Chapter Four, Results

Basic population details are summarized in Table 1.

	Whole population (43)
Gestational Age (wks)	34.09 ± 1.57
Birth weight (g)	2169 ± 668
Steroids	14 (32.6%)
C-section	27 (62.8%)
Male sex	25 (58.1%)
SNAPPE II	5 [0-18]
Apgar V	9 [7-10]
pPROM	9 (20.9%)
Clinical chorioamnionitis	3 (7%)
Surfactant	8 (18.6%)
TTN	34 (79.1%)
RDS	9 (20.9%)

Table 1: Basic Population Characteristics. Data are expressed as mean (standard deviation), median [interquartile range] and number (%). Abbreviations: CRIB-II: critical risk index for babies; SNAPPE : Score for Neonatal Acute Physiology and Perinatal Extension.

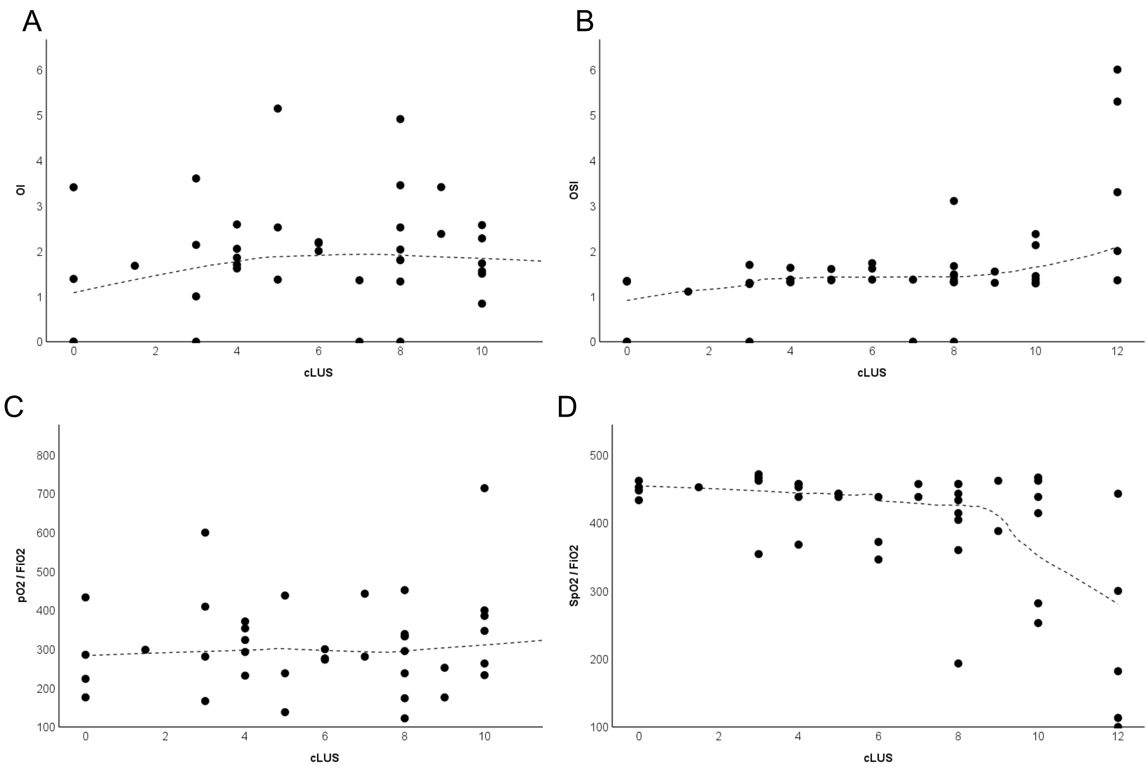
A total of 43 preterm infants (n = 43 at "Antoine Béclère" hospital, Clamart, France) were included. Demographic and clinical characteristics of the study populations are detailed in Table 1. During the study period none of the infants received volume filling or blood/ plasma transfusions, which could have influenced TFC measurement.

