Feasibility of two-dimensional ultrasound shear wave elastography of human fetal lungs and liver: A pilot study


To cite this version:


HAL Id: hal-03490074
https://hal.science/hal-03490074
Submitted on 21 Jul 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Distributed under a Creative Commons Attribution - NonCommercial| 4.0 International License
Feasibility of two-dimensional ultrasound shear wave elastography of human fetal lungs and liver: a pilot study

Short title: 2D ultrasound shear wave elastography of fetal lungs and liver

Nicolas Mottet 1, 2 *, Claire Cochet 1, Chrystelle Vidal 3, Jean Patrick Metz 1, Sébastien Aubry 4, Aude Bourtembourg 1, Astrid Eckman-Lacroix 1, Didier Riethmuller 1, Lionel Pazart 3, Rajeev Ramanah 1, 2

1 Department of Obstetrics and Gynecology, Pôle Mère-Femme, University Hospital of Besancon; University of Franche-Comte, 25000 Besançon, France

2 Nanomedicine Lab, Imagery and Therapeutics, EA4662, University of Franche-Comte, 25000 Besancon, France.

3 Centre d'investigation Clinique-Innovation Technologique 1431, INSERM, University Hospital of Besançon, 25 000 besançon, France.

4 Department of Musculoskeletal Imaging, University Hospital of Besancon, 25000 Besancon, France

* Corresponding author: ncmottet@gmail.com

Department of Obstetrics and Gynecology, Hopital Universitaire de Besancon, Boulevard Alexander Fleming, 25000 Besançon, France

All the authors have no conflict of interest related to the manuscript

© 2019 published by Elsevier. This manuscript is made available under the CC BY NC user license https://creativecommons.org/licenses/by-nc/4.0/
Abstract

Purpose: The first aim was to evaluate feasibility and reproducibility of 2-dimensional ultrasound (2D) shear wave elastography (SWE) of human fetal lungs and liver between 24 and 34 weeks of gestation. The second aim was to model fetal lung-to-liver elastography ratio (LLE ratio) and to assess its variations according to gestational age and maternal administration of corticosteroids.

Material and methods: 2D-SWE examinations were prospectively performed in fetuses of women with an uncomplicated pregnancy (group 1) and fetuses of women with a threatened preterm labor requiring administration of corticosteroids (group 2). Two 2D-SWE examinations were performed at “day 0” and “day 2” in group 1; before and 24 hours after a course of corticosteroid in group 2. Three operators performed 2 cycles of 3 measurements on the lung (regions A1, A2, A3) and the liver (regions IV, V, VI). Repeatability and reproducibility of measurements were calculated. The fetal LLE ratio was modeled from the most reproducible regions.

Results: Fifty-five women were enrolled in group 1 and 48 in group 2. For the lung, 8.6% of measurements were considered invalid and 6.9% for the liver. The most reproducible region for the lung was A3 [ICC between 0.70 (95% CI: 0.42-0.85) and 0.78 (95% CI: 0.48-0.90)] and region VI for the liver [ICC between 0.70 (95% CI: 0.40-0.85) and 0.84 (95% CI: 0.60-0.94)]. According to gestational age, a moderate positive linear correlation was found for stiffness values of A3 (R=0.56), V (R=0.46) and VI (R=0.44). LLE ratio values at “day 0” were not different between the two groups but decreased at “day 2” in group 2 (0.2; 95% CI: 0.07-0.34; *P*<0.001).

Conclusion: Quantitative fetal lung and liver stiffness measurements are possible with 2D-SWE with acceptable reproducibility.
Keywords: Elasticity imaging techniques; Sonoelastography, Shear wave; Observer variation; Fetal lung; Fetal liver

Introduction

Prenatal evaluation of fetal lung maturity (FLM) is still controversial and different approaches have been described with invasive biological tools and fetal imaging [1–10]. FLM is mainly correlated with surfactant level and different tests have been developed to quantify this production through detection of components in the amniotic fluid [1]. However, performances of these tests to predict neonatal respiratory morbidity are limited for a routine clinical application because they need invasive procedures and all have a positive predictive value ranging from 18 to 34%[2,4–6]. Thus, development of new and non-invasive methods to assess FLM is challenging to predict respiratory distress syndrome (RDS). RDS complicates 1% of all births and the incidence increases with prematurity. RDS ranges from 26% to 30% in preterm neonates before 34 weeks of gestation (WG) and from 5% to 20% after 34 WG [2,11].

