frontiers | Research Topics

Pediatric respiratory critical illness: etiology, diagnosis, and treatment

Edited by
Rujipat Samransamruajkit and Hongxing Dang

Coordinated by
Karen Ka Yan Leung

Published in
Frontiers in Pediatrics

ISSN 1664-8714
ISBN 978-2-8325-6797-5
DOI 10.3389/978-2-8325-6797-5

Generative AI statement
Any alternative text (Alt text) provided alongside figures in the articles in this ebook has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Pediatric respiratory critical illness: etiology, diagnosis, and treatment

Topic editors
Rujipat Samransamruajkit — Chulalongkorn University, Thailand
Hongxing Dang — Children's Hospital of Chongqing Medical University, China

Topic coordinator
Karen Ka Yan Leung — Hong Kong Children's Hospital, Hong Kong, SAR China

Citation
Samransamruajkit, R., Dang, H., Leung, K. K. Y., eds. (2025). *Pediatric respiratory critical illness: etiology, diagnosis, and treatment*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-6797-5

Table of contents

frontiers | Frontiers in Pediatrics

TYPE Editorial
PUBLISHED 14 August 2025
DOI 10.3389/fped.2025.1677006

OPEN ACCESS

EDITED AND REVIEWED BY
Niranjan Kissoon,
University of British Columbia, Canada

*CORRESPONDENCE
Hongxing Dang
dhxdoc@163.com

RECEIVED 31 July 2025
ACCEPTED 04 August 2025
PUBLISHED 14 August 2025

CITATION
Dang H, Samransamruajkit R and Leung KKY (2025) Editorial: Pediatric respiratory critical illness: etiology, diagnosis, and treatment. Front. Pediatr. 13:1677006. doi: 10.3389/fped.2025.1677006

Editorial: Pediatric respiratory critical illness: etiology, diagnosis, and treatment

Hongxing Dang[1]*, Rujipat Samransamruajkit[2] and Karen Ka Yan Leung[3]

[1]Children's Hospital of Chongqing Medical University, Chongqing, China, [2]Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, [3]Hong Kong Children's Hospital, Kowloon, Hong Kong SAR, China

KEYWORDS

respiratory insufficiency, bronchopulmonary dysplasia, mechanical ventilation, biomarkers, pediatric intensive care units

Editorial on the Research Topic
Pediatric respiratory critical illness: etiology, diagnosis, and treatment

Introduction

Severe respiratory diseases—including acute respiratory distress syndrome (ARDS), bronchopulmonary dysplasia (BPD), and severe pneumonia—remain leading causes of pediatric morbidity and mortality worldwide. Despite decades of advances, mortality from pediatric ARDS remains high, particularly in patients with sepsis or multi-organ dysfunction (1, 2). BPD affects more than 20% of extremely low birth weight infants and contributes to prolonged hospital stays and long-term respiratory complications (3). Globally, pneumonia remains a top cause of death among children under (4). These challenges highlight the urgent need for improved diagnostics, monitoring tools, and targeted therapies.

This Research Topic gathers nine original contributions that offer valuable insights into early assessment, physiologic monitoring, clinical management, and rare disease phenotypes in pediatric respiratory critical illness.

Innovations in early assessment and risk stratification

Qin et al. explored the use of exhaled nitric oxide (FeNO) and tidal breathing parameters to assess airway hyperresponsiveness (AHR) in infants under 3 years of age. Their findings reinforce the predictive value of FeNO >14 ppb and flow-volume ratios in suspected asthma—a significant advance for a difficult-to-test age group. This aligns with recent pediatric studies showing FeNO as a noninvasive surrogate for eosinophilic inflammation and long-term asthma risk (5).

Colak et al. introduced renal near-infrared spectroscopy ($RrSO_2$) as a physiologic marker for extubation failure in ventilated children. A >6.15% drop in $RrSO_2$ during

readiness testing had a sensitivity of 98.4% and specificity of 88.9%, suggesting it may be a reliable adjunct to respiratory monitoring in complex cases.

Respiratory support and weaning models

Ge et al. developed a multifactorial prediction model integrating P/F ratio, diaphragm ultrasound indices (DE-RSBI, DTF-RSBI), and Pediatric Critical Illness Score (PCIS). This model significantly outperformed individual variables (AUC = 0.96) in predicting successful weaning. The study supports a shift from single-parameter thresholds to multimodal, data-driven decision tools.

Banik et al. evaluated the usability of the Vayu bCPAP system in five neonatal care facilities in Bangladesh. Providers reported strong acceptability and effectiveness in treating respiratory distress syndrome (RDS), highlighting how low-cost, portable innovations can improve neonatal care in resource-limited settings.

Mechanistic insights and biomarker discovery

Li et al. reviewed the role of oxidative stress in the pathogenesis of BPD. Their analysis identified 8-hydroxy-2'-deoxyguanosine (8-OHdG) as a promising biomarker for early detection, adding to a growing body of evidence supporting biomarker-guided neonatal care (5).

Duenas-Meza et al. studied sleep-disordered breathing in children with cystic fibrosis at high altitude. They found that total sleep time with oxygen saturation <90% and <85% correlated negatively with FEV_1, reinforcing the importance of polysomnography in evaluating pulmonary function in high-altitude environments.

Case-based contributions: rare and complex presentations

Zhou et al. assessed bronchoalveolar lavage (BAL) in treating small airway diseases. Compared with conventional therapy, BAL accelerated symptom resolution, imaging recovery, and reduced re-hospitalization, suggesting a potential benefit in patients with recurrent or persistent lower airway disease.

Liu et al. described two Chinese siblings with FINCA syndrome caused by a novel NHLRC2 mutation. One child responded to long-term corticosteroids, indicating that anti-inflammatory therapy may offer benefit in genetically mediated interstitial lung disease.

Yang et al. presented a case of *Mycoplasma pneumoniae*-induced diffuse alveolar hemorrhage (DAH) complicated by hemophagocytic lymphohistiocytosis (HLH). The case demonstrated the importance of metagenomic next-generation sequencing (mNGS) and early recognition of HLH in life-threatening infections.

Conclusion

Together, these articles underscore critical advancements in pediatric respiratory critical care: Noninvasive biomarkers such as FeNO and $RrSO_2$ offer new pathways for early detection and monitoring; Multimodal prediction models outperform traditional approaches in ventilator weaning; Low-cost devices like Vayu bCPAP expand respiratory care access in LMICs; Precision recognition of rare and complex conditions facilitates timely and targeted interventions.

This Research Topic highlights how integrated, multidisciplinary efforts are shaping the future of pediatric respiratory care. We thank all contributing authors and reviewers for their valuable insights.

Author contributions

HD: Writing – original draft, Writing – review & editing. RS: Writing – review & editing. KL: Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

References

1. Wang Q, Liu Y, Fu Y, Liu C, Li J, Dang H. Analysis of predictors of mortality and clinical outcomes of different subphenotypes for moderate-to-severe pediatric acute respiratory distress syndrome: a prospective single-center study. *Front Pediatr*. (2022) 10:1019314. doi: 10.3389/fped.2022.1019314

2. Wang Q, Dang H, Fu Y, Liu C, Li J, Xu F. Risk factors for mortality in children with moderate-to-severe ARDS with concurrent hematological or immune-related diseases: a retrospective analysis. *BMC Pediatr*. (2025) 25(1):396. doi: 10.1186/s12887-025-05734-1

3. Moreira A, Noronha M, Joy J, Bierwirth N, Tarriela A, Naqvi A, et al. Rates of bronchopulmonary dysplasia in very low birth weight neonates: a systematic review and meta-analysis. *Respir Res*. (2024) 25(1):219. doi: 10.1186/s12931-024-02850-x

4. Cao L, Ji Z, Zhang P, Wang J. Epidemiology and mortality predictors for severe childhood community-acquired pneumonia in ICUs: a retrospective observational study. *Front Pediatr*. (2023) 11:1031423. doi: 10.3389/fped.2023.1031423

5. Pasha MA, Hopp RJ, Habib N, Tang DD. Biomarkers in asthma, potential for therapeutic intervention. *J Asthma*. (2024) 61(11):1376–91. doi: 10.1080/02770903.2024.2361783

frontiers | Frontiers in Pediatrics

TYPE Original Research
PUBLISHED 19 January 2024
DOI 10.3389/fped.2023.1326550

OPEN ACCESS

EDITED BY
Rujipat Samransamruajkit,
Chulalongkorn University, Thailand

REVIEWED BY
Rakesh Lodha,
All India Institute of Medical Sciences, India
Warwick Wolf Butt,
Royal Children's Hospital, Australia

*CORRESPONDENCE
Gokhan Ceylan
✉ drgokhanceylan@gmail.com

RECEIVED 23 October 2023
ACCEPTED 11 December 2023
PUBLISHED 19 January 2024

CITATION
Colak M, Ceylan G, Topal S, Sarac Sandal O, Atakul G, Soydan E, Sarı F, Hepduman P, Karaarslan U and Ağın H (2024) Evaluation of renal near-infrared spectroscopy for predicting extubation outcomes in the pediatric intensive care setting.
Front. Pediatr. 11:1326550.
doi: 10.3389/fped.2023.1326550

Evaluation of renal near-infrared spectroscopy for predicting extubation outcomes in the pediatric intensive care setting

Mustafa Colak[1,2], Gokhan Ceylan[2,3]*, Sevgi Topal[2], Ozlem Sarac Sandal[2], Gulhan Atakul[2], Ekin Soydan[2], Ferhat Sarı[2], Pinar Hepduman[2], Utku Karaarslan[2] and Hasan Ağın[2]

[1]Department of Paediatric Intensive Care Unit, Basaksehir Cam and Sakura City Hospital, Istanbul, Turkey, [2]Department of Paediatric Intensive Care Unit, Dr Behcet Uz Children's Disease and Surgery Training and Research Hospital, Health Sciences University, Izmir, Turkey, [3]Department of Medical Research, Hamilton Medical AG, Bonaduz, Switzerland

Background: In pediatric intensive care units, extubation failure following invasive mechanical ventilation poses significant health risks. Determining readiness for extubation in children can minimize associated morbidity and mortality. This study investigates the potential role of renal near-infrared spectroscopy (RrSO2) in predicting extubation failure in pediatric patients.
Methods: A total of 84 patients aged between 1 month and 18 years, mechanically ventilated for at least 24 h, were included in this prospective study. RrSO2 levels were measured using near-infrared spectroscopy before and during an extubation readiness test (ERT). The primary outcome measure was extubation failure, defined as a need for reintubation within 48 h.
Results: Of the 84 patients, 71 (84.6%) were successfully extubated, while 13 (15.4%) failed extubation. RrSO2 was found to be lower in the failed extubation group, also decrease in RrSO2 values during ERT was significantly greater in patients with extubation failure. ROC analysis indicated a decrease in ΔRrSO2 of more than 6.15% from baseline as a significant predictor of extubation failure, with a sensitivity of 0.984 and a specificity of 0.889.
Conclusion: Monitoring changes in RrSO2 values may serve as a helpful tool to predict extubation failure in pediatric patients. Further multi-center research is warranted to improve the generalizability and reliability of these findings.

KEYWORDS

extubation readiness test, mechanical ventilation, near-infrared spectroscopy, noninvasive monitoring, pediatric intensive care

1 Introduction

Invasive mechanical ventilation is a life-saving practice commonly employed in pediatric intensive care units (PICUs) (1). However, it poses significant risks such as ventilator-associated pneumonia (VAP), ventilator-associated lung injury (VILI), patient self-induced lung injury (P-SILI), ventilator-associated diaphragm damage, and long-term exposure to narcotics and sedatives (2–4). Thus, it is crucial not to keep patients intubated longer than necessary. In the process of weaning patients from mechanical ventilation support, there are some accepted criteria that determine whether a patient's condition is suitable. These criteria include the patient being hemodynamically stable, capable of achieving adequate oxygenation, and performing gas exchange within acceptable limits (5). Determining the

readiness of pediatric patients for extubation is critical to minimize morbidity and mortality associated with prolonged mechanical ventilation and extubation failure (6). Unfortunately, a significant proportion of patients who were once considered candidates for extubation require re-intubation after the extubation procedure. Rates of extubation failure in pediatric intensive care units have been reported to vary widely, from 2.7% to 22%, across studies that differ in patient populations and are influenced by the variability in study design and objectives. Despite the implementation of extubation criteria to evaluate the readiness for discontinuation of mechanical ventilation, there are instances where children may not be able to maintain effective spontaneous breathing after a planned extubation (7).

To prevent unsuccessful extubation and its associated complications, extubation readiness protocols are employed in pediatric care. However, despite the existence of various published protocols, there is a lack of standardized guidelines for integrating these practices into extubation protocols for the pediatric population. This absence of standardization can lead to significant variations in clinical practice and potential delays in weaning from invasive ventilation (8).

Near-infrared spectroscopy (NIRS) is a non-invasive technique used to continuously measure cerebral and regional oxygen saturation (rSO2). Initially used in the operating room to assess the decrease in cerebral blood flow during cardiopulmonary bypass, its use has since been extended to the critical care unit for patients at risk of multiple organ dysfunction or death (9). A correlation has been identified between the monitoring of renal-regional oxygen saturation (RrSO2) and invasive renal oxygen measurements during cardiac surgery (10). Moreover, an association has been observed between decreased rSO2 values during the extubation readiness test (ERT) and extubation failure in post-cardiac surgery patients (11). Additionally, studies involving neonates monitored after cardiac surgery have identified a significant decrease in renal NIRS values associated with extubation failure (12).

Despite the application of extubation readiness tests, extubation failure is still observed in pediatric patients. This means we need further tests or methods to avoid extubation failure. Specifically, in neonates and children monitored post-cardiac surgery, a reduction in renal NIRS levels during the preparation phase for extubation has been correlated with extubation failure. However, the relationship between renal NIRS values and extubation failure during ERT in critically ill pediatric patients other than cardiac surgery patients is not clear. This study aimed to examine the relationship between changes in RrSO2 and extubation outcomes. By doing so, we sought to shed light on the potential correlation and explore ways to minimize unnecessary intubations by reducing the risk of extubation failure.

2 Materials and method

2.1 Ethics

The study was conducted in compliance with the Helsinki Declaration, and the research protocol was approved by the ethics committee of Dr. Behcet Uz Children's Research and Training Hospital (no: 13399118-799). Informed consent was obtained from the patients' next of kin before enrollment.

2.2 Participants

The study included patients aged between 1 month and 18 years who had been mechanically ventilated with an endotracheal tube for at least 24 h before undergoing an extubation readiness test (ERT). Eligible patients were required to pass both the ERT and the cuff-leak test and exhibit no signs of upper airway obstruction. Exclusion criteria included patients who did not provide informed consent, those undergoing follow-up after cardiac surgery, patients with neuromuscular diseases, respiratory tract anomalies, renal agenesis or vascular malformations, and those with hemoglobinopathy or cyanide intoxication.

2.3 Procedure

The patients for whom extubation was planned were determined for the 9.00 pm round. Dexamethasone at a dose of 0.15 mg/kg was administered at both 00:00 and 06:00 am. The first visit was re-evaluated the next day and a cuff leak test was applied. A decision was made to extubate patients who met the criteria for extubation and successfully passed the cuff leak test. Enteral feeding was stopped at least 1 h before the extubation readiness test (ERT). At the commencement of the procedure, patients' sedatives were titrated until their State Behavioral Scale (SBS) scores were at either 0 or 1 (13). Subsequently, using an ultrasound machine, the patient's right kidneys were visualized with a 5–12 MHz frequency probe (HD 15, Philips Healthcare, Bothell, WA, USA) placed alongside the T12-L2 vertebra.

The probe of a near-infrared spectroscopy device (INVOSTM 5100, Somanetics Corporation, Troy, Michigan, USA) was then positioned on the ultrasound-visualized right kidney area. The HAMILTON MEDICAL C3 or C6 mechanical ventilator (Hamilton Medical, Bonaduz, Switzerland) was deployed either for manual adjustments of the expiratory trigger sensitivity (ETS), pressure ramp, and flow trigger or for automatic synchronization through embedded software.

Patient ID was entered in the NIRS device and recording started the NIRS device commenced recording RrSO2 values at 30-s intervals. After one hour of observation, ERT was started. During this phase, a spontaneous mode (PS) was employed with a positive end-expiratory pressure (PEEP) setting of 5 centimeters of water (cm H2O). The pressure support was adapted in relation to the internal diameter of the endotracheal tube (ETT), set at 10 cm H2O for ETT with a 3–3.5 mm internal diameter, 8 cm H2O for ETT with a 4–4.5 mm internal diameter, and 6 cm H2O for ETT with an internal diameter larger than 5 mm.

The extubation readiness test (ERT) was continued for 2 h. Throughout the extubation readiness test (ERT), patients were mandated to sustain a SpO2 above 95% and an expiratory tidal volume (VTe) of at least 5 ml/kg based on the predicted body

weight. If the recorded respiration rate exceeded the acceptable target rate for the patient's age, or minimum SpO2 or VT is not sustained, the test was deemed unsuccessful. Age-related respiratory rates were classified as follows: 20–60 /min for 1 month to 6 months, 15–45 / min for 6 months to 2 years, 15–40 /min for 2–5 years, and 10–35 /min for patients older than 5 years (6, 14).

Baseline RrSO2 before ERT and RrSO2 values during ERT data were taken from the NIRS device. Data were analyzed with Invos Analytics Tool software (Version 1.2). Whole NIRS data containing one-hour before and during ERT were documented and average values for both phases were recorded.

Ninety-one extubation tests were performed on 84 patients. Extubation readiness test failed in seven patients. These patients were re-considered for extubation the next day and extubated after a successful ERT.

2.4 Statistical analysis

Statistical analyses were conducted using SPSS version 20 (IBM Corp., Armonk, NY) and JASP (JASP Statistics, Version 0.16.3, University of Amsterdam). The normality of the study data was assessed using the Kolmogorov–Smirnoff analysis. Participants' ages, length of stay on mechanical ventilator support, PIM 3 scores, respiratory and hemodynamic measures during ERT, and Renal-rSO2 values were stratified based on extubation success, and values were presented as median and 25–75 quantiles, and the Mann–Whitney *U*-test was employed to discern statistically significant differences between the groups with successful and unsuccessful extubation outcomes. The *p*-value of less than 0.05 was considered statistically significant.

We also calculated post-power of our study and found that sample sizes of 73 and 11 achieve 92% power to detect a difference of 1.10 between the group means with standard deviations of 1.0 and 1.00 at a significance level (alpha) of 0.05 using a two-sided *z*-test. These results assume that 5 sequential tests are made using the O'Brien-Fleming spending function to determine the test boundaries.