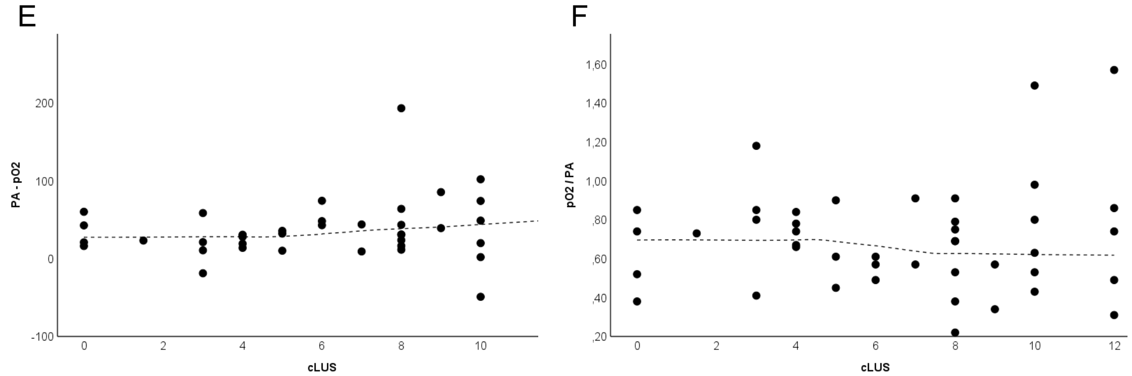
The GA range was 32 to 36 weeks (mean 34 weeks of GA), and the birth weight range was 1060 to 3200 g (mean 2169 grams). Of these, 25 were males and 18 females; 27 were born by caesarean section (C-section) and 16 by spontaneous delivery. A complete course of antenatal steroids was administered in 32.6% of babies. Mean Apgar score at 5 minutes of life was 8.72. Mean SNAPPEII was 10, 27. 9 infants (22.5%) had a prolonged precocious rupture of membranes (pPROM), and 3 (7.5%) had chorioamnionitis.

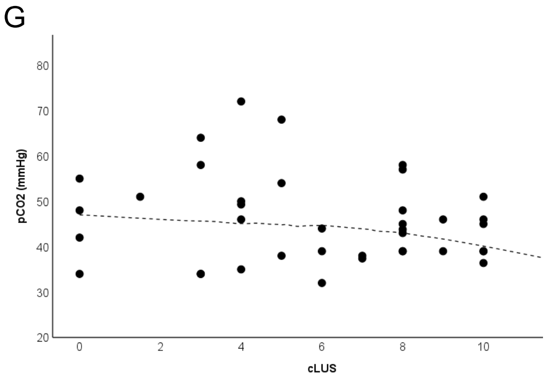
Overall, 38 infants (88.3% of the total study population) required a non-invasive respiratory support during the study period: 37 neonates (86%) were supported with CPAP, 1 (2.3%) with biPAP, and 5 (11.6%) were in spontaneous breathing. The mean (SD) mean airway pressure (MAP) was 5.25 cm H2O. A complete course of antenatal steroids was administered in 14 patients (all born at a GA less than 34 weeks). All babies who finally received surfactant, 9 out of 43 (20.9%) were diagnosed as having RDS; the other babies, 34 (79.1%) were diagnosed as having TTN. Only 1 lung ultrasound per patient was performed and was always well tolerated.

Surfactant replacement was performed in the whole population at mean 4.18 hours of life (1.5 – 8); none of the patients necessitate a second surfactant dose. All the patient received surfactant by INSURE. None of the patients had air leak complication after surfactant administration. None of the patient necessitate of MV after surfactant administration.

Mean duration of O2-therapy was 0.5 days (0 – 5). Mean NICU stay was 8 days (2-17). Mortality was 0.

