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Diagnostic accuracy of lamellar body count as a predictor of fetal lung maturity: A systematic review and meta-analysis

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ABSTRACT

Objective: This study aimed to synthesize evidence from published studies about the diagnostic accuracy of lamellar body count (LBC) as a predictor of fetal lung maturity.
Study design: We searched Medline (via PubMed), EBSCO, Web of Science, Scopus and the Cochrane Library for relevant published studies assessing the accuracy of LBC as a predictor of fetal lung maturity. Studies were classified according to the counting essays, centrifugation protocols, and the reported optimum cut off values. Data of the true positive, true negative, false positive, and false negative were extracted and analyzed to calculate the overall sensitivity and specificity of the LBC.
Results: Thirty-one studies were included in the final analysis. Fourteen studies reported data for centrifuged amniotic fluid (AF) samples, 13 studies reported data for uncentrifuged samples, and four studies did not have enough information about whether centrifugation was done. LBC showed an area under the curve >80% in diagnosing lung immaturity with variable cut off values. Pooled analysis showed that LBC a 100% specificity to exclude respiratory distress syndrome (RDS) at a cut off value of 15,000 and 100% sensitivity to diagnose RDS at a cut off value of 55,000.
Conclusion: Cases with LBC < 15,000 are considered to have lung immaturity while cases with LBC > 45,000 in centrifuged AF samples or >55,000 in uncentrifuged AF samples are likely to have mature lungs. Cases with LBC ranging between these maturity and immaturity limits should be considered for further evaluation by other lung maturity tests.
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Introduction

Inadequate surfactant in the immature fetal lungs might lead to life-threatening respiratory distress syndrome (RDS) and might require specific management strategies during at delivery [1–3]. Therefore, it is important to diagnose cases with fetal lung immaturity prior to delivery in order to provide the surfactant therapy and reduce the risks of RDS. Recently, the evaluation of fetal lung maturity (FLM) has become an important consideration in the few weeks prior to delivery.

The lecithin to sphingomyelin (L/S) ratio in the amniotic fluid (AF) has been regarded as an indicator of FLM [4]. Further research in FLM recommended that the standard diagnosis of fetal lung immaturity can be established by combined evaluation of the percentage of phosphatidylglycerol (PG) as well as the L/S ratio in AF. Because the evaluation of PG and L/S ratio is relatively expensive and time-consuming, the search for alternative indicators of FLM has continued [5–11].

Surfactant is stored in type II pneumocytes in the form of lamellar bodies. Hence, the amount of these lamellar bodies might be an indicator of surfactant production and therefore, might be used for the evaluation of FLM [12,13]. Several studies have evaluated the diagnostic accuracy of lamellar body count (LBC) as an indicator for fetal lung maturity. However, data from these studies are heterogeneous owing to the difference in centrifugation protocols, type of hematology counters, and the reported cut off values.

The aim of this systematic review and meta-analysis was to synthesize evidence from published diagnostic test accuracy studies about the diagnostic accuracy of LBC in evaluating lung maturity.

Methods

We followed the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA statement) when reporting this study, supplementary file 1 [14]. All steps were performed in strict accordance with the Cochrane Handbook of Diagnostic Test Accuracy Studies [15]. Because the study is a review, there was no need for ethical approval.

Literature search strategy

We searched Medline (via PubMed), EBSCO, Web of Science, Scopus and the Cochrane Library for relevant published studies up to December 2018, with the following search terms: 'Lamellar body count OR LBC' and 'Lung maturity OR respiratory distress'.

We selected clinical trials assessing the accuracy of LBC as a predictor of neonatal lung maturity. Two review authors independently selected eligible studies, independently extracted the data, independently assessed the quality in strict accordance with the Cochrane handbook for systematic reviews of diagnostic test accuracy studies [15]. The bibliographies of the included studies and recent reviews were hand-searched.