3 Results

A total of 84 patients were enrolled in the study, including 48 males and 36 females. The characteristics and reasons for intensive care unit hospitalization are shown in Table 1. Among the patients deemed suitable for extubation, a total of 91 ERT were conducted on 84 patients who successfully passed the cuff leak test. Of these, 77 patients were successfully extubated following the initial ERT. The remaining 7 patients underwent a second ERT the following day and were extubated following a successful outcome of this test. Of the participants, 71 (84.6%) were successfully extubated, while 13 (15.4%) failed extubation (Figures 1, 2). Participants were categorized according to extubation success, and significant differences were observed in age (months) and length of stay on mechanical ventilation, respiratory rates at the start of ERT, maximum respiratory rate during ERT, heart rate at the start of ERT, maximum heart rate during ERT and, heart rate at end of the ERT. During ERT, RrSO2 was found to be lower in the failed extubation group. No significant difference was observed between the groups in terms of the lowest oxygen saturation (SO_2) values during the ERT, as well as in the partial pressure of oxygen (PO_2) and carbon dioxide (PCO_2) in the arterial blood gas taken at the end of the ERT. Not only the comparison RrSO2 values during the ERT but also comparison of the ΔRrSO2 values between patients who required reintubation and those who were successfully extubated revealed a significant decrease in ΔRrSO2 during ERT in the extubation failure group (−10.4 [(−21.8)–(−7.2)] vs. 2.7 [(−1.7)–(5.5)], as presented in Table 2 and Figures 3, 4. The NIRS values of the seven patients who did not pass the ERT on the first day were found to be: Before ERT Renal-rSO2 at 66 (58–71), during ERT RrSO2 at 54 (49–61), and ΔRrSO2 at 14 [(−19)–(−12.9)].

TABLE 1 Demographics of the patients at the PICU admission.

Age (months)	Median (IQR) 38.5 (6–42)
Gender	
Female	36
Male	48
PIM 3 score	1.55 (1.2–2.5)
Etiology	**Number (%)**
Pneumonia	51 (65.7)
Sepsis/septic shock	13 (12.8)
Status epilepticus	11 (11.4)
Metabolic disease	8 (8.5)
Drug poisoning	1 (1.4)

PIM 3, pediatric index of mortality 3.

The ROC analysis conducted for ΔRrSO2 demonstrated that a decrease of more than 6.15% from baseline had a high predictive value for extubation failure, with a sensitivity of 0.944 and specificity of 0.769 (AOC: 0.965).

4 Discussion

Our observational study aimed to assess whether renal NIRS levels could function as an indicative factor for predicting the probability of extubation failure in pediatric patients. Successful implementation of this approach might result in decreased requirements for reintubation, consequently reducing the rates of mortality and morbidity linked to unsuccessful extubation in this specific patient group. The study compared the ΔRrSO2 values of patients who were successfully extubated to those who were reintubated. The extubation failure group had significantly lower ΔRrSO2 values during ERT (−10.4 [(−21.7)–(−7.2)] vs. 2.7 [(−1.7)–(5.5)], ($p < 0.01$). The study population underwent ROC analysis, and the cut-off value of −6.15% was determined for RrSO2. This value had a sensitivity of 0.944 and a specificity of 0.769.

Cerebral blood circulation is sensitive to changes in hydrogen ion levels, and acidosis due to increased CO2 levels in patients on respiratory support can cause cerebral artery vasodilation,

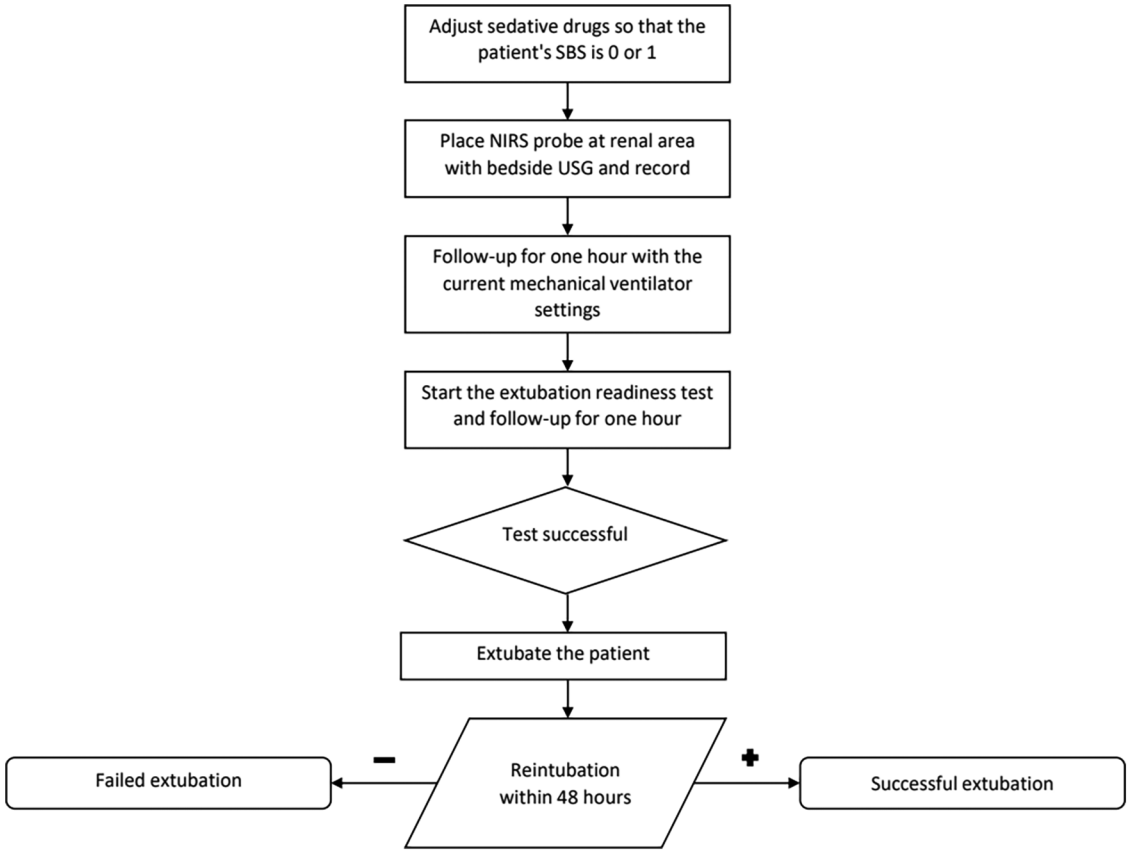


FIGURE 1
The study flow chart.

leading to increased cerebral blood flow (CBF) and decreased blood flow to other organs (15, 16). A study using an animal model of hemorrhagic shock monitored cerebral and renal blood flow using NIRS and found that the reduction in renal blood flow occurred before the reduction in cerebral blood flow (17).

Changes in CBF play an important role in stabilizing breathing patterns in the central respiratory chemoreflex. The increase in CBF promotes diffusion of CO2 from cerebrospinal and cerebral extracellular fluids to the cerebral vasculature, resulting in a decrease in hydrogen ion concentration and suppressing pH

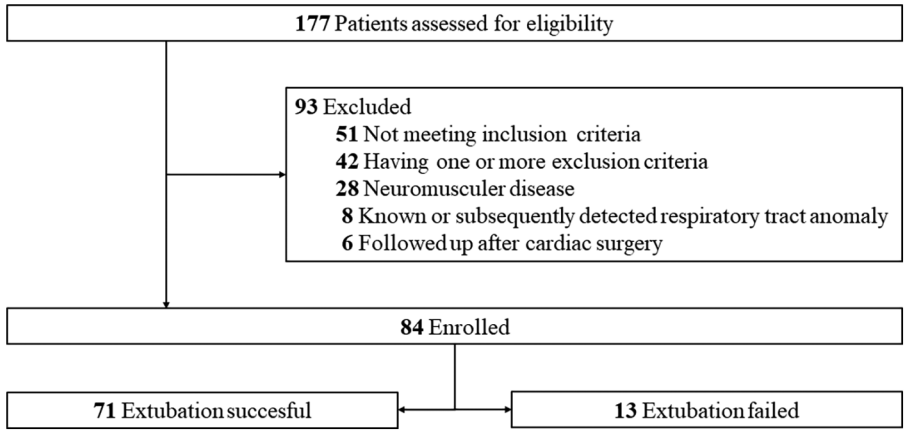


FIGURE 2
Study participants and excluded patients.

TABLE 2 Characteristics and comparison of patients according to extubation success.

	Extubation successful ($n = 71$)	Extubation failed ($n = 13$)	p Value
Age (month)	26 (8–77)	5 (3.5–13)	0.002
Ventilation days	8 (6–9)	13 (10–16)	<0.001
gPIM 3 score	1.6 (1.2–2.5)	1.5 (1.2–3.4)	0.774
During ERT SO_2 (%)			
Start of ERT	97 (96–99)	97 (96–98)	0.685
Max during ERT	98 (97–99)	97 (96–98)	0.140
Min during ERT	96 (95–97)	96 (95–98)	0.897
End of ERT	98 (95–98)	96 (95–98)	0.264
End of ERT PO_2 (mmHg)	92 (85–98)	89 (83–96)	0.237
End of ERT PCO_2 (mmHg)	42 (35–46)	44 (38–48)	0.465
During ERT pH	7.39 (7.38–7.41)	7.39 (7.37–7.41)	0.880
Respiratory rate (per min)			
Start of ERT	32 (24–37)	38 (35–41)	0.009
Max during ERT	36 (28–40)	42 (39–45)	0.002
Min during ERT	30 (22–36)	35 (30–38)	0.244
End of ERT	35 (27–41)	40 (37–42)	0.190
Heart Rate (per min)			
Start of ERT	108 (98–122)	124 (115–135)	0.002
Max during ERT	116 (105–128)	131 (122–142)	0.003
Min during ERT	107 (95–121)	115 (109–123)	0.289
End of ERT	110 (101–122)	128 (117–136)	0.002
Renal-rSO_2			
Before ERT	69 (64–73)	65 (64–69)	0.353
During ERT	70 (64–76)	57.6 (51–63)	<0.001
$\Delta RrSO_2$	2.7 (−1.7), (5.5)	−10.4(−21.8), (−7.2)	<0.001

ERT, extubation readiness test; Max, maximum; Min, minimum; PCO_2, partial carbon dioxide pressure in arterial blood gas; PO_2, partial oxygen pressure in arterial blood gas; PIM 3, pediatric index of mortality 3; rSO_2, regional oxygen saturation; SO_2, oxygen saturation (pulse oximeter) $\Delta RrSO_2$, percentage change between before ERT Renal-rSO_2 and During ERT Renal-rSO_2 Values are represented as median and IQR 1 and 3 (25th–75th percentile) consecutively.

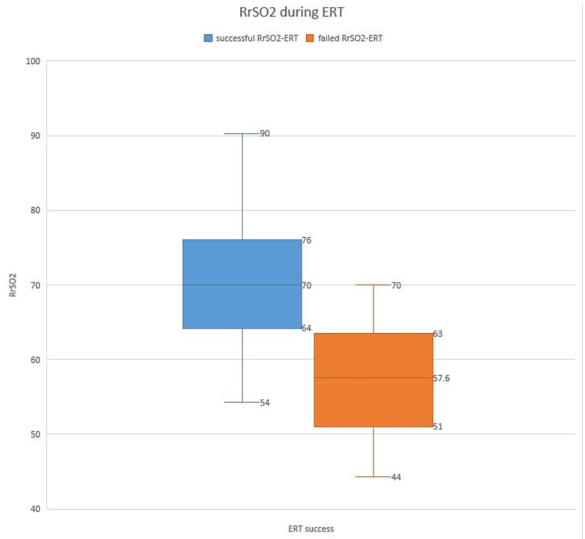


FIGURE 3
The RrSO2 during ERT.

decrease at the central chemoreceptors (18, 19). We think that, the patient did not experience tachypnea during ERT due to increased blood flow to the brain. However, the increase in cerebral blood flow resulted in decreased renal blood flow, causing a decrease in RrSO2 values. Foster et al. conducted a study on post-operative cardiac surgery patients and found that somatic rSO2 values decreased, but cerebral rSO2 values did not significantly change, which was similar to the results of this study (11).

Another on athletes also found a breakpoint in NIRS values of respiratory muscles during exercise, indicating a decrease in values when the respiratory compensation threshold was exceeded. Similar to this study, we demonstrated a decrease in renal NIRS values before the occurrence of the breakpoint (20).

In our study, the rate of extubation failure was found to be 15.4%, which is consistent with previous literature (7, 21). We observed a statistically significant difference in the median age between patients who passed the ERT and were successfully extubated vs. those who failed and were reintubated. The median age for reintubated patients was 5 months, while for successfully extubated patients it was 42 months. This highlights the importance of considering age as a potential factor when assessing the likelihood of reintubation after passing the ERT. Dexamatasone was administered to all patients who met the inclusion criteria and for whom extubation was decided. Numerous controversial studies have been conducted on the use of dexamethasone before or during the extubation of pediatric patients, specifically focusing on the reintubation rate. Nevertheless, the majority of these studies suggest a reduced occurrence of events related to upper airway obstruction in the dexamethasone group. A recent meta-analysis, incorporating 10 randomized controlled studies, concluded that corticosteroid use is deemed acceptable. As a result, we administered dexamethasone with the goal of minimizing these events, aiming to mitigate any potential impact on NIRS values within the study population (22, 23). Although our study aimed to investigate the relationship between RrSO2 levels and extubation failure in pediatric patients, we acknowledge several limitations that should be taken into account. First and foremost, our research was conducted at a single center, which may limit the generalizability of our findings to other populations and settings. Moreover, we acknowledge that there may be other confounding factors that contribute to the risk of extubation failure that we did not account for in our study design. Therefore, caution should be exercised in interpreting our results as a causal relationship between RrSO2 levels and extubation failure cannot be established without further experimental evidence. In order to improve the validity and applicability of the research findings, we suggest that future studies be conducted across multiple centers and increase the number of participants. It is essential to include patients who have undergone long-term mechanical ventilation, suffer from associated lung diseases, neuromuscular weakness after intensive care or related pathology, sedation or delirium issues, as well as congenital comorbidities such as tracheobronchomalacia, vocal cord dysfunction or diaphragmatic paralysis, as they significantly contribute to extubation failure. Additionally, there is still a contentious debate in the existing

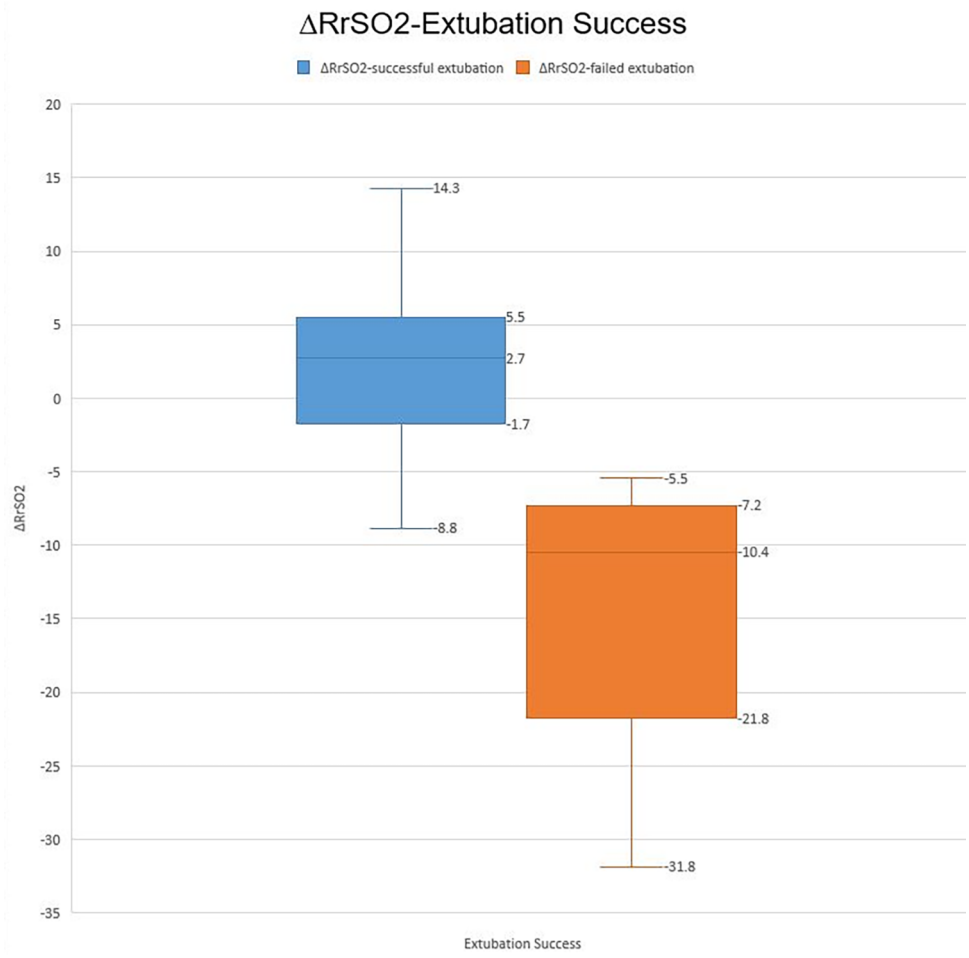


FIGURE 4
ΔRrSO2-Extubation success.

literature regarding the type of post-extubation treatments (such as oxygen therapy, high-flow nasal cannula, or nasal continuous positive airway pressure) that help patients achieve a successful extubation. The findings and limitations of this study should be considered in larger studies to advance this important observational study.

5 Conclusion

The use of RrSO2 monitoring during extubation in our study showed a significant decrease in RrSO2 values in patients who were unsuccessfully extubated, indicating its potential as a tool for identifying those at risk of extubation failure. Alongside ERT, we recommend monitoring RrSO2 before and during extubation and considering changes in ΔRrSO2 when making extubation decisions. Although our study had limitations, such as a small sample size and being conducted at a single center, future research, including multicenter studies, can provide more comprehensive understanding and improve the success rate of extubation in pediatric patients.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethical Committee of Dr. Behcet Uz Children's Research and Training Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

MC: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Writing – original draft.