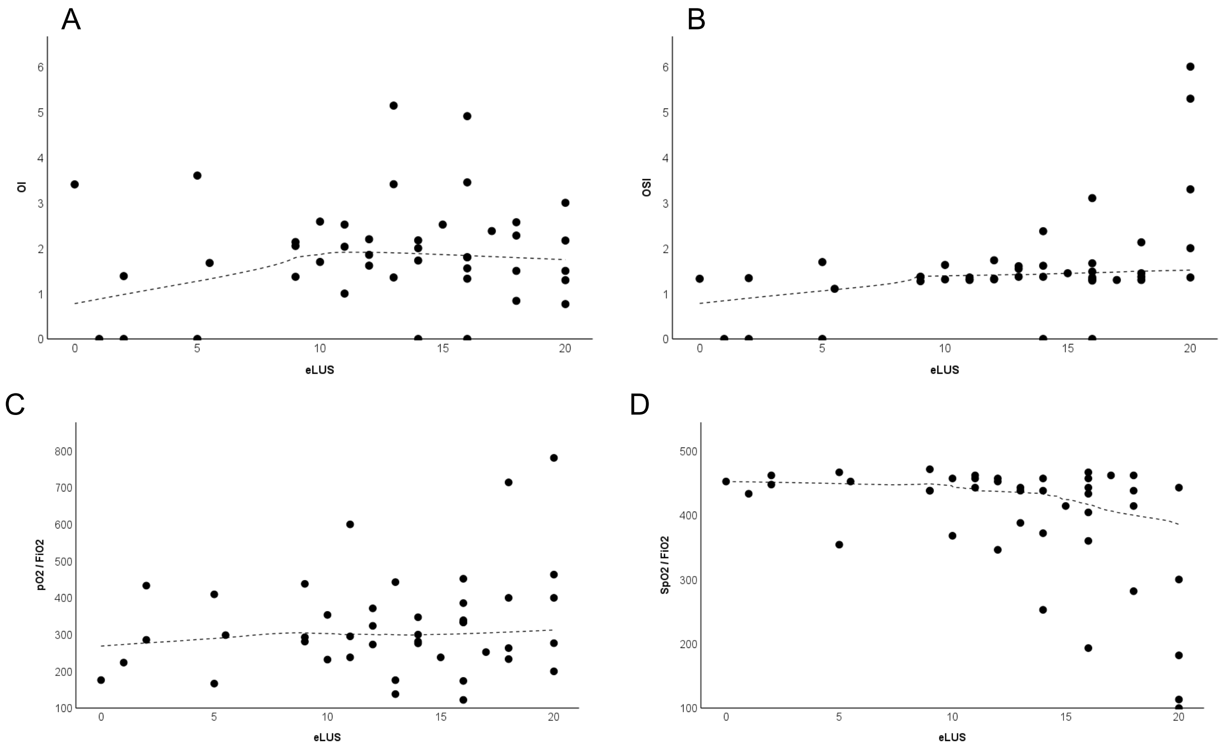






	Rho (ρ)	*p*	Adj_{GA} r	*p*
A	0.095	0.545	0.012	0.470
B	0.529	< 0.001	0.478	<0.001
C	0.210	0.177	0.276	0.077
D	-0.524	<0.001	-0.479	<0.001
E	0.200	0.199	0.107	0.498
F	0.074	0.638	0.143	0.365
G	-0.347	0.023	-0.364	0.018

Tables 2: Relationships between cLUS and oxygenation metrics. A, Oxygenation index. B, Oxygen saturation index (OSI). C, Transcutaneous partial pressure of oxygen (PtcO2) to fraction of inspired oxygen (FiO2) ratio. D, Peripheral Oxygen saturation (SpO2) to fraction of inspired oxygen (FiO2). E, Alveolar-arterial gradient. F, Arterial to alveolar ratio. G, Transcutaneous partial pressure of carbon dioxide (PtcCO2). The indices representing oxygenation are in absolute numbers. Lines represent the best-fitting data regressions. Results of correlation analysis are also shown, with ρ indicating the crude Spearman coefficient and adjusted r indicating the partial correlation coefficient adjusted for gestational age. In yellow, significant correlations.



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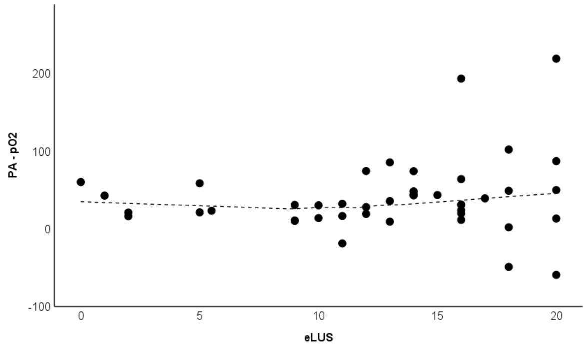

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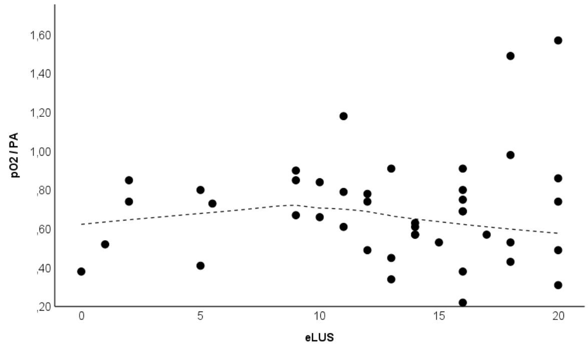

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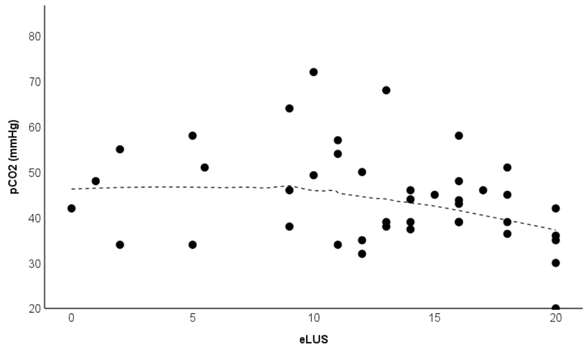


	Rho (ρ)	***p***	**Adj$_{GA}$ r**	***p***
A	0.118	0.450	0.038	0.812
B	0.494	< 0.001	0.434	0.004
C	0.223	0.151	0.290	0.062
D	-0.450	0.002	-0.399	0.009
E	0.151	0.333	0.053	0.739
F	0.109	0.487	0.181	0.252
G	-0.297	0.053	-0.313	0.044

