Study Protocol

Heterogeneity Index in Neonatologist-performed Lung Ultrasound in Neonates Receiving Respiratory Support - a pilot study

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1. Introduction

Lung ultrasound is rapidly gaining recognition as a valuable clinical tool for dynamic lung assessment.[1–3]
Recently, its application in neonatal intensive care units (NICU) has received increasing attention. Neonatologist-performed lung ultrasound (NPLUS) has proven to be a highly reliable, quick and safe diagnostic tool in neonatal intensive care.[4,5] The clinical utility of NPLUS extends to diagnosing and monitoring a range of respiratory disorders, including acute respiratory distress syndrome, transient tachypnoea of the newborn, pneumonia, pleural effusion, atelectasis, lung edema, meconium aspiration syndrome, and pneumothorax.[6–11] Unlike conventional X-ray imaging, NPLUS provides a radiation-free alternative with high specificity and sensitivity, making it an attractive option for neonatal lung assessment. [3,12,13]

2. Background

Lung ultrasound relies on interpreting ultrasound artifacts, analysing the pleura, and visualizing consolidations and pleural effusions, thereby improving the differential diagnosis of respiratory distress in neonates.
Neonates can progress from a white lung pattern (i.e. complete aeration loss due to fluid-filled alveoli) through B-lines (vertical, dynamic artifacts arising at the fluid-air interface, commonly linked to extravascular lung water or alveolar-interstitial edema) to A-lines (horizontal reverberation artifacts suggesting improved aeration and fluid clearance), marking a gradual transition toward lung aeration.
The impairment of lung aeration can be quantified based on the number and type of visualized artefacts. [14,15]
Several studies have investigated the role of NPLUS in neonatal respiratory assessment. By employing the lung ultrasound score (LUS) - a semi-quantitative tool for evaluating lung aeration and pathology - neonatologists can identify infants requiring surfactant therapy or those at risk for developing bronchopulmonary dysplasia. [5,8,16–18]
There is emerging evidence that NPLUS is not only a reliable tool for predicting the need for surfactant administration but also for differentiating between the underlying causes of respiratory distress in neonates. To date, different scoring systems have been developed for various clinical contexts. [19–21] These scores aim to provide a comprehensive assessment of lung condition by systematically screening the lung fields. We aim to use the LUS proposed Rodriguez-Fanjul [7,8,16], dividing the chest into six regions (anterior, lateral and posterior region on each side), assigning a score from 0 – 3 to each region.

Among the many advantages of this accessible semi-quantitative method, the subjectivity of the scoring system remains a limitation that may influence the evaluation of a patient's lung condition. Moreover, the standard 0–3 scoring scale of each lung area may not always capture subtle yet clinically significant changes in lung aeration over time.

To correlate fetal lung tissue characteristics with lung pathologies, gynaecologists have introduced the Heterogeneity Index (HI) as a quantitative assessment tool. [22–24]

The HI represents a quantitative ultrasound texture analysis method that has demonstrated promising diagnostic performance, with a sensitivity of 88% and specificity of 92% for evaluating fetal lung maturity and predicting respiratory distress in the newborn. [22,25–27] This technique analyses ultrasonographic fetal lung heterogeneity at the pixel level. By assessing the patterns and variations within the lung tissue, this method provides a numerical representation of lung texture, rather than a categorical score as used in current lung ultrasound scoring systems. [28,29] Although the fetal lung has different physical properties compared to the neonatal lung, as it is not yet aerated, the same method could be applied to assess the lung condition in neonates after birth. As aeration progresses, the neonatal lung generates ultrasound artifacts that, despite the significant differences in physical properties, could still be quantitatively evaluated using the HI.
This objective approach enhances the accuracy of lung ultrasound assessments and may contribute to improved decision-making in neonatal respiratory management. We aim to introduce a quantitative ultrasound analysis that can be easily implemented into clinical practice, as it might provide valid information from standard lung ultrasound.
To the best of our knowledge, this approach has not yet been applied in the field of NPLUS.

3. Objectives

This study aims to perform NPLUS in neonates receiving non-invasive or invasive respiratory support in the NICU and analyse the raw ultrasound image data to derive the HI. The HI will then be compared to the LUS to assess its clinical relevance.

Hypothesis I: It is feasible to obtain the HI through NPLUS in the study population (‘proof of concept’).

Hypothesis II: The HI correlates with the LUS. A higher HI reflects a more severe respiratory condition, characterized by greater loss of aeration, and is associated with a higher LUS. Conversely, a lower HI corresponds to better lung aeration and a lower LUS, indicating a healthier lung condition.

Hypothesis III: During the first week of life, in neonates receiving respiratory support, the HI demonstrates a dynamic change between two time points at which NPLUS is performed. A decrease in HI would indicate an improvement in the neonate’s lung condition. The decrease in the HI between the two assessment time points may be more pronounced compared to the LUS, suggesting a higher discriminative ability of the HI in detecting changes in lung condition.

Hypothesis IV: There is a correlation between HI and both the duration of respiratory support required and the applied mode of ventilation.