Fetal lung and liver signal or echogenicity are often compared to assess FLM [7–10,12]. Non-invasive assessment of FLM has been first attempted using ultrasonography quantified by gray level histogram [13–15]. These studies demonstrated imaging changes associated with lung maturation but weak correlations between echogenicity and clinical outcomes at birth. Recently, a quantitative fetal lung texture analysis has been described and demonstrated a strong correlation with gestational age, biological test on amniotic fluid and risk of neonatal respiratory morbidity [2,16]. Prediction of the pulmonary function through quantitative fetal lung stiffness measurement seems innovative and could be complementary to quantitative ultrasound texture analysis.
Two-dimensional ultrasound (2D) shear wave elastography (SWE) is the latest evolution of elastography techniques and enables assessment of tissue stiffness in real time [17]. The aim of elastography techniques is to assess the hardness of a tissue, either by objective quantification or compared to an adjacent tissue [18]. Quantitative evaluation of the stiffness is expressed either in terms of shear wave speed \( \text{m.s}^{-1} \) or Young’s modulus (kPa) \([17,19,20]\). This technique has been validated in several organs (liver, breast, placenta, cervix)[20–24], and some studies reported interesting results about exploration of non human fetal organs with 2D-SWE [25,26]. 2D-SWE could be a promising tool in fetal medicine by providing a new approach to studying changes in fetal lung tissue properties during gestation.

A pilot study is necessary to clarify applicability of 2D-SWE for exploration of human fetal lungs and liver during gestation. It seemed relevant to compare fetal lung and liver stiffness evolution during pregnancy, and a new ratio could be modeled to provide an objective quantification: the lung to liver elastography (LLE) ratio.

The primary objective of this study was to evaluate feasibility and reproducibility of 2-D SWE of human fetal lungs and liver between 24 and 34 weeks of gestation. The secondary objective was to model fetal LLE ratio and to assess its variations according to gestational age and maternal administration of corticosteroids.

**Methods**

**Study design**

A prospective case-control study was performed at the University Hospital of Besançon, (Besançon, France), Department of Obstetrics and Gynecology from April to November 2016, in strict accordance with the ethical guidelines of the Declaration of Helsinki. The « ELASTOMAP study » was approved by human ethical research committee (Comité de Protection des Personnes EST II, process number 15/494) and the French National
The study was registered on clinical trial.gov with the following number: NCT02870608. All participants provided written informed consent.

Details of the rationale and design of the trial have been published previously [27]. Fetal lungs and liver were studied by 2D-SWE between 24 and 34 weeks of gestation (WG) in two groups: women with an uncomplicated pregnancy composed the control group (group 1), and women with a threatened preterm labor requiring administration of corticosteroids were enrolled as cases (group 2). The inclusion criteria were common to both groups: pregnant women aged 18 years or older, singleton pregnancy, eutrophic fetus, signature of consent and affiliation to health insurance scheme. Exclusion criteria were common to both groups: fetal lung or liver pathologies, inclusion in another medical study, and patients under legal incapacity. Two 2D-SWE examinations were performed at “day 0”, and “day 2” in group 1, separated for 48h. A 2D-SWE examination was performed in group 2 before and 24h after a complete course of corticosteroids indicated for threatened preterm labor, respectively at “day 0” and “day 2” (Fig.1). Betamethasone course was prescribed by maternal intramuscular injection of 12 mg and repeated 24 h after the first injection. All variables (patient parameters, data items, data elements) were aggregated into electronic Case report forms.

Prenatal variables and measuring technique

A Logic® E9 system (General Electric Healthcare) with CE certification CE LNE/G-MED [CE0459] equipped with abdominal convex probe 1-6 MHz (C1-6-D probe) was used for this study. The shear wave acquisition measurement protocol was the same for each patient. Details of the protocol have been published previously [27].

Three operators participated in this study. Operator 1 was the reference operator (NM, senior clinical lecturer), and two others operators were trained: operator 2 (JPM, resident) and operator 3 (CC, intern). To assess repeatability of measurements, operators performed 2
measurements to obtain duplicates on each target regions while systematically repositioning
the probe on the target organ: three regions for the proximal lung (anterior “region A1”,
medium “region A2” and posterior portion “region A3”) and three regions for the liver (IV,
V, VI) (Fig.2). To test the inter-observer reproducibility, a second observer successively
performed measurements on 30 fetuses. All measurements were carried out directly by
ultrasound on the target organ. The second observer performed measurements just after the
first one and was blinded to the feasibility and results obtained by the first one.

The fetal LLE ratio was initially defined as the value of the proximal lung stiffness
divided by the value of the liver stiffness. Only repeatable and reproducible values of each
ROI were considered to model the fetal LLE ratio. Measurements were performed on a
homogeneous circular ROI of 5 mm diameter manually drawn. Technical failure was defined
as failure to obtain a homogeneous elastogram in greater than 50% in the sampling area. At
the end of the follow up, the operator 1 was blinded of the women’s status and reviewed all
the measurements. Measurements were considered invalid when the ROI did not match with
the requested region or when important artifacts interfered with the ROI.

