another test based on fluorescent polarization uses a ratio of surfactant and albumin rather than microviscosity; this test is prompt but special equipment is required.[17]

All these tests require amniocentesis in order to obtain a sample of AF, which although is a safe procedure,[18] nevertheless carries the risk of potential complications, i.e., premature rupture of membranes, placental detachment, premature labor, fetal or maternal bleeding; furthermore some difficulties may arise in its performance (patient refusal, oligo-anhydramnios).

In the past, several attempts have been made to introduce a noninvasive method in order to evaluate FLM. Many investigators have used diagnostic ultrasonography (US) to eliminate the potential risk of amniocentesis, i.e., the evaluation of noninvasive alternatives to classical FLM testing.

US studies were performed in order to assess FLM; they focused on fetal biometry (biparietal diameter, fetal epiphyseal ossification centers),[19-23] placental grading,[24-26] detection of freefloating particles in AF[27] and tissue characterization of fetal lung.[28]

However, all these procedures resulted to be inaccurate for the prediction of FLM;[29] in addiction several US findings are influenced by advanced gestational age (>37 weeks), when FLM has been most likely achieved.

We have suggested an US method to assess of FLM, based on the detection of several patterns of FBMs and their possible relation with FLM.[30]

Several attempts have been made to characterize FBMs by US technology. Most studies featured mainly the evaluation of breathing-related fluid flow velocities at the level of the fetal trachea and nostrils.[31-32] In our previous study on the ontogeny of normal FBMs[33] using US technique, we have observed several patterns of fetal respiratory behaviour detectable during fetal life. FBMs consist of three different patterns of respiratory activity:

- abdominal wall movements;
- thoracic wall movements (TMs);
- nasal fluid flow velocity waveforms (NFFVW).

These patterns are detectable at various gestational age and they represent different stages of development and maturation of the fetus. Abdominal movements are the first FBMs pattern to appear in the fetus; they are detectable in 60% of fetuses prior of 20 weeks' gestation; TMs is the second pattern to appear and are present in 20% of fetuses at 20-25 weeks' gestation; NFFVW are the last pattern to arise and is detectable at 25-30 weeks' gestation in 40% of human fetuses.[33]

From these observations, we proposed an US method for the assessment of FLM based on the evaluation of FBMs in utero and to use them as sonographic markers in terms of their ability to predict a mature fetal lung vis à vis biochemical and biophysical AF tests.[30]

Materials and Methods

A total of 141 women were examined in the period between April 1997-December 2000. In order to assess FLM amniocentesis was performed in 39 women coupled with US study of FBMs. The latter was performed less than 24 hours before delivery. All women delivered by cesarean section because of high-risk pregnancy (Table 1). The study was approved by the local ethical committee and an informed consent was obtained from all women.

Table 1. Characteristics of our study group

GA at amniocentesis (mean)	32,05 (wks' gestation)
GA at delivery (mean)	32,09 (wks' gestation)
Pregnancy Risk factors	• Preeclampsia (No.2) • HELLP (No.1) • IDDM (No.1) • Oligohydramnios (No.2) • Preterm Labor (No.12) • PIH (No.4) • CMV infection (No.1) • Anhydramnios (No.1) • IUGR (No.7) • PIH, IUGR (No.1) • GDM (No.1) • GDM, oligohydramnios (No.1) • IUGR, oligohydramnios (No.2) • Multiple pregnancy (No.2) (3 amniocentesis performed)

All pregnancies were dated on the basis of US measurements of crown-rump-lenght at 6-11 weeks and at 12-20 weeks' gestation. The women included were not in labor nor received steroid treatment at least one week before the study. Women with fetal malformations were excluded from the study. The US study was performed within three hours after breakfast, in order to exclude the influence of glicemia on FBMs[12,34] and lasted a minimum of 30 min (max 90 min).

The examination was performed by the same ultrasound registrar using a commercially available ultrasound system with colour flow and spectral Doppler analysis; the convex transducer frequency was adjusted at 3.5 MHz with the sample volume between 2-4 mm, the high pass filter was kept on at a low level. The insonation angle was always kept <30°.