4. Design, setting and participants

Study design: The study is designed as an observational, prospective pilot study.

Study population: This study will include neonates with a gestational age between 28+0 weeks and full term who require either non-invasive or invasive respiratory support in the NICU.

Study procedure: A trained medical professional will perform NPLUS at predefined time points after birth: *NPLUS 1* within 72 hours of birth and *NPLUS 2* within 72 hours (minimum 24 hours) after the first examination. To minimize interference with routine care, NPLUS will be performed by an independent physician who is not involved in the patient's routine clinical care. Participation in the study will not alter the standard clinical care in the NICU. Clinicians will be blinded to the NPLUS results except in cases of pneumothorax and pleural effusion.

NPLUS is performed bilaterally with longitudinal scans of the chest on the midclavicular, anterior, and posterior axillary line. The scans can be performed either with the patient in supine or prone position as the posterior axillary line is accessible from both sides of the neonate. A 12-15 MHz linear transducer is used, preferably with the preset "small parts" and an ultrasound penetration depth of 3-4 cm. The raw ultrasound images are saved as Tag Image File Format (TIFF).

Heterogeneity Index (HI): The raw ultrasound images will be reviewed by a trained medical professional. A region of interest (ROI) will be manually selected in each lung area that should be analysed. Each ROI will be a square that includes the largest possible area with lung artefacts, starting from the pleura and excluding subcutaneous tissue.
The grayscale-based analysis will be performed using a custom-made program developed in MATLAB (R2024b, Version 11.19.6.2). Texture features in the lung tissue will be related to pathophysiologic processes. The HI will then be determined by measuring the intensity of each pixel and calculating the average of all pixels within the ROI.

Lung ultrasound score (LUS): The score from 0-3 will be assigned to each scan area based on the detected pattern (0 = A-pattern: only A-lines; 1 = B-pattern: three or more B-lines, well-spaced; 2 = severe B-pattern: crowded and/ or fusing B lines with/ without consolidations limited to subpleural space; 3 = extended consolidations). [7,8,16]
To ensure inter-observer reliability, two neonatologists, blinded to the patient's identity and clinical conditions, will independently score the anonymized ultrasound images.

Inclusion criteria:

- Preterm neonates born between 28+0 and 36+6 weeks of gestation and full-term neonates (> 37+0 weeks of gestation) admitted to the NICU of the Division of Neonatology, Medical University of Graz.
- Presence of respiratory distress requiring respiratory support at the time of *NPLUS 1*.
- Written informed consent obtained from the parents prior to the measurement.

Exclusion criteria:

- Cardiopulmonary malformations
- Pleural effusion
- Pneumothorax

Sample Size: A sample size of 40 patients (20 preterm and 20 full-term neonates) will be analysed in this study. Sample size calculations cannot be performed in advance, as no data from previous studies are available. Therefore, this pilot study aims to generate data for the sample size estimation in the consecutive main study. [30]

5. Statistical analysis

Demographic data, clinical characteristics, and lung ultrasound features will be summarized descriptively. First, the Shapiro-Wilk test will be used to assess the normality of the data. Depending on the distribution, data will be presented as mean (standard deviation, SD) or median (interquartile range, IQR).

Differences in primary and secondary outcome parameters are calculated using Chi-square and Fisher's exact tests for categorical variables, and t-test or Mann-Whitney U test for continuous variables.

Additionally, correlations between HI and LUS, as well as duration of respiratory support required and the applied mode of ventilation will be analysed using either the Spearman's rank correlation coefficient or the Pearson's correlation, depending on which is more appropriate.

The correlation analyses are considered in an explorative sense; therefore, no multiple testing corrections will be performed. A value of $p < 0.05$ will be considered as statistically significant.

MATLAB (R2024b, version 11.19.6.2) will be used for statistical analysis.

6. Outcome measures

Primary Outcome Measures

- Determination of the heterogeneity index of *NPLUS 1* and *NPLUS 2*
- Determination of the LUS by Rodriguez-Fanjul [16] of *NPLUS 1* and *NPLUS 2*

Secondary Outcome Measures

- Duration of respiratory support (in hours)
- Mode of respiratory support (non-invasive versus invasive ventilation)
- Routinely obtained capillary blood gas analysis of the neonate within 6 hours before or after *NPLUS 1* or *NPLUS 2*
- Routinely obtained monitoring parameters including arterial oxygen saturation (SpO_2), heart rate (either by pulse oximetry or electrocardiography), and – if available – cerebral tissue oxygen saturation (near-infrared spectroscopy)
- SpO_2/FiO_2 ratio (S/F ratio)
- Chest X Ray findings (if performed within 6 hours before or after *NPLUS 1* or *NPLUS 2*)
- pH of the umbilical artery
- APGAR score

- Pregnancy risk factors including intra-amniotic infection, and premature rupture of the membranes, pre-eclampsia
- Prenatal corticosteroids

7. Research Plan and timetable

The study will start in spring/summer 2025.
The study is designed for an approximate running time of two years.

8. References

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