Tables 3: Relationships between eLUS and oxygenation metrics. A, Oxygenation index. B, Oxygen saturation index. C, Transcutaneous partial pressure of oxygen (PtcO2) to fraction of inspired oxygen (FiO2) ratio. D, Peripheral Oxygen saturation (SpO2) to fraction of inspired oxygen (FiO2). E, Alveolar-arterial gradient. F, Arterial to alveolar ratio. G, Transcutaneous partial pressure of carbon dioxide (pCO2). The indices representing oxygenation are in absolute numbers. Lines represent the best-fitting data regressions. Results of correlation analysis are also shown, with ρ indicating the crude Spearman coefficient and adjusted r indicating the partial correlation coefficient adjusted for gestational age. In yellow, significant correlations.

The mean hour of evaluation was 6.58, the range of evaluation was 2 to 14 hours after birth. Subgroup analysis gave similar significant correlations for SpO2 to FiO2 ratio and cLUS (ρ = −0.529; GA < .001), and oxygen saturation index (OSI) and both cLUS and eLUS (cLUS: ρ = -0.524; P < .001; eLUS: ρ = 0.494; P < .001). Thus, a significative correlation was found between pCO2 and cLUS (ρ = -0.347; P < .023) and SpO2 to FiO2 ratio and eLUS (ρ = -0.450; P < .002).

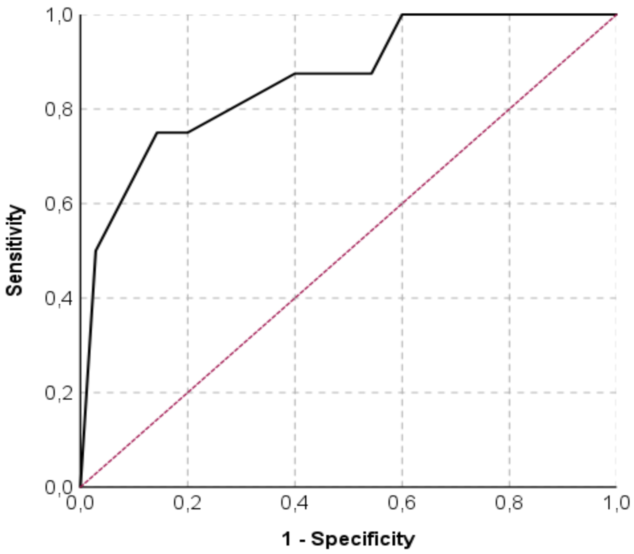
We calculated the TFC index as the ratio between TCF and the birth weight (BW) expressed in kilos and then explored the correlations between the TFC index and the main oxygenation indices.

	Rho (ρ)	*p*
A	0.119	0.446
B	0.132	0.398
C	-0.192	0.218
D	-0.112	0.473
E	0.075	0.632
F	-0.139	0.374
G	0.185	0.236

Table 4: Relationships between TFC index/Kg of BW and oxygenation metrics. A, Oxygenation index. B, Oxygen saturation index. C, Transcutaneous partial pressure of oxygen (PtcO2) to fraction of inspired oxygen (FiO2) ratio. D, Peripheral Oxygen saturation (SpO2) to fraction of inspired oxygen (FiO2). E, Alveolar-arterial gradient. F, Arterial to alveolar ratio. G, Transcutaneous partial pressure of carbon dioxide (pCO2).

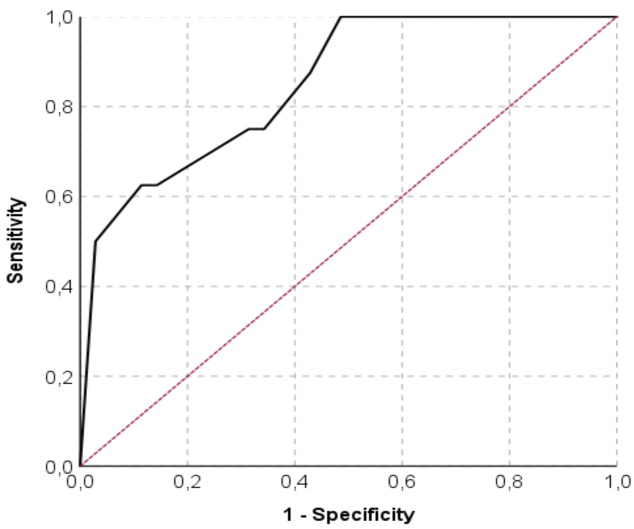
We defined a ROC curve to find the cLUS value with the best sensitivity and specificity to predict the need for surfactant. The area under the ROC curve was 0.862 (95% CI = [0.718 to 1.000], p = 0.002). The same was done for eLUS: the area under the ROC curve was 0.850 (95% CI = [0.711 to 0.989], p = 0.002).