Postnatal variables

Two pediatricians examined all the infants at birth and were blinded to the prenatal
exposure to 2D-SWE. The following data were collected: term of birth, weight, neonatal
transfer, Apgar score and evaluation of respiratory distress by Silverman score. In the two
days after birth, automated evaluation of auditory evoked potentials were recorded in case of
premature delivery before 37 WG to despite cochlear damage in the exposed fetus. After 37
WG, auditory evoked potentials were performed if otoacoustic emissions were absent. At
three months after birth, the women received a phone call to assess infant well-being and to
collect the following data: medical history, health problems, respiratory diseases or
symptoms, liver disease or symptoms and number of hospitalizations since birth.
Statistical analysis

Given the paucity of data regarding 2D-SWE on human fetal lung, the sample size calculation was based on measurements of fetal lungs in pregnant baboons. All calculation hypothesis were detailed in the published protocol [27]. Forty-two patients per group were needed to have 90% power to statistically demonstrate such a difference (0.2 versus 0.057) assuming an α risk of 0.05 and standard deviation of 0.2.

We used descriptive analysis to examine the population in the study. Qualitative variables were expressed as raw numbers, proportions and percentages and quantitative variables were expressed as means, SDs and ranges. Feasibility was evaluated by assessing the number of examinations performed and the number of examinations with interpretable results (invalid data). In order to investigate the repeatability of measurements over a short period of time, two measurements per subject were made under identical conditions by the same observer [28]. A paired Student’s t-test was used to test repeatability of measurements. Results were expressed as mean difference with 95% confidence interval, standard deviation of the differences and P value. The intraclass correlation coefficient (ICC) was used to test inter observer reproducibility with 95% CI [29]. Differences in fetal LLE ratio values between two examinations were calculated by using the Wilcoxon test. Simple linear regression was used to test correlation between stiffness values and gestational age: the Pearson correlation coefficient R and the coefficient of determination R2 were mentioned. The strength of the association was regarded as very weak for Pearson coefficient values of 0-0.19, as weak for 0.2-0.39, as moderate for 0.40-0.59, as strong for 0.6-0.79 and as very strong for 0.8-1. Concerning postnatal data, a descriptive analysis at baseline and at each follow-up was performed. Comparisons between group 1 and group 2 were performed using Student’s t test or Mann-Whitney’s U test for quantitative variable and Chi-squared test or Fisher’s exact test for qualitative variables, as appropriate.
All statistical tests were two-sided with p-values inferior to 0.05 denoting statistical significance. Statistical analysis was performed with SAS/STAT version 9.4 for Windows (The SAS Institute, Cary, NC).

**Results**

During the study period, 103 patients were included: 55 women with an uncomplicated pregnancy composed group 1, and 48 women with a threatened preterm labor requiring administration of corticosteroids were enrolled as cases in group 2 (Fig. 1). Maternal characteristics are summarized in Table 1.

A total of 540 measurements were performed on each ROI. For the proximal lung, 8.6% of measurements were considered invalid (21.1% for A1, 2.8% for A2, 2% for A3), and 6.9% were considered invalid for the liver (14.4% for IV, 3.3% for V, 2.9% for VI). The number of invalid measurements did not differ between the control group and cases for each ROI. These data were excluded for statistical analysis.

For the 3 operators, measurements on each ROI were repeatable except for region IV for both operator 1 (mean difference = 0.1; 95% CI: 0.002 - 0.206; SD = 0.65) ($P=0.04$) and operator 2 (mean difference = 0.13, 95% CI: 0.006-0.26; SD = 0.54) ($P=0.04$) (Table 2). Interobserver ICC values for stiffness values (average of duplicates) in the different ROI among the three operators are presented in Table 3.

Considering invalid data for ROI A1 and IV, absence of repeatability for region IV for two operators, and low ICC between operator 1 and 3 for ROI A2 (ICC = 0.28; 95% CI: -0.72 - 0.69), these ROIs were excluded for fetal LLE ratio modeling. Only ROI A3 for the lung and ROI V and VI for the liver were considered eligible for statistical analysis. The stiffness value for each region was calculated from the average of duplicates performed by the operator. Three ratios were modeled and compared. There were: Fetal LLE ratio 1: A3/ [(V + VI)/2]; Fetal LLE ratio 2: A3/ V, and Fetal LEE ratio 3: A3/ VI.
The median values of these three indexes were compared at “day 0” and “day 2”, and the relative differences ([(“day 0 – day 2”)]/“day 0”) were evaluated in all the cohorts (group 1 and group 2). No statistically significant differences were noted among the three indexes, and we decided to retain fetal LLE ratio 1 for the remainder of the statistical analysis. Mean stiffness values for the lung (A3) and the liver (V, VI) were respectively 2.21 +/- 0.41 [1.44-3.06] KPa, 3.74 +/- 0.86 [2.38-6.36] kPa and 3.99 +/- 0.91 [2.63-6.20] kPa in the group 1.