Study eligibility

Studies satisfying the following criteria were included in our review:

- Study design: studies that were described as clinical trials.
- Population: studies whose population was infants and neonates with a suspected RDS diagnosis
- Indicator: studies where LBC was considered as a predictor of neonatal lung maturity
- Outcome: studies where the outcome of interest was neonatal lung maturity

the diagnostic accuracy of LBC by providing numbers of true positive (TP), false positive (FP), false negative (FN), true negative (TN), sensitivity, and specificity.

We excluded studies in the following conditions: Animal experiments (not on human subjects) and studies whose full-text article was not available.

Study selection and data extraction

Eligibility screening was conducted in two steps, a) title and abstract screening for matching the inclusion criteria, and b) full-text screening for eligibility to meta-analysis. Disagreements were resolved upon the opinion of a third reviewer.

Assessment of risk of bias

Two investigators independently evaluated the methodological quality by using the tool provided by the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) to conduct quality assessment [16]. The standard QUADAS-2 form was composed of four domains: patient selection, index test, reference standard, and flow and timing. In order to evaluate the overall quality of the included studies, each domain, the risk of bias and concerns about applicability except timing domains and the flow were analyzed and rated as low risk, high risk, and unclear risk. Discussion with another reviewer resolved any disagreement. Results were summarized with Review Manager version 5.3 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark).

Data extraction

Data were extracted from each included study using a uniform online data extraction sheet. Extracted data included the following domains: (1) study design characteristics, (2) methods of processing of fluid samples and essays of lamellar body counting, (3) reported cut off values, and (4) diagnostic accuracy outcomes.

Qualitative evidence synthesis

Studies were categorized according to the centrifugation protocol into: studies with centrifuged AF samples, studies with uncentrifuged AF samples, and studies with unknown centrifugation information. Within the categories, studies were grouped according to the reported cut off values in the following intervals: less than 15,000, 15,000–20,000, 20,000–25,000, 25,000–30,000, 30,000–35,000, 35,000–40,000, 40,000–45,000, 45,000–50,000, and more than 50,000. Meta-analysis for the accuracy of LBC as a

predictor of neonatal lung maturity was done by calculating pooled estimates of sensitivity and specificity. The summary receiver operating characteristic curve (sROC) was drawn to get the area under the curve (AUC) using Review Manager software (RevMan) version 5.3. Forest plots of the pooled data were presented with 95% confidence intervals.

Results

Literature search results

Our literature search yielded 341 citations. Following title and abstract screening, 65 articles were eligible for full-text screening and were examined in detail. Finally, thirty-one studies were included in quantitative synthesis (meta-analysis) with a total of 5422 patients. The flow diagram of the literature search and study selection is shown in Fig. 1.

Characteristics of the included studies' population

A total of 5422 patients were included in our systematic review. Those patients were suspected of having RDS. The summary of the baseline characteristics of the study populations is shown in Table 1.

Methodological quality of the included studies

The quality of included studies was assessed by QUADAS-2 criteria. There was an unclear risk of bias regarding the selection of patients in three studies [17–19]. There was low risk in text index and reference standard for all included studies. There was an unclear risk of bias in flow and timing in three studies [19–21]. The included studies showed low applicability concerns and our evaluation showed that our systematic review and meta-analysis included high-quality studies.

Diagnostic accuracy of LBC in predicting lung immaturity

The pooled analysis of the diagnostic accuracy of LBC in predicting lung immaturity showed an overall sensitivity and specificity of 82.4% and 78.5%. The SROC curve is shown in Fig. 2.

Diagnostic accuracy of LBC in different cut off points

The pooled sensitivity and specificity were variable in different cut off intervals. However, the highest specificity (100%) was reached with LBC less than 15,000 and the highest sensitivity (100%) was reached with LBC more than 55,000. The forest plot of the pooled sensitivity and specificity at each cut off interval is shown in Fig. 3.