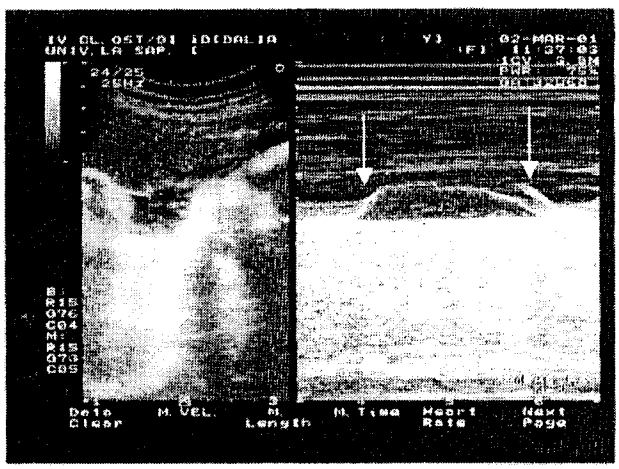

Fig. 1. Represent the detection of a TMs using M-Mode US technique. Curved arrows show the downward movement of the sternum, which represents the inspiratory phase.

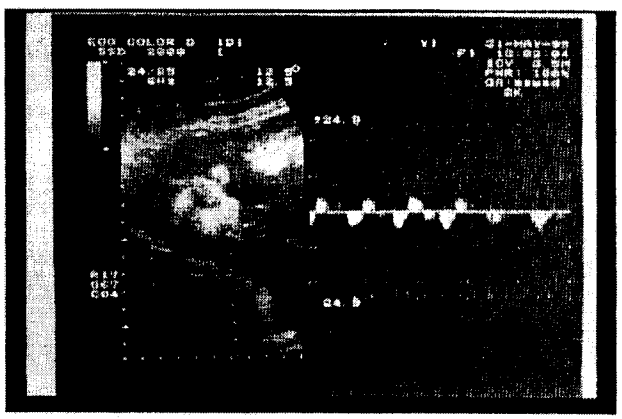

Fig. 2. (NFFVW detected at a gestational age of 21 wks) It shows the sequence of espiratory and inspiratory NFFVW, i.e., the waveform moving upwards represents the expiratory phase while that moving downwards is the inspiratory one. It this case the fetal breathing pattern (NFFVW) is irregular.

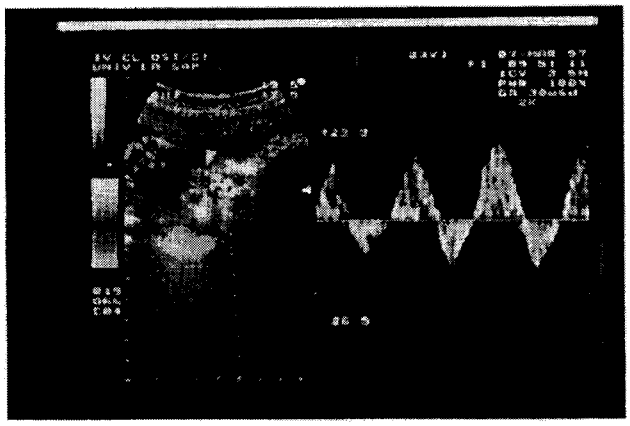

Fig. 3. (NFFVW detected at a gestational age of 31 wks.) It is shows a regular sequence of NFFVW, in which the espiratory phase, represented by the waveform moving upwards, is sequentialy followed by the inspiratory phase, represented by the waveform moving downward.

Abdominal movements and TMs were studied by real time US in sagittal plane of the fetus in order to visualize the anterior abdominal wall, the anterior thoracic wall and the spine. Using M-mode, fetal respiratory activity is visualized in the moment when abdominal wall moves upwards, thoracic wall moves inwards while fetal spine does not move. This respiratory finding reflects the inspiration phase that is determined by the contraction of the diaphragm which causes an increase in abdominal pressure so that the abdomen in a sagittal plane moves upwards; on the contrary, the inspiratory muscles when activated determine the expansion of the thoracic wall and in the sagittal plane the sternum moves inwards (Figure 1). In the expiratory phase abdominal wall moves inwards and thoracic wall moves upwards. NFFVW have been studied by pulsed color Doppler US. Once the nostrils are visualized in a sagittal plane or in a coronal plane, entering color Doppler flow moving in the nostrils can be detected. If pulsed Doppler is used at this moment, a waveform is depicted by spectral analysis and represents a synusoidal wave corresponding to inspiratory and espiratory cycles. Moreover, in the waveform analysis, the latter breathing pattern can be divided in irregular NFFVW, depicted by a synusoidal wave in which inspiration and expiration phases are irregular in amplitude and duration (Figure 2), or into regular NFFVW whereby inspiration and expiration phases are regular in amplitude and in duration (Figure 3).