Analysis of stiffness variations according to gestational age and corticosteroids were based on the reference operator’s data. Operator 1 performed examinations in 87 singleton pregnancies at a median age of 30 WG (range: 24-33.5 WG). According to gestational age, linear correlation was significant for stiffness values of ROI A3, V and VI with positive correlation coefficients (R: 0.56, 0.46 and 0.44 respectively) (Fig. 3). However, if variation of the fetal LLE ratio was assessed according to gestational age, the linear trend correlation was not significant (P = 0.6) and correlation was very weak (R= 0.08) (Fig. 4).

The fetal LLE ratio values for the first examinations at “day 0” were not significantly different between controls and cases. Operator 1 performed 2D-SWE examinations in 42 controls and 30 cases. The fetal LLE ratio values at “day 2” decrease in group 2 exposed to corticosteroids (0.2; 95% CI: 0.07 to 0.34) and increase in controls (-0.04; 95% CI: -0.29 to 0.08) (P < 0.001) Differences in fetal LLE ratio values between two examinations are presented in Figure 4.

Pediatricians examined a total of 102 children exposed to 2D-SWE in the two days following birth. There was one case of in utero fetal death secondary to uterine rupture in a woman hospitalized for threatened preterm labor at 31 WG. Infant characteristics and respiratory status at birth are summarized in Table 1. All infants in the control group were born after 37 WG, and otoacoustic emissions were present for 53 infants. Auditory evoked potentials were controlled in one case and were normal. Otoacoustic emissions were not
registered in the medical file for one case, but no additional control was mentioned. In the group 2, 23 infants were born before 37 WG, and the abnormal auditory evoked potentials were registered for one ear in one case of premature delivery at 31 WG. Otoacoustic emissions were normal for 22 infants born after 37 WG in the group 2. Otoacoustic emissions were not registered in 2 cases in the two days following birth but were normal during a secondary control after leaving the maternity hospital (Table 1). Health status was evaluated four months after birth for 78 children and is summarized in Table 1.

Discussion

Our study demonstrates that quantitative fetal lung and liver stiffness measurement with 2D-SWE is feasible with acceptable reproducibility considering average pairs of measurements. Some ROIs are more reproducible and repeatable on these organs.

We assess lung stiffness on the proximal lung to reduce presumed impact of depth on measurements and to improve the reliability of fetal LLE ratio. Indeed, Quarello et al reported variation of ICC between proximal and distal lung in pregnant baboons, respectively 0.65 (95% CI: 0.42-0.81) and 0.42 (95% CI: 0.11-0.65) [25]. Our study indicates that region A3 was the most relevant given its feasibility and acceptable reproducibility considering average pairs of measurements (ICC between 0.70 and 0.78) (Table 3). Invalid measurements were also the lowest in this region compared to both other regions. This difference can be explained by acoustic shadowing generated by fetal ribs and disturbance of share wave speed induced by cardiac movements that are more important in A1 and A2 compared to A3.

Concerning the liver, the left extremity (region IV) was not repeatable and contained many invalid measurements (14.4%) compared with ROI V and VI. These results were probably related to the close anatomical relationships between liver, stomach and bowels in these regions. It is likely that operators confused these organs with incorrect placement of the focused ultrasounds, obtaining inaccurate results. Considering average pairs of measurements,
reproducibility was acceptable for region V (ICC between 0.75 and 0.73) and region VI (ICC between 0.70 and 0.84). Thus, in the modeling process of the fetal LLE ratio, we decided to retain only A3, V and VI as the most repeatable and reproducible ROI to compare fetal lung and liver stiffness on average of two measurements. As described in the results, three indexes were modeled and compared without significant differences for evaluation of lung and liver stiffness in the whole cohort. Thus, ROI V and/or VI can used to assess fetal liver stiffness with the same performance.