GC: Conceptualization, Methodology, Project administration, Supervision, Visualization, Writing – review & editing. ST: Data curation, Investigation, Project administration, Writing – original draft. OS: Conceptualization, Data curation, Writing – review & editing. GA: Formal analysis, Investigation, Supervision, Writing – original draft. ES: Writing – review & editing. FS: Writing – review & editing. PH: Data curation, Investigation, Writing – original draft. UK: Resources, Supervision, Writing – review & editing. HA: Methodology, Project administration, Supervision, Validation, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

GC worked at Hamilton Medical AG in the Department of Medical Research.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2023.1326550/full#supplementary-material

References

1. Khemani RG, Markovitz BP, Curley MAQ. Characteristics of children intubated and mechanically ventilated in 16 PICUs. *Chest*. (2009) 136(3):765–71. doi: 10.1378/chest.09-0207

2. Ricard J-D, Dreyfuss D, Saumon G. Ventilator-induced lung injury. *Curr Opin Crit Care*. (2002) 8(1):12–20. doi: 10.1097/00075198-200202000-00003

3. Goligher EC, Dres M, Patel BK, Sahetya SK, Beitler JR, Telias I, et al. Lung- and diaphragm-protective ventilation. *Am J Respir Crit Care Med*. (2020) 202(7):950–61. doi: 10.1164/rccm.202003-0655cp

4. Tobias JD. Tolerance, withdrawal, and physical dependency after long-term sedation and analgesia of children in the pediatric intensive care unit. *Crit Care Med*. (2000) 28(6):2122–32. doi: 10.1097/00003246-200006000-00079

5. Poletto E, Cavagnero F, Pettenazzo M, Visentin D, Zanatta L, Zoppelletto F, et al. Corrigendum on: ventilation weaning and extubation readiness in children in pediatric intensive care unit: a review. *Front Pediatr*. (2022) 10:1044681. doi: 10.3389/fped.2022.1044681

6. Faustino EVS, Gedeit R, Schwarz AJ, Asaro LA, Wypij D, Curley MAQ. Accuracy of an extubation readiness test in predicting successful extubation in children with acute respiratory failure from lower respiratory tract disease*. *Crit Care Med*. (2017) 45(1):94–102. doi: 10.1097/ccm.0000000000002024

7. Baisch SD, Wheeler WB, Kurachek SC, Cornfield DN. Extubation failure in pediatric intensive care incidence and outcomes. *Pediatr Crit Care Med*. (2005) 6(3):312–8. doi: 10.1097/01.pcc.0000161119.05076.91

8. Krasinkiewicz JM, Friedman ML, Slaven JE, Lutfi R, Abu-Sultaneh S, Tori AJ. Extubation readiness practices and barriers to extubation in pediatric subjects. *Respir Care*. (2020) 66(4):582–90. doi: 10.4187/respcare.08332

9. Bhutta AT, Ford JW, Parker JG, Prodhan P, Fontenot EE, Seib PM, et al. Noninvasive cerebral oximeter as a surrogate for mixed venous saturation in children. *Pediatr Cardiol*. (2007) 28(1):34–41. doi: 10.1007/s00246-006-1379-z

10. Tholén M, Ricksten S-E, Lannemyr L. Renal near-infrared spectroscopy for assessment of renal oxygenation in adults undergoing cardiac surgery: a method validation study. *J Cardiothorac Vasc Anesth*. (2020) 34(12):3300–5. doi: 10.1053/j.jvca.2020.04.044

11. Foster CB, Spaeder MC, McCarter RJ, Cheng YI, Berger JT. The use of near-infrared spectroscopy during an extubation readiness trial as a predictor of extubation outcome. *Pediatr Crit Care Med*. (2013) 14(6):587–92. doi: 10.1097/pcc.0b013e31828a8964

12. Gradidge EA, Grimaldi LM, Cashen K, Gowda KMN, Piggott KD, Wilhelm M, et al. Near-infrared spectroscopy for prediction of extubation success after neonatal cardiac surgery. *Cardiol Young*. (2019) 29(06):787–92. doi: 10.1017/s1047951119000829

13. Curley MAQ, Harris SK, Fraser KA, Johnson RA, Arnold JH. State behavioral scale (SBS) A sedation assessment instrument for infants and young children supported on mechanical ventilation. *Pediatr Crit Care Med*. (2006) 7(2):107–14. doi: 10.1097/01.PCC.0000200955.40962.38

14. Curley MA, Arnold JH, Thompson JE, Fackler JC, Grant MJ, Fineman LD, et al. Clinical trial design–effect of prone positioning on clinical outcomes in infants and children with acute respiratory distress syndrome. *J Crit Care*. (2006) 21(1):23–32; discussion 32–7. doi: 10.1016/j.jcrc.2005.12.004

15. Kim SY, Chae DW, Chun Y-M, Jeong KH, Park K, Han DW. Modelling of the effect of end-tidal carbon dioxide on cerebral oxygen saturation in beach chair position under general anaesthesia. *Basic Clin Pharmacol Toxicol*. (2016) 119(1):85–92. doi: 10.1111/bcpt.12549

16. Sørensen H, Secher NH, Rasmussen P. A note on arterial to venous oxygen saturation as reference for NIRS-determined frontal lobe oxygen saturation in healthy humans. *Front Physiol*. (2014) 4:403. doi: 10.3389/fphys.2013.00403

17. Rhee CJ, Kibler KK, Easley RB, Andropoulos DB, Czosnyka M, Smielewski P, et al. Renovascular reactivity measured by near-infrared spectroscopy. *J Appl Physiol (Bethesda, Md.: 1985)*. (2012) 113(2):307–14. doi: 10.1152/japplphysiol.00024.2012

18. Sheel AW, Bailey EF. Mechanisms of respiratory modulation of cardiovascular control. *J Appl Physiol*. (2020) 128(1):212–3. doi: 10.1152/japplphysiol.00537.2019

19. Xie A, Skatrud JB, Morgan B, Chenuel B, Khayat R, Reichmuth K, et al. Influence of cerebrovascular function on the hypercapnic ventilatory response in healthy humans. *J Physiol (Lond)*. (2006) 577(Pt. 1):319–29. doi: 10.1113/jphysiol.2006.110627

20. Contreras-Briceño F, Espinosa-Ramirez M, Keim-Bagnara V, Carreño-Román M, Rodríguez-Villagra R, Villegas-Belmar F, et al. Determination of the respiratory compensation point by detecting changes in intercostal muscles oxygenation by using near-infrared spectroscopy. *Life*. (2022) 12(3):444. doi: 10.3390/life12030444

21. Ferguson LP, Walsh BK, Munhall D, Arnold JH. A spontaneous breathing trial with pressure support overestimates readiness for extubation in children. *Pediatr Crit Care Med*. (2011) 12(6):e330–5. doi: 10.1097/pcc.0b013e3182231220

22. Kimura S, Ahn JB, Takahashi M, Kwon S, Papatheodorou S. Effectiveness of corticosteroids for post-extubation stridor and extubation failure in pediatric patients: a systematic review and meta-analysis. *Ann Intensive Care*. (2020) 10(1):1–9. doi: 10.1186/s13613-020-00773-6

23. Butragueño-Laiseca L, Manrique Martín G, González Cortés R, Rey Galán C, Martínez de Compañón Martínez de Ma Z, Gil Antón J, et al. Multicenter randomized clinical trial comparing dexamethasone versus placebo in preventing upper airway obstruction after extubation in critically ill children. *Sci Rep*. (2022) 12(1):4336. doi: 10.1038/s41598-022-08178-0

frontiers | Frontiers in Pediatrics

TYPE Original Research
PUBLISHED 29 April 2024
DOI 10.3389/fped.2024.1359406

OPEN ACCESS

EDITED BY
Rujipat Samransamruajkit,
Chulalongkorn University, Thailand

REVIEWED BY
Gokhan Ceylan,
University of Health Sciences, Türkiye
Qalab Abbas,
Aga Khan University, Pakistan

*CORRESPONDENCE
Goutom Banik
✉ goutom.banik@savethechildren.org

†These authors have contributed equally to this work and share senior authorship

RECEIVED 21 December 2023
ACCEPTED 15 April 2024
PUBLISHED 29 April 2024

CITATION
Banik G, Halim MA, Md. Abdullah AS, Oishee I, Boyce C, Dey SK, Mannan MA, Moni SC, Shabuj MKH, Jahan I, Chowdhury RM, Afroze S, Wall S and Shahidullah M (2024) Vayu bubble continuous positive airway pressure is a promising solution with favorable treatment outcomes for respiratory distress syndrome in newborns: a qualitative study in Bangladesh. Front. Pediatr. 12:1359406. doi: 10.3389/fped.2024.1359406

Vayu bubble continuous positive airway pressure is a promising solution with favorable treatment outcomes for respiratory distress syndrome in newborns: a qualitative study in Bangladesh

Goutom Banik[1]*, M. A. Halim[2], Abu Sayeed Md. Abdullah[2], Irtifa Oishee[1], Carolyn Boyce[3], Sanjoy Kumer Dey[4], Md Abdul Mannan[4], Sadeka Choudhury Moni[4], Mohammad Kamrul Hassan Shabuj[4], Ismat Jahan[4], Rumpa Mani Chowdhury[4], Sharmin Afroze[5], Steve Wall[3†] and Mohammod Shahidullah[4†]

[1]Health and Nutrition Sector, Save the Children Bangladesh, Dhaka, Bangladesh, [2]Centre for Injury Prevention and Research, Dhaka, Bangladesh, [3]Save the Children Federation, Inc., Fairfield, CT, United States, [4]Department of Neonatology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh, [5]Department of Neonatology, Dr. M R Khan Shishu Hospital & Institute of Child Health, Dhaka, Bangladesh

Background: According to Bangladesh Demographic and Health Survey (2022), neonatal mortality, comprising 67% of under-5 deaths in Bangladesh, is significantly attributed to prematurity and low birth weight (LBW), accounting for 32% of neonatal deaths. Respiratory distress syndrome (RDS) is a prevalent concern among preterm and LBW infants, leading to substantial mortality. The World Health Organization (WHO) recommends bubble continuous positive airway pressure (bCPAP) therapy, but the affordability and accessibility of conventional bCPAP devices for a large number of patients become major hurdles in Bangladesh due to high costs and resource intensiveness. The Vayu bCPAP, a simple and portable alternative, offers a constant flow of oxygen-enriched, filtered, humidified, and pressurized air. Our study, conducted in five health facilities, explores the useability, acceptability, and perceived treatment outcome of Vayu bCPAP in the local context of Bangladesh.
Methods: A qualitative approach was employed in special care newborn units (SCANUs) of selected facilities from January to March 2023. Purposive sampling identified nine key informants, 40 in-depth interviews with service providers, and 10 focus group discussions. Data collection and analysis utilized a thematic framework approach led by trained anthropologists and medical officers.
Results: Service providers acknowledged Vayu bCPAP as a lightweight, easily movable, and cost-effective device requiring minimal training. Despite challenges such as consumable shortages and maintenance issues, providers perceived the device as user-friendly, operable with oxygen cylinders, and beneficial during referral transportation. Treatment outcomes indicated effective RDS management, reduced hospital stays, and decreased referrals. Though challenges existed, healthcare providers and facility managers expressed enthusiasm for Vayu bCPAP due to its potential to simplify advanced neonatal care delivery.

Conclusions: The Vayu bCPAP device demonstrated useability, acceptability, and favorable treatment outcomes in the care of neonates with RDS. However, sustained quality service necessitates continuous monitoring, mentoring and retention of knowledge and skills. Despite challenges, the enthusiasm among healthcare providers underscores the potential of Vayu bCPAP to save lives and simplify neonatal care delivery. Development of Standard Operating procedure on Vayu bCPAP is required for systematic implementation. Further research is needed to determine how the utilization of Vayu bCPAP devices enhances accessibility to efficient bCPAP therapy for neonates experiencing RDS.

KEYWORDS

newborns, respiratory distress syndrome, bubble CPAP, prematurity, low birth weight, neonatal mortality, Bangladesh

1 Introduction

Globally, almost one million newborns die from complications of prematurity every year. The most common severe preterm complication is respiratory distress syndrome (RDS), a primary cause of mortality in premature newborns. Worldwide, approximately 11% of all live births occur before 37 weeks of gestation (1), with preterm birth rates increasing. Survival is improving, especially in well-resourced settings, primarily due to improved healthcare and widely available and effective technology (2).

In Bangladesh, approximately 604,000 babies are born prematurely each year (before 37 completed weeks gestation), with an annual preterm birth rate of 14% (3). Complications of preterm birth account for 23,600 direct preterm child deaths per year, many from respiratory conditions (4). The bubble continuous positive airway pressure (bCPAP) device is included in Bangladesh's National Newborn Health Program (NNHP) as a key intervention of the special care newborn unit (SCANU) program to provide quality facility-based care to newborns with complications (5).

Newborns and young infants with respiratory distress from other conditions, such as Meconium Aspiration Syndrome and pneumonia, also are at heightened risk of death (6). To improve the outcomes of newborns and infants with respiratory distress, continuous positive airway pressure (CPAP) is extremely effective in well-resourced settings with reported 65% mortality decrease in RDS (7). Widely used in high-income settings, CPAP use in less-resourced settings—where needed most—is limited due to the high cost, reliance on compressed air and continuous electricity, maintenance, availability of consumables, and lack of necessary skills (8). The United States Food and Drug Administration (FDA) has granted Emergency Use Authorization (EUA) for a life-saving medical device to provide respiratory support to patients with respiratory distress in these less-resourced settings. The device, called the Vayu bubble CPAP (Vayu bCPAP), developed by Vayu Global Health, is an effective means to help save newborns and infants from preventable respiratory distress deaths. It is high-quality, easy to use, and extremely affordable, with production costs lower than other comparable devices (9, 10). The device provides adjustable inspired concentrations of oxygen (FiO2), flow rates, and pressures, as well as humidification comparable to a gold standard bCPAP device, yet does not require compressed air or electricity (11).

To manage RDS, respiratory support is needed, but necessary oxygen concentration, flow rates, and pressure are often not available in low-resource settings worldwide. In Bangladesh, commercial bCPAP devices remain out of reach for many newborns with respiratory distress due to costs, challenges for healthcare providers to achieve and maintain the requisite skills, and difficulties in maintaining well-functioning bCPAP equipment. Other common barriers include intermittent losses of electricity and lack of compressed air required for commercial bCPAP devices (10).

Considering the high maintenance issues of the other available bCPAP devices, we aimed to conduct a study to determine whether the use of the Vayu bCPAP and oxygen blender is useable and acceptable by health care providers (HCPs) in SCANUs as part of routine care for newborns with respiratory distress, primarily for preterm newborns in the local context of Bangladesh. Before initiating the study, a total of 28 Vayu bCPAP and oxygen blender devices were distributed and installed in study facilities. We organized a national-level workshop at Bangabandhu Sheikh Mujib Medical University (BSMMU) in August 2022 to sensitize the national- and local-level stakeholders and develop a plan for appropriate implementation of Vayu bCPAP in the study facilities. We also established a group of national-level master trainers for periodic monitoring and mentoring support for service providers in the study facilities. Following that, we trained a total of 50 service providers from the target facilities in using Vayu bCPAP. Periodic monitoring visits from the national level as well as mentoring were ongoing to encourage discussion and collaboration in addressing implementation challenges at the facility level. Over the implementation period (September 2022-March 2023), a total of 410 newborns received treatment with Vayu bCPAP in the SCANU settings of the study facilities.

2 Methods

2.1 Study design and setting

The study design involved qualitative methods to gather and analyze data. Qualitative research focuses on exploring the usability, acceptability and perceived treatment outcome in depth using different qualitative approaches of data gathering. We selected five different types of health facilities in consultation with Bangladesh's

NNHP of the Directorate General of Health Services (DGHS) to implement the research. The five facilities included a medical university—BSMMU in Dhaka; a medical college hospital—Sylhet Medical College Hospital (SOMCH) in Sylhet; a district hospital—Lakshmipur District Hospital (LDH) in Lakshmipur; a specialized hospital—Mohammadpur Fertility Services and Training Center (MFSTC) in Dhaka; and a private specialized hospital—Dr. MR Khan Shishu Hospital (MR Khan) in Dhaka. Each selected facility represented a unique setting within Bangladesh's healthcare landscape, ranging from tertiary care institutions like medical universities to specialized and private hospitals. By including diverse types of facilities, the study aimed to capture a broad spectrum of experiences, perspectives, and practices related to the implementation of Vayu bCPAP in Bangladesh.

2.2 Sampling and sample

The sampling process employed in this study utilized purposive sampling. Purposive sampling involves selecting individuals or groups who possess specific characteristics or experiences relevant to the research objectives. In this case, the researchers purposefully selected participants who could provide valuable insights on the usability, acceptability, and perceived treatment outcome of Vayu bCPAP. Data collection was ongoing before achieving data saturation. Data saturation refers to the point at which no new information or insights emerge from additional data collection, indicating that a comprehensive understanding of the topic has been attained. We conducted nine key informant interviews (KIIs) with facility managers, 40 in-depth interviews (IDIs) with HCPs, and 10 focus group discussions (FGDs) with nurses and support staff.

2.3 Data collection and quality assurance

The primary investigator (PI) and Co-PI of the study drafted, reviewed, and finalized the interview guidelines and consent forms. The guidelines were developed based on the following issues: relevance, efficiency, effectiveness, impact, sustainability, and coverage, capacity to use the following training, ease of use (assembly, application, monitoring, maintenance, and troubleshooting), ability to integrate use into care processes, facilitators and barriers to use (including suggested improvements), positive or negative effects on treatment outcomes, positive and negative attributes of the Vayu bCPAP system, and lessons learned that can be replicated in other facilities.

We recruited a total of four data collectors, including two anthropologists (one male and one female) and two medical doctors (one male and one female). These data collectors underwent a comprehensive two-day training on data collection, which included mock tests and role-playing activities. The training was designed to prepare them for qualitative data collection. Two teams carried out the data collection process. Each team consisted of one anthropologist and one doctor, one male and one female. Together, they worked to collect data and ensure the success of the project.

Data were collected through FGDs, IDIs, and KIIs. The teams conducted a total of 40 IDIs, five KIIs, and 10 FGDs within the five study facilities, and four KIIs at the national level (Table 1). All interviews and focus group discussions were performed in a private area, using audio recordings and paper forms by the research team.

A quality control team consisting of senior research team members was deployed for quality control checking of the study data. Quality control checking was designed to physically verify whether the data collectors effectively completed the interviews and FGDs (i.e., interviewed the right respondents and asked the right questions). The quality control team undertook quality control checking both in the presence and absence of the interviewing team. After data collection, the PI and Co-PI supervised processing of all the qualitative findings.

2.4 Data analysis

After conducting interviews and focus group discussions, the audio recordings of these sessions were transcribed into written text. Two anthropologists were responsible for independently transcribing the recordings. This approach helps ensure accuracy

TABLE 1 Respondents for the study's KIIs, FGDs, and IDIs in each facility.

Facility	KIIs (9)	FGDs (10)	IDIs (40)
MR Khan	Assistant Professor	FGD-1: Ward Boy-6 FGD-2: Senior Staff Nurse -6	Senior Staff Nurse -2/Technician-1 Medical Officer-3/Consultant-1/Ward Boy 1
MFSTC	Head of the Department	FGD-1: Senior Staff Nurse -6 FGD-2: Senior Staff Nurse -6	Technician-1/Senior Staff Nurse -1 Midwife-2/Medical Officer-2/Consultant-2
BSMMU	Chairman of Neonatology Department	FGD-1: Support Staff-6 FGD-2: Senior Staff Nurse -6	Senior Staff Nurse-3/Technician-1 Senior Medical Officer-2/Resident Medical Officer-2
SOMCH	Pediatric Consultant (In-charge)	FGD-1: Senior Staff Nurse -6 FGD-1: Senior Staff Nurse -6	Assistant Register-1 Indoor Medical Officer-1/Senior Staff Nurse-6
LDH	Pediatric Consultant (In-charge)	FGD-1: Senior Staff Nurse (Pediatric)-5 FGD-1: Senior Staff Nurse (SCANU)-5	Consultant-1 Medical Officer-1/Senior Staff Nurse-6
National	Program Manager, Integrated Management of Childhood Illness (IMCI)-1 Deputy Program Manager, IMCI-3		

and completeness in capturing the content of the discussions. Transcription was made in Bangla and subsequently translated into English. Using thematic analysis (12), major themes were identified and coded. Two research assistants coded the transcriptions independently. Coding involves systematically labeling and categorizing segments of the text based on recurring themes. The process examines the content of the transcriptions to identify ideas, perspectives, or experiences pointed repeatedly related to the use of the Vayu bCPAP device in managing respiratory distress syndrome in neonates. This step allows researchers to organize the data and identify key topics for analysis. After sorting and categorizing the responses, we chose excerpts from the transcripts to illustrate the summary statements, which were used to validate the findings. This rigorous process ensured systematic analysis and interpretation of qualitative data, enhancing the credibility and reliability of the findings.