Hematology analyzers used for lamellar body counting

Since lamellar bodies are in the same size of platelets, a hematology analyzer should be used to count the number of lamellar bodies. The literature reported four major types of hematology analyzers that were frequently used for lamellar body counting: Coulter counter, Sysmex counter, Cell-Dyn counter, and ADVIA counter. The list of hematology analyzers used in the published studies about LBC can be found in Table 1. As reported in published studies, the type and version of the hematology analyzers used in different institutions were variable. The literature reported that differences between these hematology analyzers should be considered during the interpretation of LBC [22,23]. Szallasi et al. [23] assessed the difference between the four hematology analyzers by using the least-squares regression method. Szallasi et al. [23] found that when using Coulter Gen-S as a standard reference, being used by the most of studies, the difference between the Sysmex XE-2100 and the Coulter Gen-S LBC values was -0.32, the difference between ADVIA 120 and Coulter Gen-S LBC values was -0.46, and the difference between the Cell-Dyn 3500 and Coulter Gen-S LBC values was 0.76. While most of recommendations about using LBC report cut off values as measured by Coulter counters [22,23], physicians should consider these differences when interpreting LBC values from other hematology analyzers in the clinical setting. For example, Roiz-Hernandez et al. [24] reported an optimum cut off value of 79,000 /μL based on Cell-Dyn hematology counter. Interestingly, this cut off value corresponds with the 50,000/μL cut off value on Coulter Gen-S counter considering the between-counter differences reported by Szallasi et al. [23].

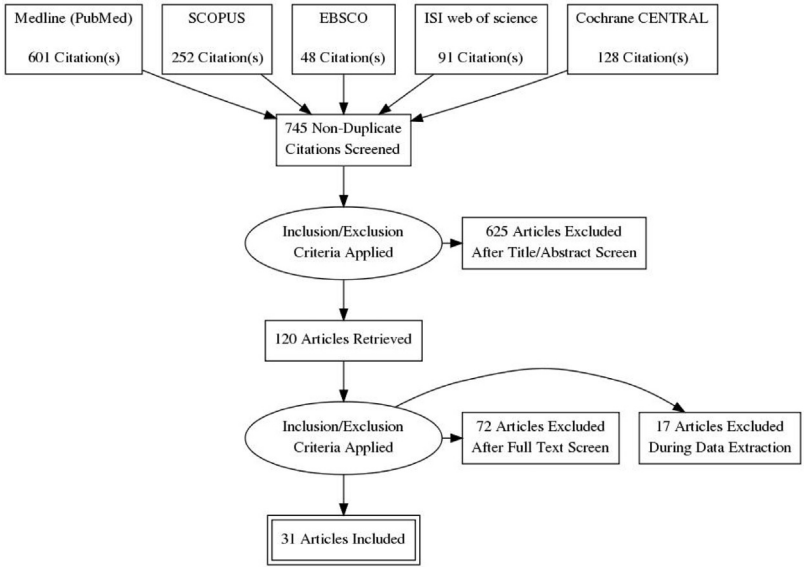

Fig. 1. The flow diagram of the literature search and study selection.

Table 1
The summary of the baseline characteristics of the study populations.