One advantage of this study was to evaluate stiffness in three tissues with different supposed biomechanical properties: normal lung, lung with increased surfactant after corticosteroids course, and liver. We noticed that 2D-SWE revealed stiffness variations according to organs: mean stiffness values for the lung (A3) and the liver (V, VI) in the group 1 were 2.30 kPa [1.30-3.40], 3.90 kPa [2.38-6.40] and 3.86 kPa [2.23-6.34], respectively. A moderate positive correlation with gestational age was found for the fetal lung. Considering that gestational age is one of the most important factors determining fetal lung maturation, increase lung stiffness during the studied period could reflect lung development and maturity. Similarly, there was a moderate positive correlation between fetal liver stiffness and gestational age. Thus, because lung and liver stiffness was increased in parallel through gestation in this study, the value of the fetal LLE ratio between 24 and 34 WG was constant with a mean value of 0.6. As a consequence, a very weak correlation was found for the fetal LLE and gestational age (R=0.08). By comparing liver and lung elasticity, gestational age-related changes in fetal lung maturation could be underestimated and masked. However, the studied period was limited between 24 and 34 WG, and the fetal LLE ratio may evolve differently after 34 WG because of surfactant synthesis by alveolar type 2 cells.

In this study, the fetal LLE ratio was interesting to compare variations of the fetal lung elasticity after a course of corticosteroids. Between two examinations, fetal lung elasticity
values decreased while those of the liver remained stable. Indeed, the relative difference of the fetal LLE ratio value was more important in group 2 exposed to corticosteroids (0.2; 95% CI: 0.07 to 0.34) compared to group 1 (-0.04; 95% CI: -0.29 - 0.08) ($P < 0.001$). These results could be explained by dispersion of shear wave speed and attenuation relevant to increase viscosity in fetal lung after corticosteroids secondary to surfactant synthesis, especially phosphatidylcholine. As dispersion increases, shear wave will be attenuated [30–32].

The use of 2D-SWE in fetal medicine is actually not recommended in current practice and must be regulated in registered research protocols. Issaoui al reviewed the potential effect of 2D-SWE on fetal tissues in a quantitative health risk assessment [33]. To date, no health agency has published recommendations about widespread use of 2D-SWE in obstetrics due to lack of data concerning impact of acoustic radiation force on particular displacement in fetal tissues[33]. However, observational and reassuring data are reported in the literature about use of 2D-SWE after 24 WG in preterm infants [21,34,35]. The “Elastomap study” is the first study to provide a collection of postnatal variables in all infants exposed in utero to 2D-SWE [36]. Indeed, vigilance data were collected at birth and 4 months later to ensure the absence of clinical effects. We did not collect severe adverse outcomes in the whole cohort, and the unique case of stillbirth is secondary to uterine rupture. The cochlea may be a particularly sensitive structure as 2D-SWE provides a mechanical pulsatile vibration [37]. In this study, neonatal hypoacusia screening tests were normal for 100 infants exposed to 2D-SWE during the prenatal period. In one case, otoacoustic emissions were not registered in the medical file, and one infant presented abnormal auditory evoked potentials for one ear in one case of premature delivery at 31 WG. These new data (medical history, health problems, exploration of olivo-cochlear reflex at birth, respiratory diseases or symptoms, and number of hospitalizations since birth) will enrich current literature concerning the use of 2D-SWE in
fetal medicine. These vigilance data are only descriptive but provide preliminary reassuring results.

This pilot study is monocentric, and limitations include impact of depth on measurements and use of a single transducer SC6-1 without comparison with others probes and machines. On the one hand, stiffness increases in both organs between 24 and 34 WG could be related to tissue density increases throughout pregnancy. On the other hand, the correlation coefficient was low, and other variables, especially depth, could influence the stiffness value. Indeed, the more pregnancy progresses, the more the distance between the probe and target organ is reduced, improving detection of the shear wave’s speed and estimation of stiffness value. Some pediatric studies reported the influence of the probe on stiffness evaluation related to the relationship between viscoelastic properties and transducer frequency. Stiffness should be interpreted according to the transmitted frequency, and the same transducer should be used for the follow-up [34,38,39]. Other studies are needed to confirm the feasibility of 2D-SWE for exploration of human fetal lungs and liver during gestation and to validate its reproducibility according to the probe and the machine.

This preliminary study contributes to clarifying the applicability of 2D-SWE for fetal lung and liver. 2D-SWE of human fetal lung and liver is feasible with acceptable reproducibility. This technique could be a new complementary tool for reliable prediction of the status of pulmonary function based on biomechanical properties. Further studies are needed to confirm the feasibility of 2D-SWE for exploration of human fetal lungs and liver during gestation and to validate its reproducibility according to the probe and the machine.