Table 4 and 5 show the reliability data for the cLUS and the eLUS respectively, to predict surfactant administration. In our population, having a cLUS score greater than 6 or a eLUS greater than 14 increases the probability to need surfactant administration with a sensitivity of 87.5% and a specificity of 54.3% using cLUS, and with a sensitivity of 87.5% and a specificity of 42.9% using eLUS.



cLUS predicting Surfactant	
AUC	0.862
Significance (p value)	0.002
95% confidence interval	0.718-1.000
Cut off	**6**
sensitivity	87.5
specificity	54.3

Table 5. cLUS predicting Surfactant



eLUS predicting Surfactant	
AUC	0.850
Significance (p value)	0.002
95% confidence interval	0.711-0.989
Cut off	**14**
sensitivity	87.5
specificity	42.9

Table 6: eLUS predicting Surfactant

The correlations between TFC and GA and between TFC and eLUS are shown below (Table 7).

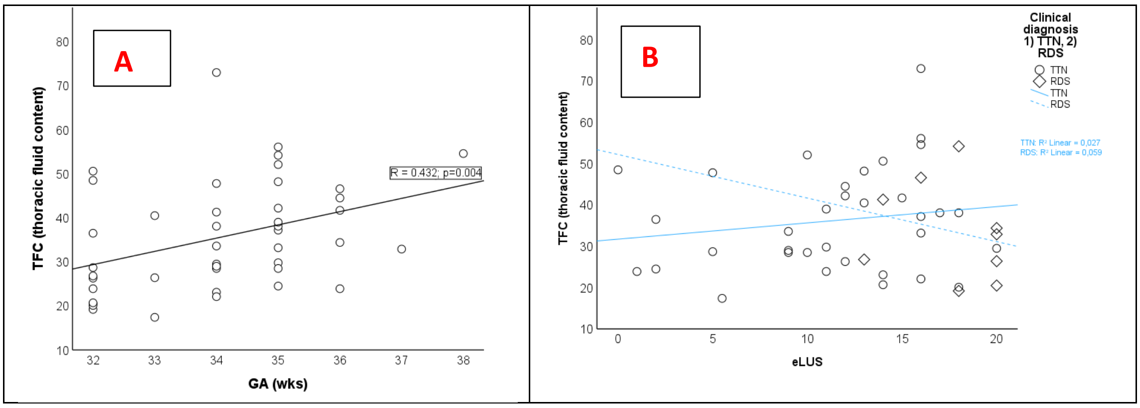


Table 7: Correlations between TFC and GA and between TFC and eLUS.

Panel A shows that the TFC increases with increasing GA (ρ=0.432, p=0.004). Panel B shows the correlation between eLUS and TFC achieved in the subgroups of respiratory diseases: TTN has a direct correlation between eLUS and TFC (ρ=0.164, p=0.027), while in RDS relationship seems almost inverse (ρ= -0.315, p=0.05).

A multivariate linear regression was performed to investigate the association between LUS and sex, gestational age, type of delivery, SNAPPE II, thoracic fluid content and clinical diagnosis. These variables statistically significantly predicted LUS, $F(8, 34) = 3.424$, $p = 0.005$; $R^2 = .446$.

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	,668[a]	,446	,316	4,511

a. Predictors: (Constant), Sex, GA, BW, type of delivery, Apgar V, SNAPPE II, TFC (thoracic fluid content), clinical diagnosis

b. Dependent Variable: eLUS

Table 8. Model Summary

The "**R**" column represents the value of *R*, the ***multiple correlation coefficient***. *R* can be one measure of the quality of the prediction of the dependent variable: in this case, LUS. A value of 0.668, indicates a good level of prediction. The "**R Square**" column represents the R^2 value (also called the coefficient of determination), which is the proportion of variance in the dependent variable that can be explained by the independent variables (technically, it is the proportion of variation accounted for by the regression model above and beyond the mean model). You can see from our value of 0.446 that our independent variables explain 44.6% of the variability of our dependent variable.

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	-60,351	23,926		-2,522	,017	-108,975	-11,727
	Sex	-,175	1,602	-,016	-,110	,913	-3,431	3,080
	GA	2,460	,808	,709	3,046	,004	,819	4,102
	BW	-,004	,002	-,524	-2,027	,051	-,009	,000
	type of delivery	,280	1,725	,025	,163	,872	-3,225	3,786
	Apgar V	-,969	,554	-,296	-1,750	,089	-2,095	,156
	SNAPPE II	-,163	,068	-,364	-2,410	,022	-,301	-,026
	TFC (thoracic fluid content)	,005	,069	,010	,066	,948	-,135	,144
	Clinical diagnosis 1) TTN, 2) RDS	6,613	1,746	,499	3,788	<,001	3,065	10,160

a. Dependent Variable: eLUS

Table 9. Coefficents

We can test for the statistical significance of each of the independent variables. This tests whether the unstandardized (or standardized) coefficients are equal to 0 (zero) in the population. If $p < .05$, you can conclude that the coefficients are statistically significantly different to 0 (zero).