Study ID	Year	Centrifugation Protocol	Study Design	Standard reference	Sample Size	Cases with RDS	Prevalence of RDS in the study	Haematology Analyzer used for counting	Cut off value	Sensitivity	Specificity
Studies with no centrifugation protocol before Lamellar Body Counting											
Chapman [1]	2004	No	Prospective Study	RDS	88	14	15.91%	ADVIA 120	35,400 25,000 21,700 17,700 6000	100% 93% 86% 71.4% 21%	67.60% 87.80% 89.20% 93.2% 100%
Ghidini [28]	2005	No	Prospective Study	RDS	102	17	16.67%	Coulter Gen S	37,000	95%	63.53%
Fernandes [25]	2006	No	Prospective Study	RDS	62	7	11.3%	DHSS pentra 60	50,000	100%	87.3%
Haymond [12]	2006	No	Prospective Study	RDS	184	12	6.52%	Coulter Gen S	50,000	92%	60%
Janicki [29]	2009	No	Prospective Study	RDS	209	5	2.39%	Cell-dyn 4000	72,000	100%	
Salim [30]	2009	No	Retrospective Analysis	RDS	75	13	17%	ADVIA 2120	28,000	72%	100%
Daniel [13]	2010	No	Prospective Study	RDS	63	34	53.97%	Sysmex XT-1800i	42,000	92%	86%
Tsuda [31]	2010	No	Prospective study	RDS	365	17	4.66%	Sysmex SF-3003	29,500	94.00%	82.40%
Tsuda [32]	2011	No	Prospective study	RDS	381	17	4.46%	Sysmex SF-3000	29,500	82.40%	76.20%
Tsuda [33]	2012	No	Prospective study	RDS	300	18	6%	Sysmex SF-3000	29,500	91.50%	83.30%
Stimac [34]	2012	No	Prospective Study	RDS	294	28	9.52%	Cell-dyn 1800	20,000	96%	88%
Zhao [35]	2013	No	Prospective Study	RDS	223	19	8.52%	Coulter Gen S Coulter LH-750 Coulter LH-500 Coulter LH-750 Coulter Ac*T diff 2 Coulter LH-750	37,950	100%	59%
Tsuda [36]	2015	No	Prospective Study	RDS/TTN	632	101	15.98%	Sysmex SF-3000	32,500 38,000 44,500 55,500	50% 71% 79% 75%	97% 80% 73% 61%
Studies with no information about centrifugation protocol before Lamellar Body Counting											
Lee [37]	1996	Unknown	Prospective Study	RDS	170	14	8.24%	Unknown	50,000	100%	80.00%
Carrillo [26]	1997	Unknown	Prospective Study	RDS	31	1	3.23%	Unknown	30,000	100%	96%
Gil 2010 [38]	2010	Unknown	Analytical cross-sectional	RDS	60	21	35.10%	Unknown	30,000	100%	73%
Visnjevac [39]	2010	Unknown	Prospective Study	RDS	232	34	14.66%	Nihon-Kohden® hematology analyzer	42,000	82%	64.60%
Babaei [40]	2013	Unknown	Prospective Study	RDS	150	59	39.3%	Sysmex Xt-1800i	19,000	0.983	0.945
Studies with no centrifugation protocol before Lamellar Body Counting											
Dalence [41]	1995	Yes	Prospective Study	RDS	130	16	12.31%	Sysmex 780	10,000 30,000	75% 100%	95% 64%
Beinlich [42]	1999	Yes	Prospective study	RDS	68	6	8.82%	Sysmex K800	30,000	83%	67%
Lewis [43]	1999	Yes	Prospective Study	L/S or PG	209	17	8.13%	Sysmex NE 1500	8000 32,000	39% 98%	100% 85%
DeRoche [44]	2002	Yes	Prospective Study	L/S or PG	90	0	0.00%	Cell counter (SKTS)	37000	100%	80%
Ross [45]	2002	Yes	Prospective Study	RDS	123	42	34.15%	Coulter Gen S Coulter MAXM Coulter Gen S Coulter MAXM	21,000 24,000 32,000 41,500	100.00% 100.00% 85.20% 87.70%	71.40% 78.60% 90.50% 90.50%
Abd El Aal [46]	2005	Yes	Prospective Study	RDS	72	32	44.2%	Cell counter (SKTS)	15,000 18,000 41,000 50,000	78.26% 78.26% 100% 100%	100% 100% 86% 74%
Khazardoost [3]	2005	Yes	Prospective Study	RDS	80	20	25%	Coulter STKR	50,000	85%	70%
Karcher [47]	2005	Yes	Prospective Study	RDS	219	13	5.94%	Sysmex XE-2100	30,000	84.60%	75.20%
Piazze [48]	2005	Yes	Retrospective Analysis	RDS	178	61	34.27%	Coulter Counter MAX	22,000	81.7%	66%
Korhonen [27]	2010	Yes	Prospective Study	L/S	70	18	25.71%	Sysmex XE-2104	6000 10,000 20,000	55% 67% 89%	100% 100% 98%