2.5 Informed consent, confidentiality, and ethics approval

The PI and Co-PI of the study supported the submission and presentation of the proposal, tools, and consents at the Centre for Injury Prevention and Research, Bangladesh (CIPRB) Ethical Review Committee (ERC) meeting. The feedback from the ERC board members was incorporated into the proposal and resubmitted to the ERC, which subsequently provided final approval of the study.

3 Results

This section provides the results of the feedback from HCPs and stakeholders in key areas such as impact, coverage, cost-effectiveness, challenges, and sustainability of Vayu bCPAP. Furthermore, the section includes recommendations on Vayu bCPAP based on the results of the study (Table 2). Overall, the results section provides valuable insights for healthcare practitioners and policymakers on the usebility, acceptability, and usability of implementing Vayu bCPAP in healthcare facilities in Bangladesh.

3.1 Introduction of Vayu bCPAP as an alternative bCPAP device

Vayu bCPAP was introduced in the SCANUs as an alternative bCPAP device where availability of a commercial bCPAP was not adequate. The device has been considered to supplement the commercial bCPAP devices to provide support to a higher number of patients with RDS.

> *"Before introducing the Vayu bubble CPAP, HCPs used the "prior commercial bCPAP device" for a long time. Then they came to know about a device that is easy to handle and is low-cost. It was on trial in different countries during the COVID-19, when a lot of people needed respiratory support."* –Key informant interviewee

> *"The Vayu device is an alternative to other bCPAP devices." Unlike other bCPAP devices," it doesn't require electricity to function. It is portable, easy to access, and cheaper in price."* – FGD respondent

TABLE 2 Distribution of interview and FGD responses according to thematic areas of the study.

Areas	Responses
Introduction of Vayu bCPAP	➢ Easy to assemble and portable. ➢ The device doesn't require electricity. ➢ Vayu bCPAP works with environmental air pressure, so a central oxygen supply is not necessary. ➢ Can be implemented in a facility with minimal resources.
Training and mentoring	➢ The training was hands-on skills and very effective. ➢ Mentors visited the hospital and addressed any issues raised. ➢ The coordination of mentors was very helpful.
Mentoring	➢ WhatsApp groups play a vital role in addressing problems faced by the users in any of the five facilities. ➢ It helps to address any issues quickly, leading to improved outcomes and patient satisfaction.
Impact of Vayu bCPAP	➢ The outcome is very good at an early stage of respiratory distress. ➢ The device has potential to reduce the need of mechanical ventilation. ➢ No perceived difference in terms of treatment outcome.
Coverage of Vayu bCPAP	➢ Referral numbers decreased after using Vayu bCPAP. ➢ Able to provide service to more patients. ➢ It improves the respiratory distress of babies.
Cost -effectiveness of Vayu bCPAP	➢ Vayu bCPAP can be operated with oxygen cylinder. ➢ Cost-effective for both the patient party and the hospital. ➢ Very effective in the case of term babies. ➢ Referral decreased after using Vayu bCPAP.
Challenges of implementation	➢ Stock out of consumables. ➢ Routine maintenance and cleaning. ➢ Continuous monitoring. ➢ Securing the position of nasal prongs and keeping this for a long time. ➢ If the flow increased slightly, the water overflowed and soaked the bed. ➢ The machine creates noise when it is in high airflow. ➢ Unavailability of nasal prongs. ➢ Accumulation of fog.
Sustainability of Vayu bCPAP	➢ Nurses need more training and continuous monitoring. ➢ Government can provide this to all levels of facilities after piloting. ➢ Compared to the "prior commercial bCPAP device" this one is very cost-effective.
Recommendations	➢ This device will be helpful in low-resource centers including sub-district level health care facilities. ➢ The consumables of the device should be available. ➢ Further study is required for the improvement of the device addressing any trouble-shooting needed.

3.2 Training on Vayu bCPAP

Respondents noted that the training model was effective, including the facilitation and cascading of training.

> *"After the orientation program, formal training was given at BSMMU where consultants from the SCANU of intervention facilities took part. In turn, they trained all other service providers of the SCANU including medical officers and nurses regarding the implementation of the Vayu bCPAP at facilities. Later, the supporting staff was given training by the nurses."* –IDI respondent

> *"The training was very effective as the researchers and engineers who were directly involved in the development of this device were present in the training and they trained them in a hands-on skill manner."* –IDI respondent

3.3 Mentoring on Vayu bCPAP

According to respondents, during the early stages of implementing a new device, the training is mostly theoretical until it is put to use in practical settings. Once the device is being used, certain issues emerge that require attention and resolution. The master trainers of BSMMU played a crucial role in providing support to the users of Vayu bCPAP. They acted as a backup for addressing any issues that arose during the regular use of the device. This ensured that the devices were being used effectively and efficiently, which ultimately improved patient care. Additionally, the WhatsApp group maintained by the Save the Children staff and delegates of the five institutions played a vital role in addressing problems faced by the users in any of the five facilities. This group facilitated communication between users and provided a platform for sharing experiences and best practices. It helped to address any issues quickly, leading to improved outcomes and patient satisfaction (Table 2).

> *"In the initial phase of introducing any device, the training to some extent remains theoretical until it starts to be used in practical fields. After starting to use the device, some problems arise which need to be addressed later. The master trainers of BSMMU act as back-up for addressing any issues arising during the regular use of the device."* –Key informant interviewee

> *"The WhatsApp group maintained by the Save the Children group and delegates of the five institutions play a vital role in addressing these problems faced by the users in any of the five facilities."* –Key informant interviewee

> *"The experts of BSMMU provide mentorship support regarding the usage of the machine. Their mentorship helped to address any issues that arise during the regular use of the device."* –IDI respondent

3.4 Impact of Vayu bCPAP

The impact of Vayu bCPAP on newborns with respiratory distress was found to be positive. Vayu bCPAP was effective in managing large numbers of patients with RDS and in reducing the need for mechanical ventilation, which is associated with complications such as lung injury and infections. The study team found no perceived difference in the treatment outcomes (e.g., shorter hospital stays) of patients on Vayu bCPAP compared to those who received standard oxygen therapy (Table 2).

> *"Unlike other bCPAP devices, it doesn't require electricity to function. It is portable, easy to access, and cheaper in price. Moreover, there is no perceived difference in the outcome between Vayu bCPAP and "prior commercial bCPAP devices."* –FGD respondent

> *"The Vayu bCPAP is an incredibly straightforward device that boasts a lightweight design, making it much easier to transport than its traditional counterparts. The device can be set up in a matter of seconds. Furthermore, this machine does not require electricity, making it even more convenient to use."* –IDI respondent

> *"As it is less expensive and doesn't need electricity, it is more acceptable. It can blend oxygen and maintain pressure, but there is no perceived difference in terms of service outcome."* –Key informant interviewee

3.5 Coverage of Vayu bCPAP

Respondents maintained that Vayu bCPAP is efficient and easy to use. The device requires minimal training, and healthcare providers can set it up quickly. Additionally, Vayu bCPAP is portable, making it easy to transport between facilities during referral. The device has potential for greater coverage and access, especially in rural areas where healthcare resources may be limited (Table 2).

> *"When we started using the device, the level of confusion reduced and gradually the acceptance of the device increased in our facility."* –FGD respondent

> *"As a result of the introduction of the Vayu bCPAP, the healthcare facility is now able to provide support to a larger number of babies who require it."* –Key informant interviewee

> *"The existing good coordination among the BSMMU, DGHS, and the Save the Children team will be helpful for the successful implementation of the Vayu bCPAP and further scale up."* –Key informant interviewee

3.6 Cost-effectiveness of Vayu bCPAP

Vayu bCPAP is a cost-effective alternative to prior commercial bCPAP devices, as well as mechanical ventilation, because the device is reusable, so costs are further reduced in the long term (Table 2).

> *"Vayu bCPAP was introduced due to its cost-effectiveness. In Bangladesh, there is always some financial constraint in the utilization of modern technologies. The available commercial bCPAP devices are very expensive. So, when they found that the cost [of] Vayu bCPAP was very cheap and was given to them at zero cost initially, they took the opportunity to increase the quantity of CPAP machines to provide support to a greater number of babies requiring respiratory support."* –Key informant interviewee

> *"As there are financial constraints in our country, low-cost devices are given priority here. Besides, the other commercial bCPAP devices were not present in sufficient numbers in different facilities due to their high price. As the Vayu bCPAP is of low cost and easy to handle, it can be very effective if supplied to district-level hospitals. Babies requiring respiratory support will benefit from this device."* –IDI respondent

3.7 Challenges of Vayu bCPAP in the facilities

One of the main challenges of implementing Vayu bCPAP identified by respondents is the need for regular maintenance and cleaning. The device requires periodic replacement of components, and healthcare providers need to be trained on how to properly clean and maintain it. Other key challenges highlighted by respondents include stock out of consumables, lack of a built-in warming system, loud noise and vibratory effect from the pressure generator, difficulty in securing the nasal prong, lack of humidity temperature recording, and the need for continuous monitoring, which is difficult due to inadequate staffing in SCANUs (Table 2).

> *"Some challenges we faced in the initial phase were regarding the acceptability of the device as it is human nature to take time to adopt new things, and for having confusion regarding the maintenance of the temperature of the babies. The service providers were not so confident in using the device. However, when they started using the device, the level of confusion reduced and gradually the acceptance of the device increased in their facility."* –Key informant interviewee

> *"Other bCPAP devices have a monitor to see the temperature of the humidified air that helps in the maintenance of temperature which is not present in the Vayu one."* –IDI respondent

> *"Placing it in the baby's cot may pose a problem as it takes up a considerable amount of space on the bed, which can be problematic for babies."* –FGD respondent

> *"The service providers were very happy as the Vayu bCPAP machine is very easy to use. They faced challenges when there was a shortage of consumables. Also, in the initial phase during the sterilization of reusable parts."* –FGD respondent

3.8 Sustainability of Vayu bCPAP in the facility

According to respondents, Vayu bCPAP was considered a sustainable solution for addressing respiratory distress in infants. The device is portable and can be easily transported between facilities. The low cost and long-term reusability of the device make it an economically sustainable solution. Additionally, Vayu bCPAP has the potential to reduce the environmental impact of respiratory care, as it reduces the need for resource-intensive mechanical ventilation (Table 2). Respondents mentioned that the following should be done to sustain use of bCPAP: ensure there are proper baseline and endline results/research, ensure the proper awareness of the usage of the tool among the caregivers, build knowledge of dos/don'ts and troubleshooting among users, and make sure all stakeholders work together in implementation.

> *"The equipment procurement cost, maintenance cost, accessories cost, cost for training of the service providers, and cost for further research should be considered for Vayu bCPAP use to be sustained in the facilities."* –Key informant interviewee

> *"The stakeholders are monitoring the program to improve the quality of service. However, for now, the focus is on learning the feasibility of this device, and if it is feasible and there is a positive outcome in the pilot phase, they will plan for its sustainability."* –Key informant interviewee

> *"The cost of consumables and the device should be made available and there should be a budget for it. Secondly, training and dissemination costs, and finally, maintenance costs. There should be a core biomedical group to maintain the device."* –IDI respondent

3.9 Recommendations of Vayu bCPAP

Respondents had several recommendations related to Vayu bCPAP. They recommended that healthcare facilities incorporate Vayu bCPAP into their respiratory care protocols, and that healthcare providers receive training and follow-up support on the proper use, maintenance, and cleaning of the device. Additionally, they felt efforts should be made to increase the availability of Vayu bCPAP in areas where it is not currently accessible (Table 2). Respondents also recommended that:

- consumables be made available at the national level,
- maintenance of Vayu bCPAP should be made easier,

- devices should be available to manufacture locally,
- devices should be improved by adding a warming capacity,
- standard operating procedures should be developed,
- training should be ongoing, and
- availability of devices throughout the country should be increased.

> *"It is essential to include and implement the Vayu bCPAP as a government program to sustain it beyond the pilot phase or to scale it to other similar facilities in Bangladesh. BSMMU has played a vital role in introducing and improving the device. However, more research activities on it are essential to gather evidence-based information on its effectiveness."* –Key informant interviewee

> *"First, the centers for implementation should be increased. Secondly, the results of the pilot phase should be shared with everyone involved in the implementation process. Comparative studies should be conducted regarding using Vayu bCPAP machine in different age groups in different conditions to learn about the outcomes. Then, training and capacity built-up. Lastly, providing the machine according to demand."* –Key informant interviewee

4 Discussion

The introduction of a new medical device into regular health services, and adoption of the device by service providers and health facilities, requires an in-depth examination of its implementation in the local context (13–15). In our study, we investigated the useability and acceptance of Vayu bCPAP by program managers, service providers, and institutions, as well as the implementation challenges of using the Vayu bCPAP device in the local context of Bangladesh.

The study found that the Vayu bCPAP device is useable and acceptable in Bangladesh SCANU settings for the management of respiratory distress across all types of service providers. The device doesn't require electricity. The outcomes of use for early stages of respiratory distress were excellent, and referral numbers decreased after implementation of Vayu. The technical simplicity and easy bedside portability and assembly of the device enhanced the early initiation of respiratory support in SCANU settings and increased the service acceptability of the new device. These study results are generally consistent with the findings of other studies conducted in other similar settings around the world (16–19).

Our study also found that Vayu bCPAP systems were easily utilized by service providers across all levels of study facilities, were well-suited for the local context, and seamlessly integrated into the SCANU settings of Bangladesh. This easy utilization and seamless integration allowed nurses to initiate required treatment immediately. If needed, nurses were able to access distant support from a pediatrician using a virtual (WhatsApp) platform. These positive changes in RDS management in SCANU settings due to the introduction of Vayu bCPAP aligned with observations in other resource-constrained settings where simple CPAP devices were introduced (20, 21).

Several studies have outlined notable challenges associated with introducing CPAP devices in low- and middle-income countries (18, 22–24). Our study reveals the major challenges of Vayu bCPAP application in Bangladesh include stock out of consumables, the loud noise from the pressure generator, difficulty in securing the nasal prong, lack of a humidity temperature recording, and the need for continuous monitoring, which is difficult due to inadequate staffing in SCANUs. Continuous monitoring and maintenance of the equipment is crucial to ensure that it is being used effectively and safely, and to prevent any potential problems (25). Currently, there are no affordable oxygen blenders routinely available and used in many low- and middle-income countries, resulting in overuse of high oxygen concentrations and causing a near epidemic of Retinopathy of Prematurity, which can result in blindness or reduced vision among former preterm babies and children. Thus, the Vayu bCPAP system, especially in combination with the low-flow oxygen blender, offers substantial benefits for preterm survival and improved long-term outcomes in low-resource settings.

The study found that hands-on training is necessary to fully understand how to use and maintain the Vayu equipment properly. It is important to have someone show the health care providers how to use the equipment, followed by practice using it until one feels confident and comfortable (26).

The study also revealed that it is important to understand the setup and mechanism of the equipment to ensure the safe and effective operation of the device (27). This knowledge can help service providers to troubleshoot and fix any problems that may arise. The device is very low maintenance since there are no motors and it does not use electricity, so most maintenance can be done by trained service providers. The building of proper skills for maintenance enables service providers to use the device effectively, reducing infants' exposure to oxygen toxicity during recovery and as they are weaned off respiratory support and high concentrations of oxygen. This feature holds great promise to reduce the incidence and severity of Retinopathy of Prematurity and blindness in the most severe cases.

Basic maintenance and cleaning are essential to ensure that the equipment is functioning optimally and to prevent any contamination or damage to the equipment. Proper maintenance and cleaning also help to extend the lifespan of the equipment (28–30). While basic training is important, ongoing training is also necessary to ensure that service providers are confident and comfortable with the maintenance and cleaning of the equipment (31, 32). To successfully assemble and operate the equipment, the service providers need both knowledge and practice (33, 34). Therefore, continuous training, routine maintenance and cleaning, the ability to manufacture locally for rapid procurement, and development of standard operating procedures for the device, are mandatory components to provide quality services. In addition, reliable availability of consumables and adequate staffing for continuous monitoring are also crucial for desired service delivery and sustainability.

This study plays a vital role in building a body of evidence regarding the utilization of Vayu bCPAP within the specific healthcare context of Bangladesh. It not only addresses the perceptions of service providers and the challenges related to ensuring high-quality service delivery, but also highlights key recommendations for formulating a comprehensive service delivery model. Such a model can serve as a blueprint for effectively implementing Vayu bCPAP in other countries that face similar healthcare challenges as Bangladesh.

Nonetheless, it's important to underscore that further scientific research is required to comprehensively evaluate the implementation and impact of Vayu bCPAP in terms of reducing newborn and infant mortality rates nationwide.

5 Strengths and limitations

This qualitative research represents a pioneering effort which aimed to assess the useability and acceptability of the Vayu bCPAP device among healthcare service providers across various types of health facilities within the specific context of Bangladesh. The study conducted in-depth interviews involving key stakeholders, including national-level decision-makers, facility managers, pediatricians, relevant service providers, and supporting staff, to comprehensively understand the critical factors associated with the introduction of this new medical device to enhance service delivery in SCANU settings. By interviewing service providers and managers at different levels, the study sought to triangulate insights, challenges, and recommendations, thereby gaining a more holistic perspective. However, it is worth noting that this approach had an unintended drawback in that it created a perception among service providers that Vayu bCPAP was primarily introduced for research purposes rather than immediate patient care. The data collection process continued until data saturation was reached and interviewees expressed satisfaction with their participation. The interviewers were rigorously trained, and the composition of the interviewing team was suitably diverse.

It is crucial to point out some limitations of this study. Firstly, the relatively short implementation period may have limited the depth of service providers' perceptions, as forming a comprehensive understanding of a new device and its effects in facilities typically demands more time. Nevertheless, the high utilization of the Vayu bCPAP device during this implementation phase contributed to an adequate grasp of participants' experiences and perceptions. Secondly, the potential for researchers' subjectivity and biases may influence data collection and analysis. To address this, the study incorporated reflexivity and implemented measures to minimize potential bias.

6 Conclusion

Vayu bCPAP is a promising solution for respiratory distress in infants, with positive impacts on patient outcomes, cost-effectiveness, and sustainability. The challenges associated with the device can be addressed through proper training and distribution strategies. The recommendations provided based on this evaluation can guide healthcare facilities in incorporating Vayu bCPAP into their respiratory care protocols, ultimately leading to better outcomes for infants with respiratory distress. Regular monitoring, hands-on and ongoing training, understanding of setup, basic maintenance and cleaning, increased confidence and preparation, and knowledge and practice on Vayu bCPAP are all very important. Overall, the positive results of using Vayu so far demonstrate its effectiveness and ease of use. However, further scientific investigation is required to ascertain its non-inferiority compared to existing bCPAP devices and to address potential implementation and scalability challenges. Development of standard operating procedures for protocolized implementation is recommended.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethical Review Committee of The Centre for Injury Prevention and Research, Bangladesh (CIPRB). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

GB: Conceptualization, Methodology, Project administration, Writing – original draft, Writing – review & editing. MH: Investigation, Methodology, Project administration, Writing – original draft. AM: Data curation, Investigation, Methodology, Writing – original draft. IO: Writing – review & editing. CB: Conceptualization, Writing – review & editing. SD: Writing – review & editing. MM: Writing – review & editing. SM: Writing – review & editing. MShab: Writing – review & editing. IJ: Writing – review & editing. RC: Writing – review & editing. SA: Writing – review & editing. SW: Conceptualization, Funding acquisition, Methodology, Writing – review & editing. MShah: Conceptualization, Methodology, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article.