**Conflict of interest**

The authors have no conflicts of interest to disclose in relation with this article.
Funding sources
This research was supported by University Hospital of Besançon, through call for project proposals: “APICHU” Ref: API/2015/60.
References


**Figure captions**

**Fig 1.** Flow chart of patient inclusion

**Fig 2. Measurement technique.** Measurements were performed on a homogeneous circular ROI of 5 mm diameter manually drawn. Three measurements were performed on the proximal lung (2a) in three regions (A1, A2, and A3) and 3 measurements on the liver (2b) in three regions (IV, V, and VI). Average elasticity (Kpa) was obtained in target regions of interest (ROI) with an automatic calculation. ROI were placed manually on the different target regions: Elastography measurements of the fetal lung on region A2 (2b) and Elastography measurements of the fetal liver on region V (2d).

**Fig 3. Analysis of the fetal LLE ratio variation and stiffness evolution (regions A3 and V) according to gestational age.** The figure shows the « FitPlot » consisting of a Scatter plots of the data overlaid with the regression line, and 95% confidence and prediction limits. a,b,c) According to gestational age, linear correlation was significant for stiffness values of regions A3, V and VI with positive correlation coefficients. d) The value of the fetal LLE ratio between 24 and 34 WG could be considered constant with a mean value of 0.6. The linear trend was not significant \( (P=0.6) \) and the correlation was very weak \( (R^2= 0.006) \). \( R^2 \) : coefficient of determination

**Fig 4.** Fetal LLE ratio values between group 1 (a) and group 2 (b). This graphic represents fetal LLE ratio values obtained by the operator 1 in both groups. The fetal LLE ratio values at “day 2” decrease in fetuses exposed to corticosteroids.

**Table 1.** Maternal and infant characteristics.

**Table 2.** Mean difference between pairs of elastographic measurements made by the three operators in different region of interest (ROI).

**Table 3.** Intraclass correlation coefficients (ICC) of elastographic measurements (average of pairs) in different region of interest (ROI).
Recruited patients (n=103)

First SWE (Day 0)
- Control group (n=55)
  - Received allocated intervention (n=55)
  - Did not receive allocated intervention (n=0)
- Patients (n=48)
  - Received allocated intervention (n=48)
  - Did not receive allocated intervention (n=0)

Second SWE (Day 2)
- Received allocated intervention (n=54)
  - Did not receive allocated intervention (n=1)
    - Declined to participate (n=1)
- Received allocated intervention (n=42)
  - Did not receive allocated intervention (n=6)
    - Delivery (n=4)
    - Early discharge at home (n=2)

Children’s health status at birth
- Analyzed (n=55)
  - Excluded from analysis (n=0)
- Analyzed (n=47)
  - Excluded from analysis (n=1)
    - In utero fetal death (n=1)

Children’s health status in the 4 months after birth
- Analyzed (n=46)
  - Lost to follow up (n=9)
- Analyzed (n=32)
  - Lost to follow up (n=15)
Region VI
R² linear = 0.19
Fetal LLE ratio