Table 1 (*Continued*)

Study ID	Year	Centrifugation Protocol	Study Design	Standard reference	Sample Size	Cases with RDS	Prevalence of RDS in the study	Haematology Analyzer used for counting	Cut off value	Sensitivity	Specificity
									30,000	100%	88%
									35,000	100%	81%
Wijnberger [49]	2010	Yes	Prospective Study	RDS	67	23	34.33%	Cell-dyn 4000	20,000	38.40%	69.60%
									20,000	75%	37.50%
Verder [50]	2010	Yes	Prospective Study	RDS	83	24	28.92%	Sysmex XE-2100	8000	75%	72%
Piazze [51]	2011	Yes	Retrospective Analysis	RDS	227	74	32.6%	Coulter Counter MAX	32,000	86%	83%

RDS, respiratory distress syndrome; L/S, lecithin/sphingomyelin; PG, phosphatidylglycerol; TTN, transient tachypnea of the newborn.

Impact of sample centrifugation on lamellar body count

Several reports in the literature showed that centrifugation might decrease the number of lamellar body count by 10% to 40%. This difference was evident in the data we extracted from published studies. Fourteen studies reported data for centrifuged AF samples, 13 studies reported data for uncentrifuged samples, and four studies did not have enough information about whether centrifugation was done. The average reported cut off value of LBC was 35,000 in the studies with uncentrifuged AF samples and 26,000 in the studies with centrifuged AF samples.

The highest diagnostic accuracy in uncentrifuged AF samples was reported by Fernandes et al. [25] who found a sensitivity of 100% and a specificity of 87.30% at a cut off value of 50,000 using the DHSS Pentra counting essay. For the centrifuged AF samples, the highest diagnostic accuracy was reported by Carrillo et al. [26] and Korhonen et al. [27] Carrillo et al. [26] found a sensitivity of 100% and a specificity of 96% at a cut off value of 30,000. Korhonen et al. [27] also reported a 100% sensitivity and 88% specificity for the same cut off value (30,000) in diabetic women and complicated pregnancies.

Discussion

This study provides evidence from published observational studies that LBC can be used as an accurate method for evaluation of FLM. The pooled analysis showed that LBC has an AUC of >80%. A cut off value of 15,000 could establish a 100% specificity in centrifuged and uncentrifuged samples. The cut off values of 45,000 and 55,000 could establish the highest sensitivities (94% and 100%) for centrifuged and uncentrifuged samples, respectively.

Our results imply that LBC < 15,000 is the threshold of immaturity; therefore, cases with LBC < 15,000 are considered to have lung immaturity. In addition, our results showed that 45,000 and 55,000 are the threshold of maturity in centrifuged and uncentrifuged samples, respectively. Therefore, cases with