The study was funded by the United States Agency for International Development (USAID), USAID Cooperative

Agreement # 720388-18-CA00002 (Grant no. 84005050), and an anonymous donor through the Saving Women and Premature Babies (SWAP) project (Grant no. 84007799).

Acknowledgments

The authors would like to acknowledge the contribution of the Bangladesh National Newborn Health Program and IMCI, Directorate General of Health Services, and Directorate General of Family Planning to this project. They express their gratitude to all the participants of the study for sharing their insights, experiences, and learnings, and for their time and patience. The authors also express their special thanks to the research team as the study could not have been accomplished without their dedication and expertise.

Conflict of interest

CB and SW were employed by Save the Children Federation, Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Blencowe H, Cousens S, Chou D, Oestergaard M, Say L, Moller AB, et al. Born too soon: the global epidemiology of 15 million preterm births. *Reprod Health*. (2013) 10 (Suppl 1):S2. doi: 10.1186/1742-4755-10-S1-S2

2. Marinonio ASS, Costa-Nobre DT, Miyoshi MH, de Cassia Xavier Balda R, Areco KCN, Konstantyner T, et al. Clusters of preterm live births and respiratory distress syndrome-associated neonatal deaths: spatial distribution and cooccurrence patterns. *BMC Public Health*. (2022) 22(1):2. doi: 10.1186/s12889-022-13629-4

3. Halim A, Dewez JE, Biswas A, Rahman F, White S, van den Broek N. When, where, and why are babies dying? Neonatal death surveillance and review in Bangladesh. *PLoS One*. (2016) 11(8):e0159388. doi: 10.1371/journal.pone.0159388

4. World Health Organization. *New global estimates on preterm birth published*. Geneva, Switzerland: World Health Organization (2018). Available online at: https://www.who.int/reproductivehealth/global-estimates-preterm-birth/en (Accessed December 18, 2023).

5. Kamyar MM, Thomas FB, Michelle D, Shu HY, Rupam S, Revathi R, et al. A low-cost Venturi ambient air-oxygen blender for neonatal oxygen therapy. *Acad J Pediatr Neonatol*. (2020) 9(1):2. doi: 10.19080/AJPN.2020.08.555808

6. Stoll BJ, Hansen NI, Bell EF, Shankaran S, Laptook AR, Walsh MC, et al. Neonatal outcomes of extremely preterm infants from the NICHD neonatal research network. *Pediatrics*. (2010) 126(3):443–56. doi: 10.1542/peds.2009-2959

7. Almeida MF, Guinsburg R, Martinez FE, Procianoy RS, Leone CR, Marba STM, et al. Perinatal factors associated with early deaths of preterm infants born in Brazilian network on neonatal research centers. *J Pediatr (Rio J)*. (2008) 84(4):300–7. doi: 10.1590/S0021-75572008000400004

8. Irwin Sherman T, Blackson T, Touch S, Greenspan J, Shaffer T. Physiologic effects of CPAP: application and monitoring. *Neonatal Netw*. (2003) 22(6):7–16. doi: 10.1891/0730-0832.22.6.7

9. Mollazadeh-Moghaddam K, Burke TF, Dundek M, Yeung SH, Sharma R, Ravi R, et al. A low-cost Venturi ambient air-oxygen blender for neonatal oxygen therapy. *Acad J Pediatr Neonatol*. (2020) 8(5):48–56. doi: 10.19080/AJPN.2020.08.555808

10. Dundek ML, Ng EK, Brazil AM, DiBlasi RM, Poli JA, Burke TF. Evaluation of a bubble CPAP system for low resource settings. *Respir Care*. (2021) 66(10):1572–81. doi: 10.4187/respcare.08948

11. Vayu. Engineering Healthcare Solutions for Global Access. Available online at: https://vayuinnovations.org/ (Accessed December 18, 2023).

12. Mihas P. Qualitative, multimethod, and mixed methods research. In: Ivankova N, editor. *International Encyclopedia of Education*, 4th ed. Amsterdam: Elsevier (2023). p. 4. Available online at: https://www.sciencedirect.com/topics/social-sciences/thematic-analysis

13. Cousins S, Richards H, Zahra J, Elliott D, Avery K, Robertson HF, et al. Introduction and adoption of innovative invasive procedures and devices in the NHS: an in-depth analysis of written policies and qualitative interviews (the INTRODUCE study protocol). *BMJ open*. (2019) 9(8):e029963. doi: 10.1136/bmjopen-2019-029963

14. Maisel WH. Medical device regulation: an introduction for the practicing physician. *Ann Intern Med*. (2004) 140(4):296–302. doi: 10.7326/0003-4819-140-4-200402170-00012

15. Bridgelal Ram M, Grocott PR, Weir HC. Issues and challenges of involving users in medical device development. *Health Expect*. (2008) 11(1):63–71. doi: 10.1111/j.1369-7625.2007.00464.x

16. Tayler A, Ashworth H, Bou Saba G, Wadhwa H, Dundek M, Ng E, et al. Feasibility of a novel ultra-low-cost bubble CPAP (bCPAP) system for neonatal respiratory support at Muhimbili National Hospital, Tanzania. *PLoS One*. (2022) 17 (12):e0269147. doi: 10.1371/journal.pone.0269147

17. Vanbinst S, Van Damme E, Raaijmakers A. Feasibility study of a low-cost bubble CPAP system in a neonatal medium care unit in Belgium. *Belgian J Paediatr*. (2023) 25 (3):158–61. https://belgjpaediatrics.com/index.php/bjp/article/view/210

18. Dada S, Ashworth H, Sobitschka A, Raguveer V, Sharma R, Hamilton RL, et al. Experiences with implementation of continuous positive airway pressure for neonates and infants in low-resource settings: a scoping review. *PLoS One*. (2021) 16(6): e0252718. doi: 10.1371/journal.pone.0252718

19. Hendriks H, Kirsten GF, Voss M, Conradie H. Is continuous positive airway pressure a feasible treatment modality for neonates with respiratory distress syndrome in a rural district hospital? *J Trop Pediatr*. (2014) 60(5):348–51. doi: 10.1093/tropej/fmu025

20. Martin S, Duke T, Davis P. Efficacy and safety of bubble CPAP in neonatal care in low and middle income countries: a systematic review. *Arch Dis Child Fetal Neonatal Ed*. (2014) 99(6):F495–504. doi: 10.1136/archdischild-2013-305519

21. Carns J, Kawaza K, Liaghati-Mobarhan S, Asibon A, Quinn MK, Chalira A, et al. Neonatal CPAP for respiratory distress across Malawi and mortality. *Pediatrics*. (2019) 144(4):10. doi: 10.1542/peds.2019-0668

22. Dewez JE, Chellani H, Nangia S, Metsis K, Smith H, Mathai M, et al. Healthcare workers' views on the use of continuous positive airway pressure (CPAP) in neonates: a qualitative study in Andhra Pradesh, India. *BMC Pediatr*. (2018) 18:1–8. doi: 10.1186/s12887-018-1311-8

23. Sessions KL, Mvalo T, Kondowe D, Makonokaya D, Hosseinipour MC, Chalira A, et al. Bubble CPAP and oxygen for child pneumonia care in Malawi: a CPAP IMPACT time motion study. *BMC Health Serv Res*. (2019) 19(1):1–8. doi: 10.1186/s12913-019-4364-y

24. McCollum ED, Mvalo T, Eckerle M, Smith AG, Kondowe D, Makonokaya D, et al. Bubble continuous positive airway pressure for children with high-risk conditions and severe pneumonia in Malawi: an open label, randomised, controlled trial. *Lancet Respir Med*. (2019) 7(11):964–74. doi: 10.1016/S2213-2600(19)30243-7

25. WHO. (2020). Children: improving survival and wellbeing. Available online at: https://www.who.int/news-room/fact-sheets/detail/children-reducing-mortality (Accessed December 18, 2023).

26. Owen LS, Manley BJ, Davis PG, Doyle LW. The evolution of modern respiratory care for preterm infants. *Lancet*. (2017) 389(10079):1649–59. doi: 10.1016/S0140-6736(17)30312-4

27. Daga BS, Joshi H, Gunjal P, Mhatre S. An innovative air-oxygen blender for continuous positive airway pressure support in resource-poor locations: a feasibility study. *J Trop Pediatr*. (2016) 63(4):269–73. doi: 10.1093/tropej/fmw085

28. Cheng M. Medical Device Regulations: Global Overview and Guiding Principles.

29. Mahfoud H, Abdellah EB, El Biyaali A. Dependability-based maintenance optimization in healthcare domain. *J Qual Maint Eng*. (2018) 24(2):200–23. doi: 10.1108/JQME-07-2016-0029

30. Khider MO, Hamza AO. Medical equipment maintenance management system: review and analysis. *J Clin Eng*. (2022) 47(3):151–9. doi: 10.1097/JCE.0000000000000538

31. Davis D, O'Brien MA, Freemantle N, Wolf FM, Mazmanian P, Taylor-Vaisey A. Impact of formal continuing medical education: do conferences, workshops, rounds, and other traditional continuing education activities change physician behavior or health care outcomes? *JAMA*. (1999) 282(9):867–74. doi: 10.1001/jama.282.9.867

32. Grimshaw JM, Shirran L, Thomas R, Mowatt G, Fraser C, Bero L, et al. Changing provider behavior: an overview of systematic reviews of interventions. *Med Care*. (2001) 39(8):II-2. https://journals.lww.com/lww-medicalcare/abstract/2001/08002/changing_provider_behavior__an_overview_of.2.aspx

33. Bahreini R, Doshmangir L, Imani A. Influential factors on medical equipment maintenance management: in search of a framework. *J Qual Maint Eng*. (2019) 25 (1):128–43. doi: 10.1108/JQME-11-2017-0082

34. Kutor JK, Agede P, Ali RH. Maintenance practice, causes of failure and risk assessment of diagnostic medical equipment. *J Biomed Eng Med Dev*. (2017) 2 (1):123. doi: 10.4172/2475-7586.1000123

frontiers | Frontiers in Pediatrics

TYPE Original Research
PUBLISHED 09 May 2024
DOI 10.3389/fped.2024.1373272

OPEN ACCESS

EDITED BY
Rujipat Samransamruajkit,
Chulalongkorn University, Thailand

REVIEWED BY
Malik Aydin,
Witten/Herdecke University, Germany
Tinashe K. Nyazika,
Liverpool School of Tropical Medicine, United Kingdom
Zhanqi Zhao,
Guangzhou Medical University, China

*CORRESPONDENCE
Lili Zhou
906698634@qq.com

RECEIVED 19 January 2024
ACCEPTED 22 April 2024
PUBLISHED 09 May 2024

CITATION
Zhou L, Peng X, Cao L, Zhang L and Xiang H (2024) Clinical efficacy of bronchoalveolar lavage in the treatment of small airway diseases in children.
Front. Pediatr. 12:1373272.
doi: 10.3389/fped.2024.1373272

Clinical efficacy of bronchoalveolar lavage in the treatment of small airway diseases in children

Lili Zhou[1]*, Xuyun Peng[1], Liqin Cao[2], Lin Zhang[3] and Huazheng Xiang[3]

[1]Department of Pediatrics, Ganzhou Women and Children' Hospital, Ganzhou, Jiangxi Province, China, [2]Department of Pediatrics, Shangyou Women and Children's Hospital, Ganzhou, China, [3]Department of Pediatrics, Jian Women and Children's Hospital, Jian, China

Objective: This study aimed to evaluate the efficacy of bronchoalveolar lavage (BAL) in the treatment of children with small airway diseases.
Methods: Children [$n = 112$; boys: 76, girls: 36 (ratio 2.1:1); age range: 1 month–10 years; median age: 12 months] with small airway diseases diagnosed by high-resolution computed tomography (HRCT) were enrolled in this study. The patients were assigned to either the BAL group (BAL and conventional therapy) or the control group (conventional therapy only). The duration of cough, fever, wheezing, hospitalization duration, disease course before admission, treatment cost, HRCT recovery time, and re-hospitalization rate were compared between the two groups.
Results: The median disease course before admission of the BAL group patients was longer than that of the controls ($p = 0.006$). The duration of cough and wheezing in the BAL group was significantly longer than that in the control group ($p = 0.012$ and $p = 0.001$, respectively). The recovery time of cough, the re-hospitalization rate, and the total expenditure incurred for the BAL group were lower than those for the control group ($p = 0.027$, $p = 0.026$, and $p = 0.000$, respectively). At 2 months after discharge, the small airway lesions were found to be absorbed in 86.2% of BAL group patients vs. 64.1% of control group patients. At 6 months after discharge, the lesions were not fully absorbed in 3.4% of the BAL group patients compared to 20.5% in the control group patients.
Conclusion: BAL is suitable for patients with a long disease course before admission, a long duration of coughing, and recurrent wheezing. BAL treatment of small airway diseases in children can promote the disappearance of clinical symptoms, accelerate the improvement of imaging, reduce the rate of re-hospitalization, and reduce the cost of treatment.

KEYWORDS

bronchoalveolar lavage, small airway disease, children, HRCT, treatment

Introduction

Small airway disease is a common group of children's respiratory system disease characterized by small airway obstruction. The small airway is relatively earlier and more easily invaded in respiratory system disease. The change of its function at the early stage of the disease, possibly induced by inflammation and mucus embolism, can be reversed. In the later stage, the small airway becomes fibrotic, deformed, narrow, or

even closed, and its function becomes irreversible. The small airway disease is defined as a disease located beyond the seventh or eighth generation of the tracheobronchial tree with a diameter of <2 mm (1). The incidence of small airway diseases has been reported as 10%–20% by Berend (2). These include pneumonia (especially interstitial pneumonia), asthma, bronchiolitis, bronchiolitis obliterans, bronchiectasis, some congenital bronchopulmonary anomalies, and early disseminated tuberculosis. These diseases are difficult to diagnose early and treat. However, with the wide application of high-resolution computed tomography (HRCT) and pulmonary function, the detection rate and awareness about small airway diseases are increasing (3). Several small airway diseases are characterized by mucus accumulation, mucus embolism, plastic bronchitis, and uneven bronchial aeration, which cannot be relieved via conventional anti-infection treatment. Instead, bronchoalveolar lavage (BAL) therapy is required for such cases. For more than 40 years now, BAL has been widely used in pediatric respiratory diseases (4). Despite its frequent use, there is a lack of contemporary literature regarding the diagnostic utility of BAL for small airway diseases in children. BAL is a safe and minimally invasive treatment, but it is also complicated and involves certain risks that limit its clinical application. Therefore, we evaluated the utility of BAL in the treatment of small airway diseases in children in this study.

Materials and methods

Study population

Inclusion criteria

The inclusion criteria are as follows: (a) children with small airway diseases diagnosed by HRCT who were hospitalized for medical treatment at the Department of Pediatric Respiratory Medicine of our hospital from January 2021 to November 2022; (b) provision of the informed consent of the child or his parents; and (c) indications for bronchoalveolar lavage treatment and identification of pathogens.

Exclusion criteria

The exclusion criteria are as follows: (a) patients with contraindication of bronchoscopy; (b) patients with incomplete clinical data; and (c) patients with primary diseases such as those of the heart, brain, blood vessels, and hematopoietic system.

Diagnostic criteria

Small airway lesions are mainly bronchiolar lesions. The main HRCT features of small airway lesions are bronchial wall thickening, tree-in-bud sign, mosaic sign, and air trapping (5).

Study groups

Children with small airway diseases who had indications for BAL therapy were grouped according to their parents' willingness to choose BAL therapy. Those who opted for BAL therapy were included in the BAL group and received BAL treatment along with conventional treatment; the control group included patients who were not willing to accept BAL treatment and received conventional treatment only. Based on their clinical symptoms, the children in the conventional treatments group were treated with anti-infection, oxygen inhalation, antipyretic, asthma relieving, atomization, phlegm reduction, sputum aspiration and elimination, nutrition, and so on.

Study contents

The duration of fever, cough, and wheezing; the recovery time of fever, cough, and wheezing; days of hospitalization; treatment cost; course of disease before admission; CT recovery time; re-hospitalization rate; and blood routine of the two groups were recorded. CT was performed at the time of hospital admission and then at 1, 2, 3, and 6 months after discharge. No more checks were performed after CT results returned to normal. Chest imaging was performed to examine whether the lesion showed no absorption, partial absorption, or complete absorption.

Procedure for BAL

No contraindications for BAL were determined. Family members of the children signed informed consent forms. Preoperative routine examinations included blood routine tests, bleeding and clotting time records, and electrocardiograms. Patients were instructed to fast and abstain from water for 4–6 h before surgery. The patients were asked to inhale 2.5 mg of terbutaline atomizing solution 30 min before the surgery. Then, 5 mg of dexamethasone was injected to prevent laryngeal edema. The entire process was recorded using Japan Fujieneng EB-270S (outside diameter 4.9 mm) and Eb-270p (outside diameter 3.8 mm) pediatric electronic bronchoscopes, an EPX-2200 image processor, a universal light source, and an image display system. After anesthesia, the bronchoscope was inserted into the airway through the glottis, passing through the nasal cavity and throat. We observed the tracheal carina, each lobe, and segmental bronchus, as well as the lesions identified in the HRCT scan along the direction of the lens. Then, normal saline at 37°C was used for irrigation in stages using 0.50–1.00 ml/kg each time, and the inflammation or sputum supposition was brushed and rinsed according to the situation of the site. The douche solution was inhaled into a sterile container for inspection, and the intraoperative situation of the child was closely monitored. After the surgery, fasting and water prohibition were maintained for 2 h. We also looked for potential complications such as fever, hemoptysis, or dyspnea. The number of BAL treatments for each child was determined as deemed necessary.

Ethical approval

The study protocol was approved by the Ethics and Research Council of Women and Children's Hospital of Ganzhou (2022-117) on 27 December 2022. The data were collected from the patients anonymously.

Statistical analyses

The data were analyzed by using the SPSS 20.0 software package. Continuous variables were reported as the median (range) and compared using Student's *t*-test or the non-parametric Mann–Whitney *U*-test. The categorical variables were presented as numbers (%) and compared using the χ^2 test. $p < 0.05$ was considered to indicate statistical significance.

Results

Demographic and clinical information

This study was retrospective. A total of 112 patients [boys: 76, girls: 36 (ratio 2.1:1); age range: 1 month–10 years; median age: 12 months] were enrolled. The re-hospitalization rate was 32 (28.6%). The pathogen positive rate was 74 (66.1%). The main viral infections were respiratory syncytial virus and rhinovirus. The main bacterial infections were *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhal*. The pathogens and the main discharge diagnosis are listed in Table 1.