R^2 linear = 0.006
<table>
<thead>
<tr>
<th></th>
<th><strong>Group 1</strong></th>
<th><strong>Group 2</strong></th>
<th><strong>P</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal characteristics</strong></td>
<td>n = 55</td>
<td>n = 48</td>
<td></td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>29.82 ±5.26 [20 - 42]</td>
<td>29.30 ± 5.54 [20 - 42]</td>
<td>0.63</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.33 ± 6.14 [19.43 - 50.78]</td>
<td>25.83 ± 3.99 [18.44 – 30.04]</td>
<td>0.41</td>
</tr>
<tr>
<td>Administration of corticosteroids</td>
<td>0 (0/55; 0%)</td>
<td>48 (48/48; 100%)</td>
<td>/</td>
</tr>
<tr>
<td>Gestational age (&lt;28)</td>
<td>21 (21/55; 38.2%)</td>
<td>12 (12/48; 25%)</td>
<td></td>
</tr>
<tr>
<td>Gestational age (28-31)</td>
<td>19 (19/55; 34.5%)</td>
<td>11 (11/48; 22.9%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Gestational age (≥31)</td>
<td>15 (15/55; 27.3%)</td>
<td>25 (25/48; 52.1%)</td>
<td></td>
</tr>
<tr>
<td><strong>Infant characteristics</strong></td>
<td>n = 55</td>
<td>n = 47</td>
<td></td>
</tr>
<tr>
<td><strong>in the 2 days following birth</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age at birth</td>
<td>38.95 ± 2.30 [35 - 41]</td>
<td>35.14 ± 3.30 [29 - 41]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Birth weight</td>
<td>3268.56 ± 500.90 [2200 - 4455]</td>
<td>2413.72 ±742.58 [725 - 3775]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median Apgar 1 min</td>
<td>10 ±0 [2 - 10]</td>
<td>10 ±2 [5 - 10]</td>
<td>0.20</td>
</tr>
<tr>
<td>Median Apgar 5 min</td>
<td>10 ±0 [6 - 10]</td>
<td>10 ±0 [8 - 10]</td>
<td>0.65</td>
</tr>
<tr>
<td>Spontaneous ventilation</td>
<td>48 (48/55; 87.2%)</td>
<td>31 (31/47; 66%)</td>
<td></td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>7 (7/55; 12.7%)</td>
<td>15 (15/47; 31.9%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Mechanical ventilation with intubation</td>
<td>0</td>
<td>1 (51/47; 2.1%)</td>
<td></td>
</tr>
<tr>
<td>Normal otoacoustic emissions</td>
<td>53 (52/53; 98.1%)</td>
<td>24 (24/24; 100%)</td>
<td>0.34</td>
</tr>
<tr>
<td>Normal auditory evoked potentials</td>
<td>1 (1/1; 100%)</td>
<td>22 (22/23; 95.6%)</td>
<td>0.83</td>
</tr>
<tr>
<td><strong>Infant characteristics</strong></td>
<td>n = 46</td>
<td>n = 32</td>
<td></td>
</tr>
<tr>
<td><strong>in the 4 months following birth</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>6.63 ± 1.35 [3.76 – 10.57]</td>
<td>5.26 ± 1.67) [1.4 – 7.77]</td>
<td>0.004</td>
</tr>
<tr>
<td>Hospitalizations</td>
<td>Yes= 2 / No = 44</td>
<td>Yes= 4 / No= 28</td>
<td>0.22</td>
</tr>
<tr>
<td>Asthma</td>
<td>0 / 46</td>
<td>0 / 32</td>
<td>&gt; 0.99</td>
</tr>
<tr>
<td>Cough</td>
<td>10 / 46</td>
<td>6 / 32</td>
<td>0.75</td>
</tr>
<tr>
<td>Sputum</td>
<td>5 / 46</td>
<td>3 / 32</td>
<td>1</td>
</tr>
<tr>
<td>Bronchiolitis</td>
<td>8 / 46</td>
<td>3 / 32</td>
<td>0.51</td>
</tr>
<tr>
<td>Extra respiratory disease</td>
<td>0 / 46</td>
<td>1 / 32</td>
<td>0.41</td>
</tr>
</tbody>
</table>

BMI: Body mass index.
Quantitative variables are expressed as mean ± standard deviation; numbers in brackets are ranges. Qualitative variables are expressed as raw numbers; numbers in parentheses are proportions followed by percentages.