Unstandardized coefficients indicate how much the dependent variable varies with an independent variable when all other independent variables are held constant. Only two variables added statistically significantly to the prediction.

Consider the effect of clinical diagnosis. The unstandardized coefficient, B_1, for clinical diagnosis is equal to 6.6 (see Coefficients table). This means that in patients with RDS, there is a possible increase in LUS of 6 compared to patients with TTN. Consider the effect of GA. The unstandardized coefficient, B_1, for GA is equal to 2.5. This means that for each week increasing in GA, there is an increase in LUS of 2.5.

Chapter Five, Discussion

To the best of our knowledge, we determined the TFC for the first time in a preterm population consisting of both moderately and late preterm neonates (born between 32 and 36 weeks of gestation). In the study outcomes, our findings partially depend on GA. We already knew that the LUS score is significantly correlated with oxygenation indices, with similar correlations in babies with a GA of 34 or greater or a GA less of 34 weeks [180]. Lung ultrasonography has already been used to provide qualitative diagnosis in neonatal critical care [111], [167], [185]; however, our findings showed that the LUS score may also be able to describe oxygenation, independently of the type of respiratory condition or GA. Moderately and late preterm infants may face respiratory challenges due to an overlap of the two more frequent pulmonary conditions of preterm age (TTN and RDS), and quantitative lung ultrasound is confirmed to be a valuable, non-invasive, and easy-to-use tool for evaluating gas exchange and assessing lung health even in this population. More into details, with the present study, we assess that, in preterm infants born 32-36 GA and presenting with respiratory distress, there is a significant correlation between both cLUS and eLUS, with OSI, SpO2/FiO2 ratio, and PtcCO2, and that was irrespective of the condition (RDS or TTN). Considering the oxygenation metrics (Table 2 and table 3), OSI is an important and more comprehensive measure of oxygenation because it includes Paw. Our findings are consistent with the correlation between LUS and several measures of oxygenation in neonates with various GAs from earlier studies [180]. The correlation between PtcCO2 and lung aeration is in contrast with a recent study; Pezza et al. [199] speculated that their finding was likely due to the restrictive and relatively mild nature of RDS and TTN.

Because LUS semiology uses artifacts due to the presence of air in the lung parenchyma [112], [178], essentially this correlation describes lung aeration. Consistent results have been found in adult patients with other respiratory conditions.[170], [268]

Surfactant replacement is currently guided only by FiO2 cutoff levels [18], which may lead to late administration or possibly unnecessary treatment. Both situations are potentially harmful because late surfactant replacement is less efficacious [14], and giving surfactant when it is not needed may be invasive and seems to increase lung inflammation in animal models. [269] A LUS cutoff level with high specificity and sensitivity could allow us to screen infants who need surfactant replacement at an early age and those who are at risk for unnecessary surfactant administration. This finding is consistent with a recent meta-analysis of early versus delayed surfactant administration, which concluded that mortality, air leaks, and chronic lung disease were decreased in babies treated early. [14]

In this study, we demonstrated good diagnostic accuracy using semiquantitative lung ultrasound for predicting surfactant replacement in moderately and late preterm neonates with RDS. In our population, a cLUS greater than 6 or a eLUS greater than 14 increased the probability to need surfactant administration with a sensitivity of 87.5% and a specificity of 54.3% using cLUS and with a sensitivity of 87.5% and a specificity of 42.9% using eLUS. Therefore, LUS can be used to accurately exclude the need for the first surfactant dose in this “inhomogeneous” cohort of patients, which could be affected by two completely different pathologies. However, Brat et al. [180] tested LUS in a general newborn population (130 newborns with a GA between 30 and 36 weeks of GA), stating that diagnostic accuracy is significantly lower in late-preterm and term infants than in more preterm infants. This was consistent with more recent studies that stated that the diagnostic accuracy of LUS in predicting surfactant need is stunning in preterm infants <28 weeks of GA. [30] . This is most likely due to the homogeneity of this subgroup, which consists of babies exclusively affected by RDS. On the contrary, babies with greater GA have different clinical diagnoses. Therefore, in the current study, we aimed to verify whether the determination of TFC adds a deeper meaning to LUS in moderately and late preterm infants. The fact that this particular cohort of patents may present with various respiratory disorders (i.e. TTN), [82] different degrees of surfactant injury, [31] and varying extents of the disease process, with a restrictive or mixed pattern [32], entails a great pathophysiological inhomogeneity and the necessity of different therapeutic approaches (i.e. surfactant therapy versus fluid restriction).