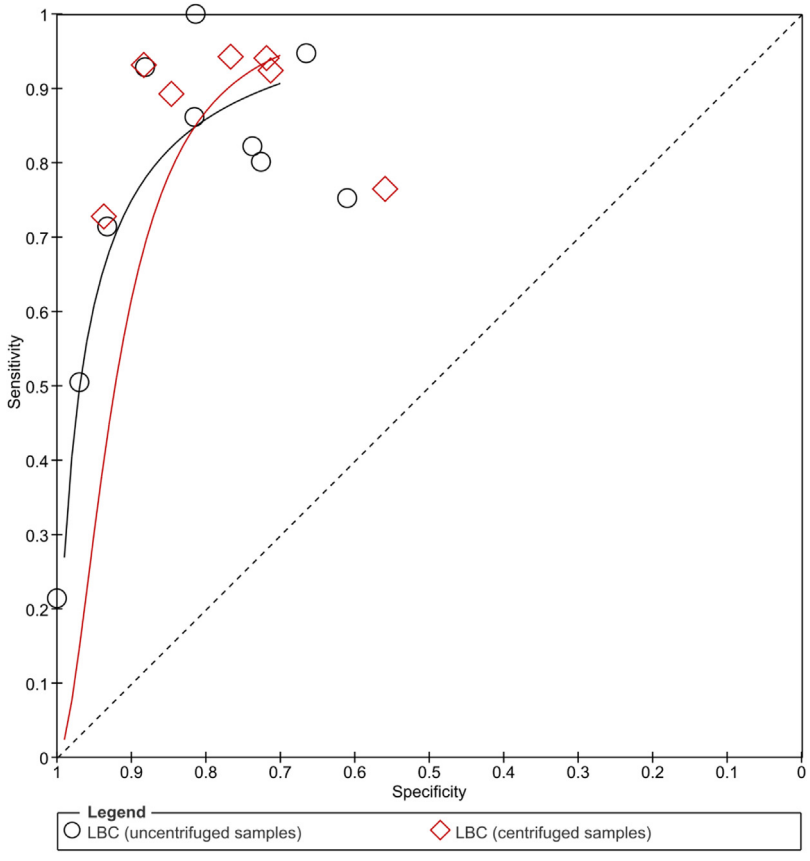

Fig. 2. The SROC curve for the LBC at different cut-off points.

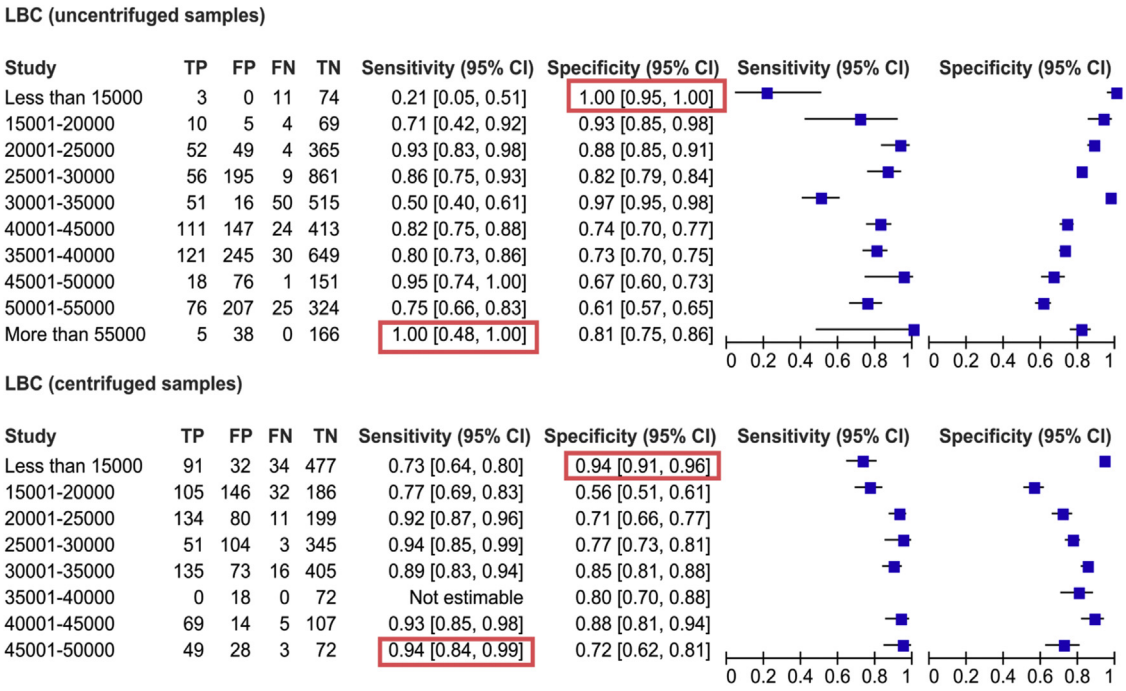

Fig. 3. shows the forest plot of the sensitivity and specificity of LBC in predicting neonatal RDS.