We considered positive NFFVW if at least 10 respiratory cycles regular or irregular were continuously detected, conversely the evaluation was judged negative when NFFVW were absent or present less than 10 continuous respiratory cycles.

When NFFVW were detected and judged positive, the M-mode was switched on and TMs were identified with a real-time M-Mode imaging on longitudinal scanning, thus enabling the visualisation of the anterior fetal chest wall echoes. TMs were considered positive if intermittent chest wall movements were present for at least 30 sec. per episode; we considered the presence of TMs as waveforms signals, corresponding to the anterior wall of the fetal chest, while no waves originated from the motion of the fetal spine; this last finding is necessary to differentiate FBMs from body movements. During each inspiratory movement, the anterior chest wall echoes moved inwards and the anterior abdominal wall echoes moved outwards, i.e., in the opposite direction. Whenever rhythmic diaphragmatic movements were identified by B mode real-time visualization, the probe

was directed to the fetal chest wall in order to evaluate the presence of TMs by switching the M-mode on. If after 30 minutes of US evaluation neither NFFVW nor TMs were identified, we considered the results negative. For all the evaluations performed we found the following combinations: presence of both NFFVW and TMs, absence of both NFFVW and TMs and presence of TMs alone.

We have hypothesised the contemporary presence of NFFVW and TMs as indicators of FLM. In the same sonographic session, an AF sample was collected by conventional US guided amniocentesis and FLM tests were performed by the determination of L/S, the presence of phosphatidylglycerol (PG) and by lamellar bodies count, as previously reported.[35,36] The operator that analysed the AF specimen was unaware of the results of the US study.

Neonatal outcome was followed until 7 days after birth. Newborns were classified as having RDS if they needed oxygen therapy (high FiO_2) and had typical clinical and radiologic signs of the disease.

Results

Gestational age at the time of amniocentesis of women who delivered within one week from amniocentesis ranged between 26.4 weeks and 35.4 weeks' gestation. Fourteen neonates affected by RDS had a mean gestational age at the time of amniocentesis of 28.6±2.3 weeks, whereas 25 neonates not affected by RDS had a mean gestational age of 32.1±1.8 weeks' gestation. In the latter group there was no difference for gender, sex (male = 13, female = 12); this was the same for the NRDS group (male = 8, female = 6). Two cases showed absent NFFVW at 33 weeks' gestation and the presence of TMs, and FLM tests showed mature L/S, absence of PG and immature lamellar bodies count. In our study group we observed that the association between TMs and NFFVW detected by US was strongly correlated with a normal neonatal outcome, while the presence of TMs not associated with NFFVW was idicative of NRDS. We considered the fetal lung to be mature when we detected at the same time the presence of regular or irregular NFFVW and TMs. Fetal lung immaturity was defined as the absence of NFFVW or the absence of synchronous TMs and NFFVW. For statistical analysis we considered the association between FBMs studied by US with conventional FLM biochemical and biophysical tests, and the correlation with neonatal outcome (RDS present/absent) for the assessment of diagnostic accuracy (sensitivity, specificity, positive predictive value [PPV] and negative predictive value [NPV]). We estimated true positive results if the neonates developed clinical and radiological signs of RDS within 7 days from birth, as reported by neonatologists. We found a statistically significant correlation when NFFVW episodes correlated with the presence/absence of NRDS and with the presence of absence/presence of FLM (Chi-square = 19.899, $p<0.001$) (Tables 2, 3). The diagnostic accurancy between the US detection of regular NFFVW and the prediction of FLM and NRDS is reported in Table 4.

