Assessing the likelihood of needing a substance called surfactant for newborns born near their expected delivery date using lung ultrasound (ULTRA Study)

STATISTICAL ANALYSIS PLAN

MAIN ANALYSES

For the accuracy of lung ultrasound score (LUS) to be non-inferior to that observed in more preterm neonates, receiver operator characteristics (ROC) analysis should give a similar area under the curve (AUC). We anticipate an AUC of 0.93 (95% confidence interval: 0.86-0.99) as this was originally found in preterm neonates (*Brat R. JAMA Pediatr 2015*) and we consider AUC=0.80 as null hypothesis. The proportion of late preterm and term neonates with respiratory failure needing surfactant treatment will be set at 20%, as previously reported (*Brat R. JAMA Pediatr 2015*). With power=80% and α=0.05, the needed sample size turned will be 145 patients (29 positive and 116 negative cases) *(Hanley JA. Radiology 1982).*

Basic population data will be compared between patients with and without surfactant administration using χ^2, Fisher, Student or Mann-Whitney test. Full ROC analysis will be performed and derived diagnostic accuracy parameters (sensitivity, specificity, positive and negative likelihood ratios, positive and negative predictive values, global accuracy, positive and negative post-test probability) will be calculated with their 95% confidence interval (CI). We will evaluate LUS as: 1) replacement test to be used with both the highest sensitivity and specificity as possible, and 2) triage test, that is with the highest sensitivity, irrespective of specificity *(Hayen A. Journal of Clinical Epidemiology 2010).*

ROC analysis will be performed for the whole population and for two pre-specified subgroups represented by late preterm and term patients (i.e. with gestational age comprised between 34^{+0} and 36^{+6} or $\geq 37^{+0}$, respectively) to investigate the effect of gestational age, as suggested by recent methodological literature *(Hayen A. Journal of Clinical Epidemiology 2010).* AUC will be compared between subgroups and with those originally reported in more preterm babies

(Brat R. JAMA Pediatr 2015 and De Martino L. Pediatrics 2018) and with the summary AUC obtained by the latest meta-analysis *(Capasso L. Pediatr Pulmonology 2023)* using the Hanley technique *(Hanley JA. Radiology 1982).*

The relationship between LUS and gas exchange will be investigated with Spearman correlation coefficient and adjusted for gestational age using linear regression *(Hayen A. Journal of Clinical Epidemiology 2010)*. Analyses were performed with SPSS 29 (Armonk-NY, USA) and MedCalc 13.3 (Ostend, Belgium) and $p<0.05$ were considered significant.

ADDITIONAL ANALYSES

For a subset of patients where the following additional measurements were performed, additional analyses will be realised. These analyses will be performed only for those patients receiving these measurements before surfactant administration (if any) within the framework of their routine clinical care. Therefore, the pragmatic nature of the study, nested within the clinical care, will stand as described above.

1. In some centres, not only the classical LUS but also the extended LUS will be calculated (based on 5 scanned chest areas per each hemithorax) *(Loi B. Am J Resp Crit Care Med 2021).* LUS and eLUS will be compared in terms of AUC (and derived accuracy variables); AUC comparison will be global and for partial zones using the Hanley method (*Hanley JA. Radiology 1982).* The relationship between eLUS and gas exchange metrics will also be investigated similarly to what is described above. The aim will be to clarify if LUS is sufficient or the eLUS is better to guide surfactant therapy early after birth.

2. In some centres, the thoracic extra-vascular fluid content, estimated non-invasively by electrical cardiometry *(Boet A. J Perinatol 2016)* will be considered. This variable will be studied in relation to the lung aeration (LUS, eLUS) and to gas exchange and vital parameters trying to understand better the pathophysiology of respiratory failure in late preterm/term neonates. Correlation analyses followed by linear regressions adjusted by gestational age and other possible confounders will be performed. AUC of total fluid content will be compared with those of lung ultrasound scores. Simple univariate analyses will also be performed dividing the population between those who received surfactant or not and according to other basic variables. The aim will be to clarify if respiratory failure is preferentially cause by excessive fluid accumulation, inflammation or mere surfactant deficiency.