LBC > 45,000 in centrifuged AF samples or >55,000 in uncentrifuged AF samples are unlikely to have lung immaturity. Cases with LBC ranging between these maturity and immaturity limits should be considered for further evaluation by other lung maturity tests.

It worth notice that published studies assessing the accuracy of LBC in detecting FLM suffer from a lot of methodological variabilities. Some studies reported data from twin pregnancies while other investigators excluded twin pregnancies from LBC evaluation. Others excluded AF samples contaminated with meconium or blood while others did not. The interval from AF sample collection till delivery was not the same in all studies.

These variabilities among studies make it difficult to estimate the optimum cuff off value with the highest sensitivity and specificity. Therefore, in the present study, we were conservative that such optimum cut off value is difficult to obtain from heterogeneous data. However, we could determine a threshold of maturity and a threshold of immaturity that is concordant with all data from published studies. Cases with LBC less than the immaturity threshold are likely to have immature lungs and cases with LBC above the maturity threshold are unlikely to have immature lungs. Applying these thresholds in the clinical setting will prevent unnecessary evaluation of FLM by L/S ratio and PG which is expensive in time and cost. Moreover, previous researchers have recommended that each laboratory could establish their own optimum cut off value which is concordant with their hematology counter, timing of AF sample collection, and the centrifugation protocol applied in their institution.

In 2001, Wijnberger et al. [28] compared the performance of the L/S ratio and the LBC in the prediction of neonatal RDS by analyzing six studies. The study concluded that LBC performs slightly better than the L/S ratio ($P = 0.13$) while in another retrospective cohort study of LBC at a gestational age <30 weeks, the L/S ratio has an additional value over the LBC, the specificity of the LBC was 30%, and the addition of the L/S ratio increased the specificity to 60%. Therefore, the use of LBC alone to assess FLM above 30-week gestation seems to be sufficient [29].

In 2009, Janicki et al. [30] sought to determine the LBC threshold for FLM with the Cell-Dyn 4000 hematology analyzer. Of the 209 patients meeting study criteria, 120 had diabetes. Five neonates experienced RDS, all born to non-diabetic mothers with LBC values less than 72,000/ml with a sensitivity of 100%, a false positive rate of 18%. Abd El Aal [31], Fernandes [25], and Lee [32] reported a sensitivity of 100% at a cut of value of 50,000/ml which is in line with our findings. Korhonen [27] reported specificities of 100% and 98% at cut of values of 10,000/mL and 20,000/mL; our pooled analysis showed that LBC below 15,000/mL is indicators for lung immaturity. Our results are in line with the large body of evidence in the literature suggests that LBC is a quick, readily available, and beneficial test to indicate lung maturity [33–36].

Strength points and limitations

Our meta-analysis has several strength points: (a) we determined search methods and performed a comprehensive search using many electronic databases; (b) in our systematic review we followed PRISMA checklist when reporting this manuscript; and (c) all steps were done in strict correspondence with Cochrane handbook of systematic reviews of diagnostic test accuracy studies. However, the limitation of our meta-analysis is the heterogeneity of the sample populations of included studies and the laboratory methods.

Conclusion

In conclusion, cases with LBC < 15,000 are considered to have lung immaturity while cases with LBC > 45,000 in centrifuged AF samples or >55,000 in uncentrifuged AF samples are likely to have mature lungs. Cases with LBC ranging between these maturity and immaturity limits should be considered for further evaluation by other lung maturity tests.

Conflict of interest

The authors report no conflict of interest.

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None to declare.

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