**P < 0.05** is significant (Student’s t test or Mann-Whitney’s U test for quantitative variable; Chi-squared test or Fisher’s exact test for qualitative variables, as appropriate)
<table>
<thead>
<tr>
<th>Measurements</th>
<th>Sample size</th>
<th>Mean difference (KPa)</th>
<th>95% CI</th>
<th>Standard deviation</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Region A1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operator 1</td>
<td>125</td>
<td>-0.05</td>
<td>-0.13 to 0.04</td>
<td>0.41</td>
<td>0.26</td>
</tr>
<tr>
<td>Operator 2</td>
<td>51</td>
<td>0.07</td>
<td>-0.02 to 0.162</td>
<td>0.32</td>
<td>0.12</td>
</tr>
<tr>
<td>Operator 3</td>
<td>12</td>
<td>0.13</td>
<td>-0.03 to 0.30</td>
<td>0.26</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Region A2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operator 1</td>
<td>158</td>
<td>0.01</td>
<td>-0.04 to 0.07</td>
<td>0.38</td>
<td>0.69</td>
</tr>
<tr>
<td>Operator 2</td>
<td>71</td>
<td>0.04</td>
<td>-0.05 to 0.13</td>
<td>0.39</td>
<td>0.41</td>
</tr>
<tr>
<td>Operator 3</td>
<td>28</td>
<td>-0.03</td>
<td>-0.26 to 0.20</td>
<td>0.60</td>
<td>0.79</td>
</tr>
<tr>
<td><strong>Region A3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operator 1</td>
<td>162</td>
<td>0.04</td>
<td>-0.02 to 0.09</td>
<td>0.38</td>
<td>0.19</td>
</tr>
<tr>
<td>Operator 2</td>
<td>73</td>
<td>-0.02</td>
<td>-0.11 to 0.07</td>
<td>0.38</td>
<td>0.59</td>
</tr>
<tr>
<td>Operator 3</td>
<td>26</td>
<td>-0.01</td>
<td>-0.20 to 0.18</td>
<td>0.48</td>
<td>0.93</td>
</tr>
<tr>
<td><strong>Region IV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operator 1</td>
<td>159</td>
<td>0.10</td>
<td>0.002 to 0.21</td>
<td>0.65</td>
<td><strong>0.04</strong></td>
</tr>
<tr>
<td>Operator 2</td>
<td>72</td>
<td>0.13</td>
<td>0.01 to 0.26</td>
<td>0.54</td>
<td><strong>0.04</strong></td>
</tr>
<tr>
<td>Operator 3</td>
<td>24</td>
<td>-0.13</td>
<td>-0.45 to 0.18</td>
<td>0.75</td>
<td>0.40</td>
</tr>
<tr>
<td><strong>Region V</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operator 1</td>
<td>160</td>
<td>-0.03</td>
<td>-0.13 to 0.07</td>
<td>0.65</td>
<td>0.52</td>
</tr>
<tr>
<td>Operator 2</td>
<td>72</td>
<td>-0.03</td>
<td>-0.16 to 0.11</td>
<td>0.56</td>
<td>0.66</td>
</tr>
<tr>
<td>Operator 3</td>
<td>23</td>
<td>-0.08</td>
<td>-0.50 to 0.34</td>
<td>0.97</td>
<td>0.68</td>
</tr>
<tr>
<td><strong>Region VI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operator 1</td>
<td>127</td>
<td>-0.03</td>
<td>-0.14 to 0.09</td>
<td>0.66</td>
<td>0.61</td>
</tr>
<tr>
<td>Operator 2</td>
<td>63</td>
<td>-0.02</td>
<td>-0.16 to 0.11</td>
<td>0.54</td>
<td>0.74</td>
</tr>
<tr>
<td>Operator 3</td>
<td>24</td>
<td>0.11</td>
<td>-0.20 to 0.42</td>
<td>0.74</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Operator 1 was the reference operator (senior clinical lecturer), operator 2 was resident and operator 3 was intern. Bold indicates significant differences.
<table>
<thead>
<tr>
<th>Measurements</th>
<th>Number of subjects</th>
<th>Single measure ICC [95% CI]</th>
<th>Average measure ICC [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Region A1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operators 1 and 2</td>
<td>28</td>
<td>0.57 [0.26-0.77]</td>
<td>0.73 [0.42-0.88]</td>
</tr>
<tr>
<td>Operators 1 and 3</td>
<td>13</td>
<td>0.68 [0.26-0.89]</td>
<td>0.81 [0.42-0.94]</td>
</tr>
<tr>
<td><strong>Region A2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operators 1 and 2</td>
<td>36</td>
<td>0.65 [0.40-0.80]</td>
<td>0.78 [0.58-0.89]</td>
</tr>
<tr>
<td>Operators 1 and 3</td>
<td>22</td>
<td>0.16 [-0.26-0.53]</td>
<td>0.28 [0.72-0.69]</td>
</tr>
<tr>
<td><strong>Region A3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operators 1 and 2</td>
<td>36</td>
<td>0.54 [0.26-0.73]</td>
<td>0.70 [0.42-0.85]</td>
</tr>
<tr>
<td>Operators 1 and 3</td>
<td>23</td>
<td>0.64 [0.32-0.82]</td>
<td>0.78 [0.48-0.90]</td>
</tr>
<tr>
<td><strong>Region IV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operators 1 and 2</td>
<td>37</td>
<td>0.48 [0.19-0.69]</td>
<td>0.65 [0.33-0.81]</td>
</tr>
<tr>
<td>Operators 1 and 3</td>
<td>23</td>
<td>0.68 [0.55-0.92]</td>
<td>0.81 [0.38-0.85]</td>
</tr>
<tr>
<td><strong>Region V</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operators 1 and 2</td>
<td>36</td>
<td>0.60 [0.34-0.77]</td>
<td>0.75 [0.50-0.87]</td>
</tr>
<tr>
<td>Operators 1 and 3</td>
<td>23</td>
<td>0.57 [0.23-0.79]</td>
<td>0.73 [0.37-0.88]</td>
</tr>
<tr>
<td><strong>Region VI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operators 1 and 2</td>
<td>33</td>
<td>0.54 [0.25-0.74]</td>
<td>0.70 [0.40-0.85]</td>
</tr>
<tr>
<td>Operators 1 and 3</td>
<td>21</td>
<td>0.72 [0.43-0.88]</td>
<td>0.84 [0.60-0.94]</td>
</tr>
</tbody>
</table>

Operator 1 was the reference operator (senior clinical lecturer), operator 2 was resident and operator 3 was intern. Bold indicates significant differences.