Comparison between the BAL group and control group

The BAL group included 35 cases (boys:girls: 2.18:1; median age: 10 months), while the control group included 77 cases (boys:girls: 2.08:1; median age: 13 months). No significant difference was noted in terms of age, sex, hospitalization days, fever duration, and the recovery time of fever and wheezing between the two groups ($p > 0.05$). The total cost in the BAL group was shorter than that in the control group ($p = 0.000$). The median disease course before admission in the BAL group was longer than that in the control group ($p = 0.006$). The duration of coughing and wheezing in the BAL group was longer than that in the control group ($p = 0.012$ and $p = 0.001$, respectively). The re-hospitalization rate, the recovery time from coughing, and the total cost of treatment for the BAL group were significantly lower than those for the control group ($p = 0.026$, $p = 0.027$, and $p = 0.000$, respectively). No significant differences were noted in the levels of white blood cells, neutrophils, lymphocytes, hemoglobin, platelets, and C-reactive protein between the two groups (details are provided in Table 2).

Absorption of small airway lesions

Of the total, 68 children were followed up and re-examined with HRCT after discharge, including 29 in the BAL group and 39 in the control group. Details are provided in Table 3. The χ^2 test revealed that $\chi^2 = 17.797$, and $p = 0.000$ indicated a statistically significant difference at 1 month after discharge (see Table 4). The children with incomplete imaging absorption were reviewed 2 months after the discharge. The χ^2 test showed that $\chi^2 = 20.179$, and $p = 0.000$ indicated a statistically significant difference (see Table 5). Incomplete absorption after 6 months was noted in one (3.4%) case in the BAL group and eight (20.5%) cases in the control group.

TABLE 1 Demographic and clinical information of 112 patients.

Index	$N = 112$
Sex (male/female)	76/36
Age (months)	12 (1–120)
Re-hospitalization	32 (28.6%)
Pathogen positive	74 (66.1%)
Viral infection	15 (13.4%)
Bacterial infection	23 (20.5%)
Mycoplasma pneumoniae infection	10 (8.9%)
Single infection	48 (42.9%)
Co-infection	26 (23.2%)
Diagnosis	
Bronchiolitis	7 (6.3%)
Bacterial pneumonia	12 (10.7%)
Viral pneumonia	14 (12.5%)
Mycoplasma pneumoniae	10 (8.9%)
Chronic pneumonia	2 (1.8%)
Aspiration pneumonia	2 (1.8%)
Severe pneumonia	16 (14.3%)
Unclassified pneumonia	30 (26.8%)
Bronchopulmonary dysplasia	3 (2.7%)
Bronchiolitis obliterans	4 (3.6%)
Bronchial asthma	8 (7.2%)
Persistent bacterial bronchitis	1 (0.9%)
Bronchial foreign body	1 (0.9%)
Gastroesophageal reflux	1 (0.9%)

TABLE 2 Comparison between BAL and control groups.

	BAL group ($n = 35$)	Control group ($n = 77$)	*p*
Sex (male)	24 (68.57%)	52 (67.53%)	0.55
Number of re-hospitalization times	5 (14.29%)	27 (35.06%)	0.026
Course of disease before admission (days)	30 (1–150)	14 (1–180)	0.006
Days of hospitalization	7 (2–14)	7 (2–26)	0.98
Age (months)	10 (2–120)	13 (1–80)	0.18
Duration of fever (days)	2 (0–9)	2 (0–17)	0.73
Recovery time of fever (days)	2 (0–6)	2 (1–13)	0.51
Duration of cough (days)	36 (0–159)	19 (0–183)	0.012
Recovery time of cough (days)	5 (1–13)	6 (1–19)	0.027
Duration of wheezing (days)	10 (0–93)	2 (0–72)	0.001
Recovery time of wheezing (days)	5 (1–14)	4 (1–23)	0.81
Total cost (yuan)	7,891 (4,653–16,387)	9,107 (1,998–58,809)	0.000
White blood cells at admission ($\times 10^9$/L)	12.12 (4.77–28)	12.88 (4.26–21.9)	0.89
Neutrophils at admission (%)	34.4 (15–78.3)	42.1 (10–91.3)	0.071
Lymphocytes at admission (%)	58.8 (12–79.2)	44.4 (6.1–77.7)	0.067
Hemoglobin at admission (g/L)	126 (98–152)	124 (68–148)	0.60
Platelets at admission ($\times 10^9$/L)	356 (201–667)	355 (7–606)	0.31
C-reactive protein (mg/L)	1.63 (0.1–40)	2 (0.1–51)	0.21

10.3389/fped.2024.1373272

TABLE 3 Recovery time of HRCT in BAL and control groups.

Group	Number	Completely absorbed				Partially absorbed
		1 month after discharge	2 months after discharge	3 months after discharge	6 months after discharge	More than 6 months after discharge
BAL group	29	1	24	1	2	1
Control group	39	6	19	5	1	8
Total	68	7	45	16	3	9

TABLE 4 Comparison of pulmonary CT absorption between the two groups at 1 month after discharge.

Group	Number	CT absorption of the lung at 1 month after discharge			
		Completely absorbed	Partially absorbed	Not absorbed	Absorption rate
BAL group	29	1	24	4	25 (86.2%)
Control group	39	6	21	12	27 (69.2%)
Total	68	7	45	16	52 (76.5%)
χ^2					17.797
p					0.000

TABLE 5 Comparison of pulmonary CT absorption between the two groups at 2 months after discharge.

Group	Number	CT absorption of lung at 2 months after discharge			
		Completely absorbed	Partially absorbed	Not absorbed	Absorption rate
BAL group	28	24	1	3	25 (89.3%)
Control group	33	19	5	9	24 (72.7%)
Total	61	43	6	12	49 (80.3%)
χ^2					20.179
p					0.000

Discussion

The small airway disease cannot be satisfactorily treated by using the conventional treatment methods alone, and the chances of recurrence are high. In this study, fiberoptic bronchoscopy was used to perform BAL in children with small airway diseases. During the surgery, the lens directly reached the lesion site and removed the secretions and sputum suppositories in the small airways, which facilitated the rapid reduction of clinical symptoms. The study indicated that BAL was suitable for patients with long disease courses before admission, long durations of coughing, and recurrent wheezing episodes. It was reported that BAL treatment was effective in persistent bacterial bronchitis, which was a common cause of chronic wet cough in preschool children (6). It also confirmed that BAL could effectively reduce recurrent wheezing in young children (7). Several important factors, such as inflammatory factors, chemokines, cytology, and infectious microorganism etiology, can be analyzed by testing alveolar lavage fluid, which can be helpful for the diagnosis, observation, and prognosis of respiratory diseases (8). Based on our results, BAL did not reduce fever and wheezing time but shortened the coughing time. Minqing et al. reported that BAL treatment could effectively shorten the fever remission time and hospital stay duration and provide cough relief (9). The inconsistency in the literature may be attributed to the difference in the inclusion criteria. Currently, only a few pieces of literature analyze small airway diseases separately. Some patients with refractory pneumonia or severe pneumonia have small airway lesions (10). The present results showed that the re-hospitalization rate of the BAL group was significantly shorter than that of the control group. The literature on the re-hospitalization rate of BAL treatment is limited to date. The total cost incurred by the BAL group patients was significantly lower than that of the control group patients, which agrees with the research findings of Carr et al. (11). The present results showed that the small airway lesions were absorbed 2 months after discharge in the BAL group and after 6 months in the control group, indicating that BAL played a positive role in improving small airway lesions. Moreover, the recovery time of HRCT in the BAL group was significantly shorter than that in the control group. There are only a few literature reports on the imaging recovery time after alveolar lavage treatment.

In conclusion, BAL is a rapid and highly efficient treatment approach for small airway diseases in children. Our findings highlight the need to focus on small airway diseases in children, which is a common disease that is easily overlooked in clinics, and to provide a theoretical basis for its treatment.

However, this study is limited by the lack of lung function testing to evaluate the recovery of small airway diseases because the study patients were relatively young and could not

independently complete the routine ventilation tests to fully reflect small airway lesions. Further research on lung functions would contribute to the complete understanding of the efficacy of the present treatment approach. This study included a relatively small number of children, and the children were from a unit of our hospital; thus, there may be some case-selective bias.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Ethics and Research Council of Women and Children's hospital of Ganzhou (2022-117) on 27 December 2022. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

LZho: Writing – original draft. XP: Conceptualization, Writing – review & editing. LC: Data curation, Writing – review & editing. LZha: Formal Analysis, Writing – review & editing. HX: Data curation, Investigation, Supervision, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The authors thank all colleagues in the Department of Respiratory Medicine for collecting the clinical data. The authors also thank all the families for their enrollment in this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Carr TF, Altisheh R, Zitt M. Small airways disease and severe asthma. *World Allergy Organ J.* (2017) 10:20. doi: 10.1186/s40413-017-0153-4

2. Berend N. Small airways disease. *Aust N Z J Med.* (1983) 13:393–7. doi: 10.1111/j.1445-5994.1983.tb04491.x

3. Zhang Y, Zhang H, Su X, Wang Y, Gao G, Wang X, et al. Analysis of influencing factors and a predictive model of small airway dysfunction in adults. *BMC Pulm Med.* (2023) 23(1):141. doi: 10.1186/s12890-023-02416-5

4. Wood RE, Fink RJ. Applications of flexible fiberoptic bronchoscopes in infants and children. *Chest.* (1978) 73(5):737–40. doi: 10.1378/chest.73.5_Supplement.737

5. Qin Y, Wang J, Han Y, Lu L. Deep learning algorithms-based CT images in glucocorticoid therapy in asthma children with small airway obstruction. *J Healthc Eng.* (2021) 2021:5317403. doi: 10.1155/2021/5317403

6. Marcella G, Melissa P, Arianna G, Emanuela P, Luca B, Andrea P, et al. When the cough does not improve: a review on protracted bacterial bronchitis in children. *Front Pediatr.* (2020) 8:433. doi: 10.3389/fped.2020.00433

7. Schellhase DE, Fawcett DD, Schutze GE, Lensing SY, Tryka AF. Clinical utility of flexible bronchoscopy and bronchoalveolar lavage in young children with recurrent wheezing. *J Pediatr.* (1998) 132:312–8. doi: 10.1016/S0022-3476(98)70451-2

8. Molina-Moliana M. "Bronchoscopy role in interstitial lung disease". In: Díaz-Jimenez JP, Rodriguez A, editors. *Interventions in Pulmonary Medicine*. New York: Springer-Science + Business Media (2013). p. 431.

9. Minqing P, Ping J, Tingting W, Caifeng X, Ruiying H, Ailing S, et al. Effect of bronchoalveolar lavage on the clinical efficacy, inflammatory factors, and immune function in the treatment of refractory pneumonia in children. *Transl Pediatr.* (2021) 10:921–8. doi: 10.21037/tp-21-89

10. Xiangtao W, Weihong L, Xinquan S, Yali X, Tuanjie W, Xiaowen Z, et al. Timing of bronchoscopy and application of scoring tools in children with severe pneumonia. *Ital J Pediatr.* (2023) 49:44. doi: 10.1186/s13052-023-01446-3

11. Carr JA, Phillips BD, Bowling WM. The utility of bronchoscopy after inhalation injury complicated by pneumonia in burn patients: results from the national burn repository. *J Burn Care Res.* (2009) 30:967–74. doi: 10.1097/BCR.0b013e3181bfb77b

frontiers | Frontiers in Pediatrics

TYPE Original Research
PUBLISHED 04 July 2024
DOI 10.3389/fped.2024.1344709

OPEN ACCESS

EDITED BY
Rujipat Samransamruajkit,
Chulalongkorn University, Thailand

REVIEWED BY
Oguz Dursun,
Akdeniz University, Türkiye
Zhongheng Zhang,
Sir Run Run Shaw Hospital, China

*CORRESPONDENCE
Li Hu
drhuli@163.com

RECEIVED 26 November 2023
ACCEPTED 18 June 2024
PUBLISHED 04 July 2024

CITATION
Ge H, Zhang A, Teng Y and Hu L (2024) Evaluation of the combined predictive value of multiple indicators based on diaphragmatic ultrasound using logistic regression and ROC curve in weaning from mechanical ventilation in pediatric patients.
Front. Pediatr. 12:1344709.
doi: 10.3389/fped.2024.1344709

Evaluation of the combined predictive value of multiple indicators based on diaphragmatic ultrasound using logistic regression and ROC curve in weaning from mechanical ventilation in pediatric patients

Hejia Ge[1], Ailian Zhang[1], Yiqun Teng[1] and Li Hu[2]*

[1]Department of Pediatrics, The Second Affiliated Hospital of Jiaxing University, Jiaxing, China,
[2]Department of Anesthesiology, The Second Affiliated Hospital of Jiaxing University, Jiaxing, China

Background: Conventional single indicators have low sensitivity and specificity for predicting weaning from mechanical ventilation in pediatric patients, necessitating the establishment of a combined prediction model for predicting weaning outcomes.
Objectives: To explore the combined predictive value of PaO_2/FiO_2 Ratio (P/F ratio), diaphragm excursion-rapid shallow breathing index (DE-RSBI), diaphragm thickening fraction-rapid shallow breathing index (DTF-RSBI), and Pediatric Critical Illness Score (PCIS) in weaning from mechanical ventilation in pediatric patients.
Methods: Sixty critically ill pneumonia pediatric patients requiring mechanical ventilation treatment from July 2022 to June 2023 at the Second Affiliated Hospital of Jiaxing University were selected. They all underwent a spontaneous breathing trial (SBT) and were divided into the weaning success group (42 cases) and weaning failure group (18 cases) based on the weaning outcome. Parameters including total duration of illness, mechanical ventilation duration, heart rate (HR), P/F ratio, diaphragm excursion (DE), DE-RSBI, diaphragm thickening fraction (DTF), DTF-RSBI, and PCIS were included in univariate and multivariate logistic regression analyses to determine independent factors affecting pediatric weaning success. Receiver operating characteristic (ROC) curves were plotted to evaluate the predictive value of P/F ratio, DE-RSBI, DTF-RSBI, PCIS alone or in combination for weaning success.
Results: Comparing P/F ratio, DE, DE-RSBI, DTF, DTF-RSBI and PCIS, there were statistically significant differences ($P < 0.05$). Through collinearity analysis and binary logistic regression analysis,P/F ratio [OR = 0.777, 95% CI (0.641,0.941)], DE-RSBI [OR = 1.694, 95% CI (1.172, 2.447)], DTF-RSBI [OR = 1.057, 95% CI (1.002, 1.114)], and PCIS [OR = 0.661, 95% CI (0.445, 0.982)] were identified as independent factors affecting successful weaning($P < 0.05$).The regression equation was: Logit$P$ = 73.299−0.253 P/F ratio + 0.525DE-RSBI + 0.055DTF-RSBI-0.43PCIS.The sensitivity of the combined indicator Logit(P) in predicting successful weaning from mechanical ventilation in pediatric patients was 88.9%, with a specificity of 95.2% (optimal cutoff value of 0.511), and the area under the ROC curve (AUC) was 0.960 [95% CI (0.915, 1.000)]. The AUC of the combined prediction model for predicting pediatric weaning was greater than that of P/F ratio, DE-RSBI, DTF-RSBI and PCIS alone (Z values = 9.129, 2.061, 2.075, 8.326, $P < 0.05$).

Conclusions: In mechanically ventilated pediatric patients, the combined prediction model has better predictive value for weaning success compared to using P/F ratio, DE-RSBI, DTF-RSBI, or PCIS alone.

KEYWORDS

mechanical ventilation, diaphragm ultrasonography, weaning, logistic regression, ROC curve

Introduction

In the Pediatric Intensive Care Unit (PICU), mechanical ventilation is a commonly used life-support intervention for children with respiratory failure (1). However, prolonged mechanical ventilation can lead to pulmonary complications such as ventilator-associated pneumonia and lung injury (2, 3). Therefore, safely and timely weaning from mechanical ventilation poses a significant challenge for clinical pediatricians. In fact, the decision to wean involves considering multiple factors, including oxygenation status, respiratory drive, and respiratory muscle function.

Currently, decisions regarding weaning are primarily based on laboratory test results and clinical assessments, such as blood gas analysis and spontaneous breathing trials. In recent years, the use of diaphragmatic ultrasound has become increasingly widespread and is considered a non-invasive, real-time tool for assessing diaphragm function. Studies have shown that diaphragmatic ultrasound can predict the recovery of diaphragm function and weaning outcomes in patients (4). However, the sensitivity and specificity of single indicators for predicting weaning are less than ideal (5). Therefore, there is a clinical need for an effective, rapid, and straightforward method for predicting weaning.

The aim of this study is to apply logistic regression to construct a combined prediction model and investigate its clinical value in weaning prediction.

Materials and methods

Study population

Sixty pre-school-aged patients underging mechanical ventilation in the PICU at the Second Affiliated Hospital of Jiaxing University from July 2022 to June 2023 were selected for this study. They were categorized into the weaning success group and weaning failure group based on the outcome of the extubation process. Informed consent was obtained from all guardians. Inclusion criteria: Invasive mechanical ventilation for more than 48 h; Meeting the Berlin criteria for acute respiratory distress syndrome, with an P/F ratio <300 mm Hg (6). Exclusion criteria: Patients with nasal intubation, non-invasive ventilation, or tracheostomy; Unplanned extubation (including accidental or self-extubation); History of diaphragmatic paralysis, chest trauma, cervical spinal cord injury, neuromuscular junction disorders, pneumothorax, and mediastinal emphysema; End-stage cancer patients; Presence of mediastinal emphysema, pneumothorax, or closed chest drainage.

Study methods

After significant improvement in the patient's condition, a Spontaneous Breathing Trial (SBT) was conducted using a low-pressure spontaneous breathing mode for weaning. The ventilation mode was changed to Pressure Support Ventilation (PSV), with a pressure support level $\leq$7 cm H_2O with or without Positive End-expiratory Pressure (PEEP) $\leq$ 5 cm H_2O. Oxygen concentration was maintained, and SBT was initiated after suctioning. The SBT duration was 120 min, and successful weaning was defined as the patient maintaining spontaneous breathing without the need for reintubation or non-invasive ventilation within 48 h after extubation. Weaning failure was defined as reintubation, advanced oxygen therapy, or death within 48 h after extubation. The criteria for SBT failure were based on the 2009 standards of the American Pediatric Critical Care Research Group (7).

General information recording

Record general data for both groups, including age, duration of illness, duration of mechanical ventilation, heart rate (HR), and respiratory rate (RR), the specific situation of the weaning failure group were recorded; PaO_2/FiO_2 Ratio (P/F ratio): Arterial blood gas analysis was performed to obtain PaO_2 and FiO_2, and P/F ratio was calculated as PaO_2/FiO_2; Pediatric Critical Illness Score (PCIS):PCIS was assessed in preparation for weaning.

Observation indicators and methods for diaphragmatic ultrasound before SBT

Diaphragmatic Excursion (DE): The patient's head was elevated by 20°–40°, and an edge ultrasound machine (Sonosite, USA) with a 10 MHz linear array probe was placed at the junction of the mid-clavicular line or anterior axillary line and the lower border of the rib cage. The liver was used as a window, and the probe was directed towards the head and back to visualize the lower third of the diaphragm. DE (cm) was calculated as the distance from the baseline to the diaphragm at the end of inhalation minus the distance at the end of exhalation.

Diaphragmatic Thickening Fraction (DTF): Using the M-mode, the right diaphragm was continuously observed in the 8–10 intercostal space in the right mid-axillary line. Diaphragmatic thickness at end-inspiration (DTi) and end-expiration (DTe) were measured, and DTF (%) was calculated as (DTi - DTe)/DTe * 100%. The average DTF was calculated over 3–5 respiratory cycles for each patient.