Table 2. Occurrence of NRDS in relation with NFFVW

	NRDS	No-NRDS
NFFVW	0	20
No-NFFVW	14	5

Table 3. Correlation between FLM and NFFVW

	FLM	No-FLM
NFFVW	20	0
No-NFFVW	2	17

Table 4. Diagnostic accuracy NFFVW for the prediction of FLM and NRDS

	FLM	NRDS
Sensitivity	100%	100%
Specificity	91%	80%
Negative predictive value	100%	100%
Positive predictive value	89%	73%

Discussion

FBMs are episodic and they reflect the development and maturation of central nervous system (CNS); their detection correlates with fetal well being.

In fetal life, lung growth depends on physical factors, i.e., AF volume, thoracic and lung compliance, volume of fetal pulmonary liquid (FPL) and the presence of breathing movements.[37] Fetal lung maturaty reflects the compliance of the lung and is composed of two factors: elastic properties, i.e., elastance and compliance, and the presence of pulmonary surfactant .

FBMs produce significant changes in intrathoracic pressure that may influence lung development and maturity.[5, 12, 37]

During fetal life we have studied the occurrence of the above mentioned FBMs patterns at different gestational ages.[33] The first breathing activity is represented by abdominal movements, then appear TMs; the third pattern to occur is NFFVW. These patterns of FBMs should reflect different stages of maturation and prepare the fetus to the extrauterine life. The detection of NFFVW reflects FLM; but it is also important to underline that FBMs patterns strongly enhance the adaptability of the fetus to accomplish the onset of regular breathing at birth.[12, 38]

It is well known that the ontogeny of FBMs may follow the pattern of maturity of sympathetic/ parasympathetic systems. From 30 weeks onwards abdominal and thoracic movements are present most of the time, whereas NFFVW is the most "immature" fetal breathing pattern, increasing from 52.4% at 31-35 wks and reaching 91.2% at 36-40 weeks' gestation.[33] As previously described, the sympathetic system predominates over the parasympathetic until approximately 30 weeks. Afterwards, both systems equilibrate, indicating an increasing maturity of the CNS. This matches the trend of fetal heart rate (FHR), which, by the same gestational period, falls into the normal range until delivery.[12, 39] Cosmi E.V. et al.[40, 41] observed a similar pattern of maturation of breathing patterns in preterm neonates during gestation by recording thoracic and abdominal breathing movements with mercury strain-gauges applied one at the level of the nipples and the other on the umbilicus. The overall FLM may resamble a trend similar to FBMs and FHR, and seem to be independent from gestational age, because some fetuses may reach pulmonary maturity even in early gestation, as we have observed in this study when FLM is evaluated by conventional AF tests. TMs are always present when similar peaks of inspiratory expiratory waveforms in NFFVW patterns are detected. When irregular peak waveforms are

found in NFFVW patterns, TMs excursions discriminated FLM from FL immatury. TMs excursions alone account for a high specificity (i.e., correlation with true negative values: 88%). We hypothesise that the contemporaneous presence of NFFVW and TMs represent a direct correlation between the maturity of CNS and functional and anatomic maturity of the lung as shown also by biochemical FLM tests.

Other attempts have been made to suggest a noninvasive technique alternative to amniocentesis and AF tests for FLM, but they have resulted to be complicated; furthermore, they need a technology often not available. Our alternative method to conventional biochemical and biophysical FLM tests in AF, is based on a commercial US machine with Doppler flow and spectral image analysis, which is available almost in every obstetric unit. Moreover, Doppler assessment of NFFVW has been shown to be reliable regarding the study of FBMs and of FPL in the human fetus.[32] Considering the diagnostic accuracy of certain biochemical and biophysical tests for FLM performed in our laboratory (sensitivity 83% for L/S and 94% for LBs),[36] the results of diagnostic accuracy with our US method for the evaluation of FLM can be defined as satisfactory (sensitivity: 100%, specificity: 80%) and to be a preliminary step for subsequent study.

These results strongly encourage further evaluation of non invasive methods as reliable approaches for the appraisal of FLM and the risk to develope RDS. Our method is comparable to the AF tests, i.e., L/S, PG and LBs count, while it avoid the need of amniocentesis, particularly in cases where AF sampling is impossible to be performed (e.g., PPROM associated with anhydramnios). Other situations may arise where this new approach may be useful, particularly when the amniocentesis is refused by the women or when there are laboratory equipment difficulties.

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