Therefore, we tested the statistical significance of each of the independent variables on eLUS and we found that unstandardized coefficient for clinical diagnosis was equal to 6.6. This means that in patients with RDS, there is a possible increase in eLUS of 6 compared with patients with TTN. Second, we found that unstandardized coefficient for GA is 2.5. This means that for each one week that increases in GA, LUS increases by 2.5 points.

LUS has a diagnostic accuracy comparable to that of biological tests used to measure surfactant availability or quality, whereas chest radiography is known to have a lower diagnostic accuracy than lung ultrasound. [157], [270]–[272]. Moreover, a lung ultrasound is quick, radiation free, minimally invasive, and has the characteristics of a point-of-care technique; a LUS calculation is easy and does not require any biological sample collection or treatment. In practice, it is easy to perform, whereas amniotic, gastric, or tracheal fluids may be too viscous to be analyzed.

Because LUS is an easy, quick, and radiation-free technique, multiple looks are always possible; thus, it is an ideal candidate for use as a screening tool to identify babies needing surfactant. Conversely, chest radiography is well known to lack diagnostic accuracy in this regard.[114] Because no other technique is easily available at the bedside, LUS is confirmed to fill an empty space in neonatal critical care imaging.

It is important to continue improving our knowledge of LUS. LUS may help to identify infants that require surfactant, adding some accurate instrumental to a well-defined clinical administration policy [61]; when we choose to give surfactant, it is important, as well as to give it as early as possible, to know the pathophysiological process we are trying to treat; this attitude allows us not only to wait less for further oxygenation worsening, which is potentially dangerous especially at low GA, but also to limit potentially harmful effects and costs.

We have chosen to report the threshold of LUS score with the highest sensitivity to not miss any surfactant administration, and a cutoff value of 6 for cLUS and 14 for eLUS allowed 87.5% sensitivity in this regard. Further studies should continue to increase the clinical value of the LUS score. For example, an earlier LUS or a repeated examination (ie, after 1 and 2 h after the first LUS) might have a higher diagnostic accuracy or might allow evaluation of the disease evolution and reduce false-positive results.

In the present study, we introduced a non-invasive tool to assess extravascular lung fluid and reveal the presence of excessive fetal lung fluid, which is one of the most frequent pathophysiological mechanisms contributing to respiratory failure in moderately and late preterm infants. We obtained this with EC, which has been confirmed to be a non-invasive, and easy-to-use tool even for neonatal populations, as already assessed by previous studies. From the analysis of the results, we can infer that TFC increases with increasing GA ($p=0.004$) and we can conclude that in TTN there is a direct correlation between eLUS and TFC ($\rho=0.164$, $p=0.02$), the higher the pulmonary water content, the greater the eLUS, as the component of interstitial edema responsible for the B pattern increases. This is no longer valid in babies affected by RDS, where the relationship seems almost inverse ($\rho= -0.315$, $p=0.05$), the higher the pulmonary water content, the greater the interstitial component, but the lower the eLUS (absence of alveolar pattern zones in alveolar-interstitial syndromes with high pulmonary water content). Consistently, in the subgroup analysis of infants born <34 weeks of GA, TFC proved to be significantly inversely correlated with LUS in patients born at 34 weeks of GA or more which were affected by RDS. Such a result, to the best of our knowledge, has never been described and opens further important considerations.

A previous study speculated that some patients with TTN also have a relative surfactant deficiency (given their low median LBC) [31], but lung ultrasound alone cannot measure the relative loss of lung aeration due to surfactant deficiency versus the presence of extravascular lung fluid or lung tissue inflammation. Based on these considerations, we speculate that TFC, if studied in a wider cohort of preterm infants, could help reduce the number of patients who inappropriately receive surfactant, thereby reducing the risks and increasing suitability of this therapy, which should be administered based on solid pathophysiological reasons.

The main strength of our study is that it is based on a formal protocol for respiratory management, with well-defined and standardized criteria for CPAP and surfactant use

[18], [262] applied in a population of moderately and late preterm neonates with good perinatal care (as revealed by the relatively high Apgar score). This study was conducted in a NICU with extensive experience in the use of lung ultrasounds. Therefore, we did not repeat certain analyzes described in other articles, such as an inter-operator concordance for lung ultrasound image interpretation or a suitability analysis [180], [264], [273]. Conversely, these strong points may also represent relative weaknesses because our results may only be applied in similar settings. However, lung ultrasound is known to have a steep learning curve and is easy to learn [274]. Other study limitations may be the fact that oxygenation was studied with transcutaneous monitoring rather than with arterial blood gas analysis. However, transcutaneous measurement is recognized to be accurate if it is performed according to available clinical guidelines. [266] Moreover, arterial blood gas analysis is invasive and not feasible in all infants. Noninvasive monitoring is currently the most common policy for preterm infants. Lung ultrasound is a minimally invasive technique, and we did not want to combine it with an invasive procedure.