Diaphragmatic Excursion - Rapid Shallow Breathing Index (DE-RSBI) and Diaphragmatic Thickening Fraction - Rapid Shallow Breathing Index (DTF-RSBI): DE-RSBI was calculated as RR/DE, and DTF-RSBI was calculated as RR/DTF.

Statistical methods

Statistical analysis was conducted using SPSS 21.0 (IBM, Armonk, NY, USA). Normally distributed continuous data were presented as means ± standard deviation. Independent sample *t*-tests were employed for group comparisons. Count data were expressed as frequencies and percentages, and group comparisons were performed using the χ^2 test or Fisher's exact test.

Single-factor analysis was conducted for two groups. Indicators with statistically significant differences in single-factor analysis were treated as independent variables, and successful weaning was treated as the dependent variable in logistic regression. First, regression applicability conditions were verified (linearity between continuous independent variables and log-transformed dependent variables, and the presence of multicollinearity between independent variables). After verification, univariate receiver operating characteristic curves (ROC) were plotted for successful weaning, with the optimal cutoff value used as the threshold for binary classification. Subsequently, multivariate logistic stepwise regression was conducted to construct a joint prediction model (variable selection criteria set at entry = 0.10, removal = 0.15), with outliers removed and re-regression performed if necessary. Regression coefficient (b) testing, model goodness-of-fit testing using the Hosmer-Lemeshow test ($P > 0.05$ indicating good fit), and odds ratio (OR) analysis were conducted.

The Box-Tidwell test was utilized to assess the linear relationship between continuous univariate and log-transformed dependent variables. Linear regression was employed to ensure tolerance >0.1 or variance inflation factor <10, indicating no multicollinearity. Outliers were defined as values exceeding 3 times the standard deviation. The Wald test evaluated regression coefficients (b). The predictive value of weaning success was evaluated using Receiver Operating Characteristic (ROC) curves, with the area under the ROC curve (AUC) compared using the non-parametric Delong method. Statistical significance was set at $P < 0.05$.

Results

Univariate analysis of factors affecting weaning success and failure

This study included a total of 60 pediatric patients, among whom 18 experienced weaning failure. Weaning failure cases comprised: reconnected to mechanical ventilation after spontaneous breathing trial ($n = 12$), non-invasive ventilation within 48 h ($n = 2$), and reintubation within 48 h ($n = 4$). There were no statistically significant differences ($P > 0.05$) between the weaning success and failure groups in terms of disease duration, mechanical ventilation time, age, HR and MAC. However, significant differences were observed ($P < 0.05$) in P/F ratio, DE, DE-RSBI, DTF DTF-RSBI, and PCIS between the two groups, as shown in Table 1.

Collinearity diagnosis and multivariate binary logistic regression

There was a linear relationship between continuous independent variables and the log-transformed values of the dependent variable. Among the independent variables, there was multicollinearity between DE and DE-RSBI, as well as between DTF and DTF-RSBI. DE-RSBI and DTF-RSBI were calculated based on DE and DTF, respectively, so DE and DTF were excluded for further analysis. There were no outliers. Logistic stepwise regression analysis showed that P/F ratio, DE-RSBI, DTF-RSBI, and PCIS were independently associated with weaning success. The combined predictive model showed a high goodness of fit ($P = 0.967$), and all regression coefficients (b) were statistically significant. The model equation is expressed as Logit$P$ = 73.299–0.253 P/F ratio + 0.525 DE-RSBI + 0.055 DTF-RSBI-0.43 PCIS, as shown in Table 2 and Table 3.

Predictive performance of P/F ratio, DE-RSBI, DTF-RSBI, PCIS, and the combined prediction model for weaning

In the weaning success group, P/F ratio, DE-RSBI, DTF-RSBI, PCIS, and the combined predictive model had sensitivities and specificities of 76.2%, 88.9%, 88.9%, 69.0%, 89.7%, and 66.7%, 81.0%, 85.7%, 66.7%, 95.2%, respectively. The AUC values for P/F ratio, DE-RSBI, DTF-RSBI, PCIS, and the combined prediction model were 0.749, 0.853, 0.880, 0.700, and 0.960, respectively. The AUC of the combined prediction model was significantly greater than the AUC of each single indicator ($Z = 9.129$, 2.061, 2.075, 8.326, $P < 0.05$), as shown in Table 4 and Figure 1. The nomogram of the combined prediction model is shown in Figure 2.

Discussion

According to relevant studies (8, 9), approximately 30% of children in the PICU require mechanical ventilation treatment for approximately one week, and 20%–23% of mechanically ventilated children may face the risk of weaning failure. Weaning failure has been a hot topic and a challenging issue for PICU physicians. Premature weaning may increase the burden on the respiratory and cardiovascular systems of children, leading to weaning failure and is an independent risk factor for increased mortality in mechanically ventilated children. However, unnecessarily prolonging mechanical ventilation not only increases healthcare costs but also elevates the risk of ventilator-associated complications (10, 11). Therefore, by analyzing factors influencing weaning and exploring reasonable predictors of weaning, clinicians can better determine the timing of weaning

10.3389/fped.2024.1344709

TABLE 1 Univariate analysis of factors affecting the success and failure of aircraft retrieval.

Group	Number	Duration ($\bar{x} \pm s$, days)	Mechanical ventilation time ($\bar{x} \pm s$, days)	Age ($\bar{x} \pm s$, years)	P/F ratio ($\bar{x} \pm s$, mm Hg)	HR ($\bar{x} \pm s$, 次/min)	MAC ($\bar{x} \pm s$, mm Hg)	DE ($\bar{x} \pm s$, dm)	DE-RSBI [$\bar{x} \pm s$, beats/(min-dm)]	DTF ($\bar{x} \pm s$)	DTF-RSBI ($\bar{x} \pm s$, beats/min)	PCIS ($\bar{x} \pm s$, scores)
Weaning success group	42	10.86 ± 3.95	8.64 ± 3.28	3.64 ± 2.28	203.81 ± 10.82	105.14 ± 12.68	67.45 ± 9.01	1.45 ± 0.26	16.88 ± 2.71	0.31 ± 0.05	82.95 ± 20.66	92.90 ± 3.00
Weaning failure group	18	10.6 ± 4.57	9.00 ± 4.39	2.67 ± 2.09	196.33 ± 9.48	103.78 ± 89.48	64.89 ± 9.29	1.02 ± 0.11	21.54 ± 2.76	0.21 ± 0.02	109.34 ± 18.10	90.33 ± 3.96
t value		0.211	−0.348	1.556	2.743	0.409	1.001	6.864	−6.06	7.559	−4.698	2.757
P value		0.834	0.729	0.125	0.008	0.684	0.321	<0.001	<0.001	<0.001	<0.001	0.008

and improve its success rate, ultimately enhancing healthcare quality and reducing resource wastage.

Studies have shown a close association between diaphragmatic injury caused by mechanical ventilation and weaning failure (12, 13). Diaphragmatic ultrasound is an effective tool to assist physicians in assessing diaphragmatic injury. Parameters such as DE and DTF are highly correlated with diaphragmatic function assessment and can be used to predict weaning outcomes. Research by En-Pei Leefound a significant decrease in DE and DTF within the first 24 h of mechanical ventilation in 31 pediatric patients (14). After extubation, there was a significant difference in DTF between successful and unsuccessful extubation groups, with DTF <17% showing a correlation with extubation failure. Mistri S found that children experience progressive diaphragmatic atrophy after mechanical ventilation (15). Yang discovered that there was no statistically significant difference in DE between the weaning success and failure groups, but DTF, as a predictive indicator, had a sensitivity of 82%, specificity of 81%, and an AUC of 0.89, making it a good predictor (16). Research by Fossat G found that the ratio of RSBI and RSBI/DE could not predict weaning success, but combining ultrasound DE and DTF as predictive indicators might be worth further investigation (17). The study by Song J revealed that the sensitivity of DE-RSBI is 89.2%, specificity is 56.9%, with an AUC of 0.813.While DTF-RSBI shows a sensitivity of 67.6%, specificity of 93.2%, with an AUC of 0.859 (18). Despite the limited research on the ultrasound indicators of diaphragm function, DE-RSBI and DTF-RSBI, in predicting extubation outcomes in mechanically ventilated pediatric patients, existing studies have confirmed that high DE-RSBI and DTF-RSBI are independent risk factors for extubation failure in adults (19). DE-RSBI and DTF-RSBI have also been shown to have diagnostic efficacy superior to a single rapid shallow breathing index. These studies confirm the feasibility of using DE-RSBI and DTF-RSBI to predict extubation outcomes, providing a theoretical basis for our research. Our study further demonstrated that although DE and DTF are independent risk factors for extubation failure, they exhibit significant collinearity with DE-RSBI and DTF-RSBI. Therefore, our study retained DE-RSBI and DTF-RSBI as independent risk factors for extubation failure and conducted ROC curve analysis, with DE-RSBI showing a sensitivity of 88.9%, specificity of 81.0%, and an AUC of 0.853, and DTF-RSBI showing a sensitivity of 88.9%, specificity of 85.7%, and an AUC of 0.88. Importantly, the cutoff values for DE-RSBI and DTF-RSBI in children are significantly higher than in adults due to the higher respiratory rate in children and lower DE and DTF compared to adults. Therefore, ultrasound measurement of diaphragm parameters DE-RSBI and DTF-RSBI can provide guidance for the selection of the optimal timing for clinical extubation, thus improving the prognosis of pediatric patients.

Dynamic monitoring of the P/F ratio can effectively reflect the body's oxygen deficiency status and pulmonary ventilation and gas exchange function, making it widely used in clinical practice (20). The study by Sunitha Palanidurai found that when PEEP is greater than 5 mmHg, the area under the ROC curve for predicting extubation outcomes using the P/F ratio is 0.659. When using 135 mmHg as the threshold, the sensitivity and specificity for

TABLE 2 Logistic regression univariate analysis of independent variables and logit transformation of dependent variables, and multicollinearity test among independent variables.

Independent variable	Linearity between independent variable and logit-transformed dependent variable		Multicollinearity among independent variables	
	Wald value	*P* value	Tolerance	Variance inflation factor
P/F ratio	0	0.99	0.852	1.174
DE-RSBI	0.183	0.669	0.75	1.333
DTF-RSBI	0.943	0.331	0.727	1.376
PCIS	0.698	0.403	0.857	1.167

TABLE 3 Multivariate binary logistic regression analysis.

Variable	*B*	*SE*	Wald χ^2 value	*P* value	OR value (95% CI)
P/F ratio	−0.253	0.1	6.430	0.011	0.777 (0.639, 0.944)
DE-RSBI	0.525	0.187	7.846	0.005	1.691 (1.171, 2.441)
DTF-RSBI	0.055	0.027	4.078	0.043	1.056 (1.002, 1.114)
PCIS	−0.43	0.205	4.392	0.036	0.650 (0.435, 0.972)

predicting extubation are 59.5% and 65.5%, respectively (21). However, this study found that P/F ratio had a sensitivity and specificity of 76.2% and 66.7%, respectively, with an AUC of 0.749, which is slightly lower than the previous results. This discrepancy may be due to differences in the study population. Combined with the common occurrence of multi-organ dysfunction in critically ill children with ARDS, using P/F ratio as a single predictor of weaning outcomes may lack sensitivity and specificity.

The PCIS is a scoring system used to assess the severity of illness in children. It helps physicians accurately assess the child's condition, formulate treatment plans, and effectively prevent and treat complications, thereby improving the child's prognosis. Lower PCIS indicate a more severe condition and may be associated with a higher risk and greater degree of multi-organ damage. Multi-organ dysfunction is a major cause of death in critically ill children, and PCIS can reflect the degree of organ damage and the risk of death to varying degrees. Fang C found that as the PCIS upon admission decreased, the proportion of mechanically ventilated children and the duration of mechanical ventilation increased (22). Critically ill children upon admission have a higher likelihood of respiratory system involvement and an increased likelihood of requiring mechanical ventilation due to hypoxia. However, this study found that PCIS had limited accuracy as a single predictor, with a sensitivity and specificity of 69.0% and 66.7%, respectively, and an AUC of 0.7.

Compared to predicting outcomes based on a single indicator, combined prediction can improve sensitivity and specificity to some extent. This study's logistic regression analysis revealed that P/F ratio, DE-RSBI, DTF-RSBI, and PCIS were all independent risk factors for pediatric mechanical ventilation weaning. The combined prediction model, P/F ratio, DE-RSBI, DTF-RSBI, and PCIS, all demonstrated a certain predictive performance for weaning success. The combined indicator's sensitivity for predicting pediatric mechanical ventilation weaning success was 88.9%, with a specificity of 95.2% and an AUC of 0.960. Compared to using P/F ratio, DE-RSBI, DTF-RSBI, or PCIS individually, the combined prediction model showed higher predictive capabilities, surpassing the overall diagnostic performance when any single indicator was used. It can be widely applied in clinical practice to facilitate timely and accurate weaning, reducing the risk of severe weaning-related complications.

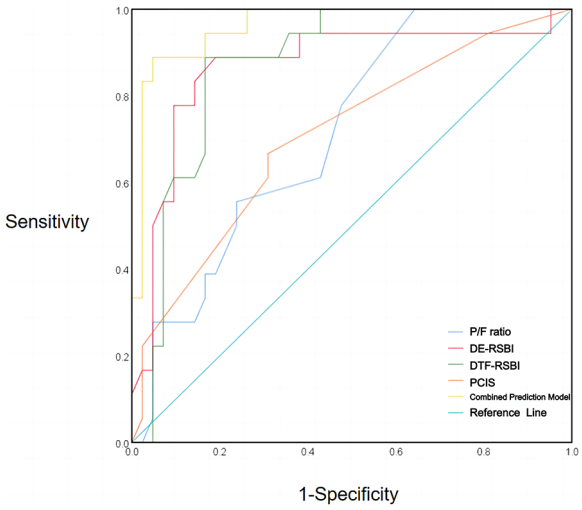


FIGURE 1
ROC curves of P/F ratio, DE-RSBI, DTF-RSBI, and PCIS for extubation prediction alone and in combination.

TABLE 4 JP/F rationt predictive performance of the P/F ratio, DE-RSBI, DTF-RSBI, PCIS, and the combined predictive model for aircraft retrieval.

Index	AUC	95% CI	Sensitivity (%)	Specificity (%)	Cut off value	*P* value
P/F ratio	0.749	(0.624, 0.875)	76.2	66.7	199.5	<0.01
DE-RSBI	0.853	(0.735, 0.970)	88.9	81.0	19.55	<0.01
DTF-RSBI	0.880	(0.792, 0.968)	88.9	85.7	90.00	<0.01
PCIS	0.700	(0.554, 0.846)	69.0	66.7	93.0	0.015
Combined predictive model	0.960	(0.915, 1.000)	88.9	95.2	0.511	<0.01

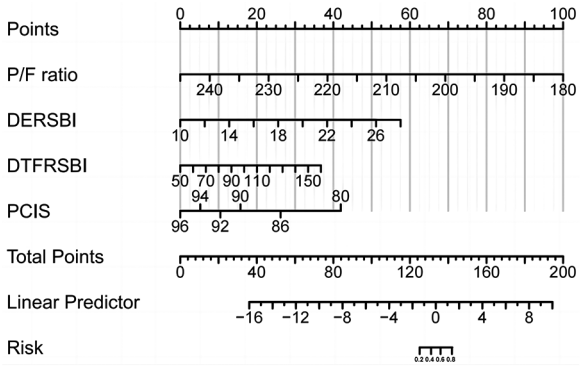


FIGURE 2
Risk nomogram conducted by logistic regression.

Identifying factors affecting weaning outcomes via diaphragm ultrasound is crucial, yet causal relationships are often unknown. Causal inference methods can illuminate these connections, aiding clinical management (23). Predictive models highlight factors like clinical characteristics and disease status, but fail to establish causality. Causal inference involves accounting for confounders like underlying diseases and treatments to ensure accuracy. Challenges include temporal precedence and unobserved confounders, addressed with advanced statistical methods. In further research, causal inference will be conducted, as it provides deeper insights, guiding treatment decisions and resource allocation, thus significantly impacting clinical practice. Additionally, multicenter studies and external validation will be conducted.

Conclusions

P/F ratio, DE-RSBI, DTF-RSBI, and PCIS are independent risk factors for pediatric weaning from mechanical ventilation. While individual diaphragmatic ultrasound parameters hold value, a combined prediction model using P/F ratio, DE-RSBI, DTF-RSBI, and PCIS offers higher predictive value for pediatric weaning.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

HG: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing, Software. AZ: Investigation, Methodology, Software, Writing – original draft. YT: Methodology, Project administration, Resources, Validation, Visualization, Writing – review & editing. LH: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article.