Furthermore, the use of different probes may influence the details of lung ultrasound findings and the score calculation. [178] We used a micro-linear, high-frequency probe in our population of extremely preterm infants because they have small lungs, and this probe provides high resolution in this setting. [170], [180], [181] Probes of a larger size or lower frequencies have been previously used for neonatal lung ultrasounds [170], [180], [181] and the effect of varying probes deserves to be investigated; appropriate LUS cutoff values should be preliminarily calculated for each type of probe. We do not have data on INSURE failure because this was beyond of our scope. Therefore, we do not know whether lung ultrasound can be used to predict lung failure. Because we have an aggressive noninvasive ventilation policy that uses multiple techniques, it is likely that most failures were not due to a respiratory cause. In any case, this is an intriguing issue that deserves to be investigated in dedicated diagnostic studies. However, our findings are sufficient to design a study to verify if a personalized, LUS-guided surfactant replacement may be used to provide clinical benefits beyond an earlier surfactant administration, and we are working on it. However, large studies are needed to validate the use of this technique for this purpose.

Our study is based on a relatively small population treated according to a fixed protocol based on low oxygen thresholds for surfactant administration,[18] which may affect generalizability of the results. The group of babies with GA more than 34 weeks was inhomogeneously affected by RDS because older babies mainly had TTN. Our results should be replicated in larger groups of preterm infants with RDS, and it will be especially important to do so in a larger population of late preterm infants, where surfactant administration could be overestimated and potentially more dangerous (GA is in direct correlation to pulmonal compliance). Conversely, we need further studies focused on different respiratory conditions diagnosed according to well-defined criteria to evaluate the usefulness of the LUS score in more mature babies or in those affected by conditions other than RDS.

These findings were produced in a well-selected population after exclusion of all respiratory disorders other than RDS and TTN; the collected data were unavailable before bedside estimations of ultrasound assessed lung aeration and non-invasive gas exchange. Thus, our data describe the evolution of the approach to RDS and TTN during modern neonatal care based on optimal perinatal care, integrated clinical definitions, and early CPAP and surfactant administration (if any). These data are useful for discriminating between the two most common neonatal respiratory disorders at the bedside and provide reference values for lung aeration, gas exchange, and lung fluid content.

Chapter Six, Conclusions

In conclusion, our data confirm previous studies that demonstrated a direct correlation between ultrasound-assessed lung aeration and oxygenation in several types of respiratory failure in neonates and older patients.[25] In fact, they show a significative correlation between LUS and oxygenation parameters, even in an inhomogeneous cohort such moderately and late preterm, which is known to be affected from both TTN (approximately 4 out of 5 infants in our cohort) and RDS (the remaining 1 out of 5 infants in our cohort). OSI, one of the most important oxygenation parameters, was significantly correlated with LUS in our population. The presence of a correlation between PtcCO2 and lung aeration, which is in contrast with a recent study conducted on lower mean GAs [199], is likely due to the restrictive nature of both RDS and TTN, which frequently overlap in this specific cohort of patients. All these findings confirm that LUS is well correlated with oxygenation status in the revised cohort of patients, showing good reliability in predicting surfactant administration. Based on our data, we can state that LUS accurately excludes the need for surfactant replacement in CPAP-treated moderately and late preterm neonates with RDS. A cLUS cutoff value of 6, and/or an eLUS of 14 shows optimal sensitivity for predicting the need for the first surfactant dose. These findings demonstrate the increasing need to incorporate LUS assessment to differentiate between the two most frequent conditions in moderately and late preterm infants.

The evaluation of the TFC made by EC to measure extravascular lung fluid can help discriminate between the two main pathophysiological mechanisms (primary surfactant deficiency and excess of extravascular lung fluid). These two mechanisms might coexist in moderately and late preterm, especially in those patients with severe or long-lasting TTN [167] or with RDS. On the basis of these considerations, we speculate that, if studied in a wider cohort of preterm infants, TFC could improve the suitability of this therapy by decreasing the number of patients who inappropriately receive surfactant, thereby reducing its risks, and promoting its administration based on solid pathophysiological reasons.

Author Contributions

Conceptualization: Francesco Vinci, Daniele De Luca, Barbara Loi.

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Validation: Daniele De Luca, Luca Antonio Ramenghi.

Writing – original draft: Francesco Vinci.

Writing – review & editing: Daniele De Luca, Barbara Loi, Luca Antonio Ramenghi.

Acknowledgements

Dedicated to Eden's mom and dad.

We thank all the parents and caregivers of infants who participate in the study.

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