This study was funded by Zhejiang Provincial Department of Education General Research Project (Y202249915); Jiaxing Science and Technology Plan Project (2021AD30107).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Colleti J Jr, Azevedo RT, de Oliveira Caino FR, de Araujo OR. Prolonged mechanical ventilation in children: review of the definition. *Pediatr Crit Care Med.* (2021) 22(11):e588–e93. doi: 10.1097/PCC.0000000000002773

2. Girona-Alarcon M, Bobillo-Perez S, Sole-Ribalta A, Cuadras D, Guitart C, Balaguer M, et al. Risk score for developing ventilator-associated pneumonia in children: the RISVAP study. *Pediatr Pulmonol.* (2022) 57(7):1635–42. doi: 10.1002/ppul.25929

3. Wang XX, Sha XL, Li YL, Li CL, Chen SH, Wang JJ, et al. Lung injury induced by short-term mechanical ventilation with hyperoxia and its mitigation by deferoxamine in rats. *BMC Anesthesiol.* (2020) 20(1):188. doi: 10.1186/s12871-020-01089-5

4. Sik N, Citlenbik H, Ozturk A, Yilmaz D, Duman M. Point of care diaphragm ultrasound: an objective tool to predict the severity of pneumonia and outcomes in children. *Pediatr Pulmonol*. (2021) 56(6):1666–72. doi: 10.1002/ppul.25352

5. Baptistella AR, Sarmento FJ, da Silva KR, Baptistella SF, Taglietti M, Zuquello RA, et al. Predictive factors of weaning from mechanical ventilation and extubation outcome: a systematic review. *J Crit Care*. (2018) 48:56–62. doi: 10.1016/j.jcrc.2018.08.023

6. Papazian L, Aubron C, Brochard L, Chiche JD, Combes A, Dreyfuss D, et al. Formal guidelines: management of acute respiratory distress syndrome. *Ann Intensive Care*. (2019) 9(1):69. doi: 10.1186/s13613-019-0540-9

7. Elisa P, Francesca C, Marco P, Davide V, Laura Z, Fabrizio Z, et al. Ventilation weaning and extubation readiness in children in pediatric intensive care unit: a review. *Front Pediatr*. (2022) 10:867739. doi: 10.3389/fped.2022.867739

8. Shah AJ, Wai K, Sharron MP, Mize M, Cohen J, Basu S. Diaphragmatic thickening fraction by ultrasound in mechanically ventilated pediatric patients: pilot observations during spontaneous breathing trials. *J Ultrasound Med*. (2022) 41(12):3043–50. doi: 10.1002/jum.16035

9. Charernjiratragul K, Saelim K, Ruangnapa K, Sirianansopa K, Prasertsan P, Anuntaseree W. Predictive parameters and model for extubation outcome in pediatric patients. *Front Pediatr*. (2023) 11:1151068. doi: 10.3389/fped.2023.1151068

10. Kilba MF, Salie S, Morrow BM. Risk factors and outcomes of extubation failure in a South African tertiary paediatric intensive care unit. *South Afr J Crit Care*. (2022) 38(1):26–32. doi: 10.7196/SAJCC.2022.v38i1.513

11. Chongcharoenyanon T, Samransamruajkit R, Sophonphan J. Corrigendum: epidemiology, risk factors and outcomes of prolonged mechanical ventilation with different cut-points in a picu. *Front Pediatr*. (2023) 11:1237299. doi: 10.3389/fped.2023.1237299

12. Zhang Z, Tao J, Cai X, Huang L, Liu C, Ren H, et al. Clinical characteristics and outcomes of children with prolonged mechanical ventilation in picus in mainland China: a national survey. *Pediatr Pulmonol*. (2023) 58(5):1401–10. doi: 10.1002/ppul.26332

13. Dionisio MT, Rebelo A, Pinto C, Carvalho L, Neves JF. Ultrasound assessment of ventilator-induced diaphragmatic dysfunction in paediatrics. *Acta Med Port*. (2019) 32(7-8):520–8. doi: 10.20344/amp.10830

14. Lee EP, Hsia SH, Hsiao HF, Chen MC, Lin JJ, Chan OW, et al. Evaluation of diaphragmatic function in mechanically ventilated children: an ultrasound study. *PLoS One*. (2017) 12(8):e0183560. doi: 10.1371/journal.pone.0183560

15. Mistri S, Dhochak N, Jana M, Jat KR, Sankar J, Kabra SK, et al. Diaphragmatic atrophy and dysfunction in critically ill mechanically ventilated children. *Pediatr Pulmonol*. (2020) 55(12):3457–64. doi: 10.1002/ppul.25076

16. Xue Y, Zhang Z, Sheng CQ, Li YM, Jia FY. The predictive value of diaphragm ultrasound for weaning outcomes in critically ill children. *BMC Pulm Med*. (2019) 19(1):270. doi: 10.1186/s12890-019-1034-0

17. Fossat G, Daillet B, Desmalles E, Boulain T. Does diaphragm ultrasound improve the rapid shallow breathing Index accuracy for predicting the success of weaning from mechanical ventilation? *Aust Crit Care*. (2022) 35(3):233–40. doi: 10.1016/j.aucc.2021.05.008

18. Song J, Qian Z, Zhang H, Wang M, Yu Y, Ye C, et al. Diaphragmatic ultrasonography-based rapid shallow breathing index for predicting weaning outcome during a pressure support ventilation spontaneous breathing trial. *BMC Pulm Med*. (2022) 22(1):337. doi: 10.1186/s12890-022-02133-5

19. Spadaro S, Grasso S, Mauri T, Dalla Corte F, Alvisi V, Ragazzi R, et al. Can diaphragmatic ultrasonography performed during the T-tube trial predict weaning failure? The role of diaphragmatic rapid shallow breathing Index. *Crit Care*. (2016) 20(1):305. doi: 10.1186/s13054-016-1479-y

20. Muniraman HK, Song AY, Ramanathan R, Fletcher KL, Kibe R, Ding L, et al. Evaluation of oxygen saturation index compared with oxygenation Index in neonates with hypoxemic respiratory failure. *JAMA Netw Open*. (2019) 2(3):e191179. doi: 10.1001/jamanetworkopen.2019.1179

21. Palanidurai S, Phua J, Chan YH, Mukhopadhyay A. P/FP ratio: incorporation of peep into the pao(2)/fio(2) ratio for prognostication and classification of acute respiratory distress syndrome. *Ann Intensive Care*. (2021) 11(1):124. doi: 10.1186/s13613-021-00908-3

22. Fang C, Mao Y, Jiang M, Yin W. Pediatric critical illness score, clinical characteristics and comprehensive treatment of children with severe Mycoplasma Pneumoniae pneumonia. *Front Surg*. (2022) 9:897550. doi: 10.3389/fsurg.2022.897550

23. Zhang Z, Jin P, Feng M, Yang J, Huang JJ, Chen L, et al. Causal inference with marginal structural modeling for longitudinal data in laparoscopic surgery: a technical note. *Laparosc Endosc Robot Surg*. (2022) 5(4):146–52. doi: 10. 1016/j.lers.2022.10.002

frontiers | Frontiers in Pediatrics

TYPE Case Report
PUBLISHED 10 July 2024
DOI 10.3389/fped.2024.1404872

OPEN ACCESS

EDITED BY
Rujipat Samransamruajkit,
Chulalongkorn University, Thailand

REVIEWED BY
Clemax Couto Sant'Anna,
Federal University of Rio de Janeiro, Brazil
Jonathan Soldera,
University of Caxias do Sul, Brazil

*CORRESPONDENCE
Li-Li Luo
439614013@qq.com
Li-Na Qiao
qiaolina@scu.edu.cn

†These authors have contributed equally to this work and share last authorship

RECEIVED 21 March 2024
ACCEPTED 06 June 2024
PUBLISHED 10 July 2024

CITATION
Yang M, Liu Z-Q, Wang Y, Luo L-L and Qiao L-N (2024) Successful treatment of diffuse alveolar hemorrhage secondary to *Mycoplasma pneumoniae* complicated with hemophagocytic lymphohistiocytosis in children: a case report and non-systematic literature review.
Front. Pediatr. 12:1404872.
doi: 10.3389/fped.2024.1404872

Successful treatment of diffuse alveolar hemorrhage secondary to *Mycoplasma pneumoniae* complicated with hemophagocytic lymphohistiocytosis in children: a case report and non-systematic literature review

Min Yang[1,2,3], Zhong-Qiang Liu[1,2,3], Yang Wang[1,2,3], Li-Li Luo[1,2,3*†] and Li-Na Qiao[1,2,3*†]

[1]Department of Pediatrics, West China Second University Hospital, Sichuan University, Chengdu, China, [2]Key Laboratory of Birth Defects and Related Diseases of Women and Children of Sichuan University, Ministry of Education, Chengdu, Sichuan, China, [3]NHC Key Laboratory of Chronobiology, Sichuan University, Chengdu, China

Background: After quarantine-related measures were completely lifted in China, the respiratory infection rate of children caused by *Mycoplasma pneumoniae* (MP) increased significantly, and MP infection may lead to rare severe intra- and extrapulmonary manifestation. Hemophagocytic lymphohistiocytosis (HLH) and diffuse alveolar hemorrhage (DAH) are life-threatening clinical syndromes. Timely recognition may contribute to timely treatment and an improved prognosis. Currently there are no reports of children with DAH secondary to MP infection complicated with HLH.

Case presentation: We successfully treated a previously healthy school-aged child who was admitted to the pediatric intensive care unit with fever, cough, drowsiness, and progressive dyspnea. HLH was confirmed by clinical and testing criteria, DAH was indicated by computed tomography scan of the chest, and *Mycoplasma* antibody detection and endotracheal aspirates pathogen metagenomic next-generation sequencing (mNGS) confirmed MP infection. After invasive mechanical ventilation, antibiotics, and glucocorticoid treatment, the patient recovered well and was discharged. At follow-up, she did not experience any more initial symptoms. For the fourth consecutive month, all indexes remained normal.

Conclusion: mNGS can be considered for identifying the causative agent of infection in patients with DAH and/or HLH. The clinical manifestations of DAH in children may only present as acute hypoxic respiratory failure, significantly decreased hemoglobin without bleeding elsewhere, and chest imaging findings may assist in the diagnosis of DAH. When MP infection is associated with hemocytopenia, HLH should be considered.

KEYWORDS

diffuse alveolar hemorrhage, hemophagocytic lymphohistiocytosis, *Mycoplasma pneumoniae*, children, case report

Introduction

Mycoplasma pneumoniae (MP) is a major pathogen of respiratory infection in school-aged children in China, which can lead to abnormal clinical intra- and extrapulmonary manifestation through direct injury and abnormal immune response of the host. As a potentially fatal disease, MP-hemophagocytic lymphohistiocytosis (HLH) is a form of related hematological manifestation induced by MP through immune dysregulation. Only a few cases were reported before the global quarantine-related measures were lifted (1–4). After the quarantine-related measures were lifted, no relevant reports were currently reported through PubMed, Web of Science, Embase, Medline, and other databases. Diffuse alveolar hemorrhage (DAH) is a potentially life-threatening syndrome with a poor overall prognosis (5). Through the above database search, only one case of DAH secondary to MP has been reported to date (6).

Here, we reported a rare case of DAH secondary to MP-HLH in children. In addition, the literature in this paper was also reviewed to provide clues for early identification of rare severe intra- and extrapulmonary manifestation, so as to timely intervene and improve the prognosis.

Case presentation

A girl, aged 9 years and 5 months, was admitted to the Pediatric Intensive Care Unit (PICU) of West China Second Hospital of Sichuan University in November 2023. Her chief complaint was "fever for 5 days, cough and drowsiness for 3 days, and shortness of breath for half a day." Only fever with no other symptoms occurred on the first and second days, with the highest temperature of 38.5°C (101.3°F) on day 1 and 39.5°C (103.1°F) on day 2, and was treated with oral antipyresants. The fever persisted, and cough and drowsiness occurred on day 3. After emergency treatment with cefoperazone sodium sulbactam for anti-infection, glutathione for liver protection, and gamma globulin (12.5 g, 0.5 g/kg) support on days 4 and 5, there was no improvement, and shortness of breath (56 breaths/min) occurred on day 5. The patient was previously healthy without family history, and no family members had a similar medical history. On admission, physical examination revealed poor mental response and slight irritability, a respiratory rate of 53 breaths/min, heart rate of 134 beats/min, pulse oxygen saturation (SPO_2) of 90% without oxygen inhalation, slight edema of bilateral eyelids, nares flaring, three depression sign was positive, no rales heard in both lungs, and no abnormalities found in physical examination of the heart, abdomen, and nervous system. A routine blood test showed thrombocytopenia [platelets (PLT) 69,000/μl] and anemia [hemoglobin (Hb) 9.8 g/dl]. The decrease was 1.9 g/dl within 24 h, white blood cells (WBC) were decreased (3,500/μl, neutrophil count 1,870/μl). Serum C-reactive protein (CRP) was 119.8 mg/L, liver enzymes were elevated [aspartate aminotransferase (AST) 241 U/L; alanine aminotransferase (ALT) 372 U/L], lactate dehydrogenase triglyceride (LDH) was 1378 IU/L, triglyceride (TG) was 3.01 mmol/L, and coagulation function screening was negative. A pharyngeal swab of molecular diagnostic testing was negative for MP. The patient was initially diagnosed with severe pneumonia with respiratory failure and suspected HLH.

Upon admission, the patient received a high-flow nasal cannula (HFNC) for assisted ventilation, meropenem (40 mg/kg, q8h, for 5 days) combined with oral azithromycin (10 mg/kg, qd, for 5 days, two courses) empiric antibiotic treatment. Five hours after admission, the patient's dyspnea did not improve with the HFNC parameters (FiO_2 60%, flow 8 L/min) and chest computed tomography (CT) revealed diffuse alveolar hemorrhage (Figure 1A). We used invasive ventilator-assisted ventilation after tracheal intubation (a few hemorrhagic substances were visible in the tracheal tube without bleeding in other parts), hemostatic treatment with plasma, ethylsulfonamide and platelet transfusion, and glucocorticoid therapy [methylprednisolone, intravenously guttae (ivgtt), 2 mg/kg/day for 5 days, 1 mg/kg/day, ivgtt, for 5 days; after that, oral prednisone was gradually reduced and discontinued] after bone marrow aspiration. After the treatment, the dyspnea was relieved (respiratory rate of 29 breaths/min, SPO_2 98%, no nares flaring, three depression sign was negative) and no hemorrhagic substance was visible in the tracheal tube. On the third day after admission, the MP antibody IgM (colloidal gold method, agglutination method) was positive, and the titer was >1:1280. Metagenomic next-generation sequencing (mNGS) of endotracheal aspirates was MP (amplified sequence number 98493, high confidence), which supported us to continue treatment of mycoplasma infection. On the fourth day after admission, the tracheal tube was removed and HFNC was used to assist ventilation. On the fifth day after admission, the patient's CRP returned to normal (7.2 mg/L), meropenem was discontinued and replaced with cefoperazone sodium and sulbactam sodium. However, the detection of fibrinogen was 1.76 g/L, D-dimer was significantly increased [>40 mg/L, fibrinogen equivalent units (FEU)], and we added a small dose of low molecular weight heparin to anticoagulant therapy. During the course of the disease, the bone marrow smear test revealed increased hemophagocytic macrophages, and the highest serum ferritin was 5,861.4 μg/L and the highest LDH was 2922 U/L (normal, 120–246 U/L). Cytokines were increased (soluble interleukin-2 receptor, IL-2, 2,888.1 U/ml, IL-8 46.2 pg/ml, IL-10 41.68 pg/ml, TNF-α 25.71 pg/ml). The patient had an H score of 236, with a 98%–99% probability of HLH (H score >169 had a sensitivity of 93% and specificity of 86% for the diagnosis of HLH) (7). Tests for Epstein–Barr virus (EBV) and cytomegalovirus (CMV) were negative. Therefore, we reconfirmed the consideration that HLH was secondary to MP and continued glucocorticoid therapy. On the 11th day after admission, the patient's body temperature was normal for 8 days, the CT lung window showed the lesions in both lungs were significantly reduced and faded (Figure 1B), and the patient was discharged. After 1 month of regular oral prednisone, she did not experience any more of those initial symptoms. At the 4-month follow-up, all indicators remained normal. The timeline of disease progression and treatment is summarized in Figure 2.

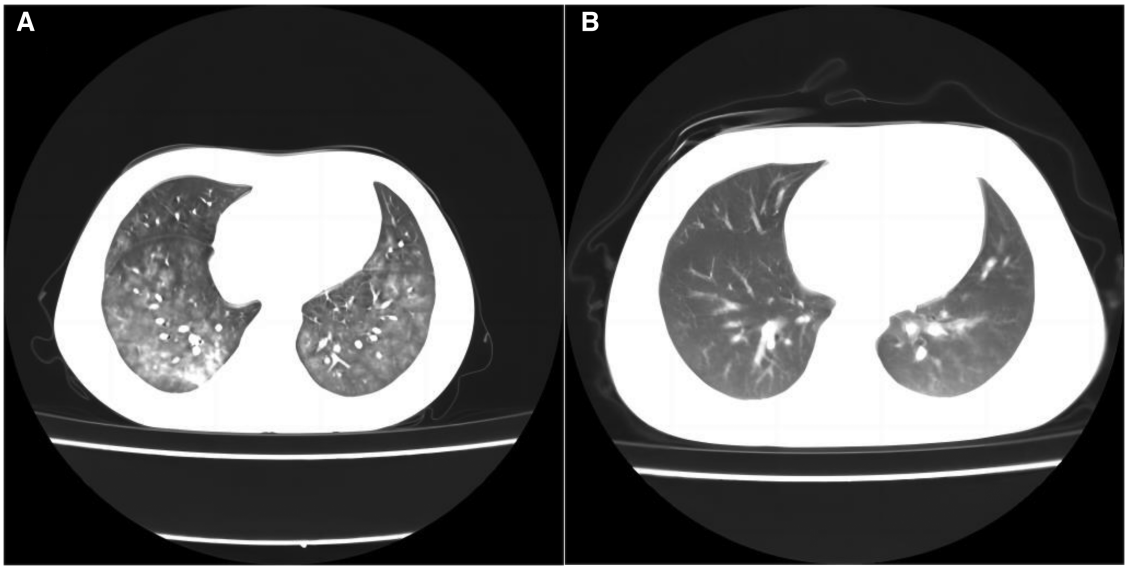


FIGURE 1
Chest CT scanning of the patient. CT lung window on admission showed diffuse small ground-glass opacities in both lungs with patchy opacities in the right lower lobe, and diffuse alveolar hemorrhage was considered (A). The CT lung window on the 11th day after admission showed a little inflammation in both lungs, and the lesions in both lungs were significantly reduced and faded (B).

System review

A review of the literature between 1998 and 2024 was undertaken using databases such as PubMed, Web of Science, Embase, and Medline. The search employed the keywords "diffuse alveolar hemorrhage," "hemophagocytic syndrome/hemophagocytic lymphohistiocytosis," "diffuse alveolar hemorrhage," "Mycoplasma pneumonia," and "Children." The relevant information was extracted from the selected articles, including the first author's name, year of publication, country of study, age range of patients, underlying disease, etiology, clinical manifestation, relevant examination, treatment, hospital length of stay, in-hospital mortality, cause of death, and follow-up time. In Table 1, we summarize the data of one published article, which revealed in detail the treatment of pediatric patients with MP-associated DAH (6). Seven published articles are summarized in Table 2, focusing on pediatric patients with HLH secondary to MP (1, 2, 4, 8–11).

Discussion

Currently, after the quarantine-related measures were lifted, there was a surge of endemic MP infections in children in countries such as the United States, Switzerland, Sweden, England, Slovenia, and China (12). MP is the most important pathogen of community-acquired pneumonia in children aged over 5 years in China and has even become one of the most important pathogens of respiratory infections (13, 14). As a pathogen without a cell wall, the infection rate of MP has again increased rapidly (15), which may affect the population who have not been exposed to MP in the past 3 years through the two main pathogenesis mechanisms of direct pathogen injury and abnormal host immune response, and lead to rare severe intra- and extrapulmonary manifestations (16). The most common intra- and extrapulmonary manifestations were plastic bronchitis, pulmonary embolism, necrotizing pneumonia, and acute attack of asthma. The main extrapulmonary manifestations were nervous system involvement and skin mucosal damage (14). Both DAH as an intrapulmonary manifestation of MP infection and/or HLH as an extrapulmonary manifestation are very rare in children. Children with infection-associated DAH have high mortality in the acute phase (4, 5, 11, 17). DAH is a clinical syndrome with a wide range of causes. Children with DAH often have complex, critical conditions and rapid progression, which can lead to rapid respiratory failure. The mortality rate of DAH in the acute phase was high, up to 75%, especially for DAH caused by infection (5, 17).

Typical clinical manifestations of DAH include hemoptysis (67%), anemia, and hypoxic respiratory failure (5, 18). Before the deadline, only one case of DAH secondary to MP infection has been reported in children (6). That patient was admitted with hypoxic respiratory failure. At 48 h after admission, she showed the above three symptoms and was treated with invasive ventilator respiratory support and veno-venous extracorporeal membrane oxygenation (VV-ECMO). During the course of the disease, she acquired a fungal infection. She was discharged successfully after 17 days in hospital and no abnormality was found after 45 days of follow-up. In our case, the child was also admitted with hypoxic respiratory failure. When dyspnea was not significantly relieved on the day of admission, the patient was changed to invasive ventilator respiratory support, and the time of tracheal intubation was not more than 4 days. There was no fungal infection during the course of the disease, and the patient

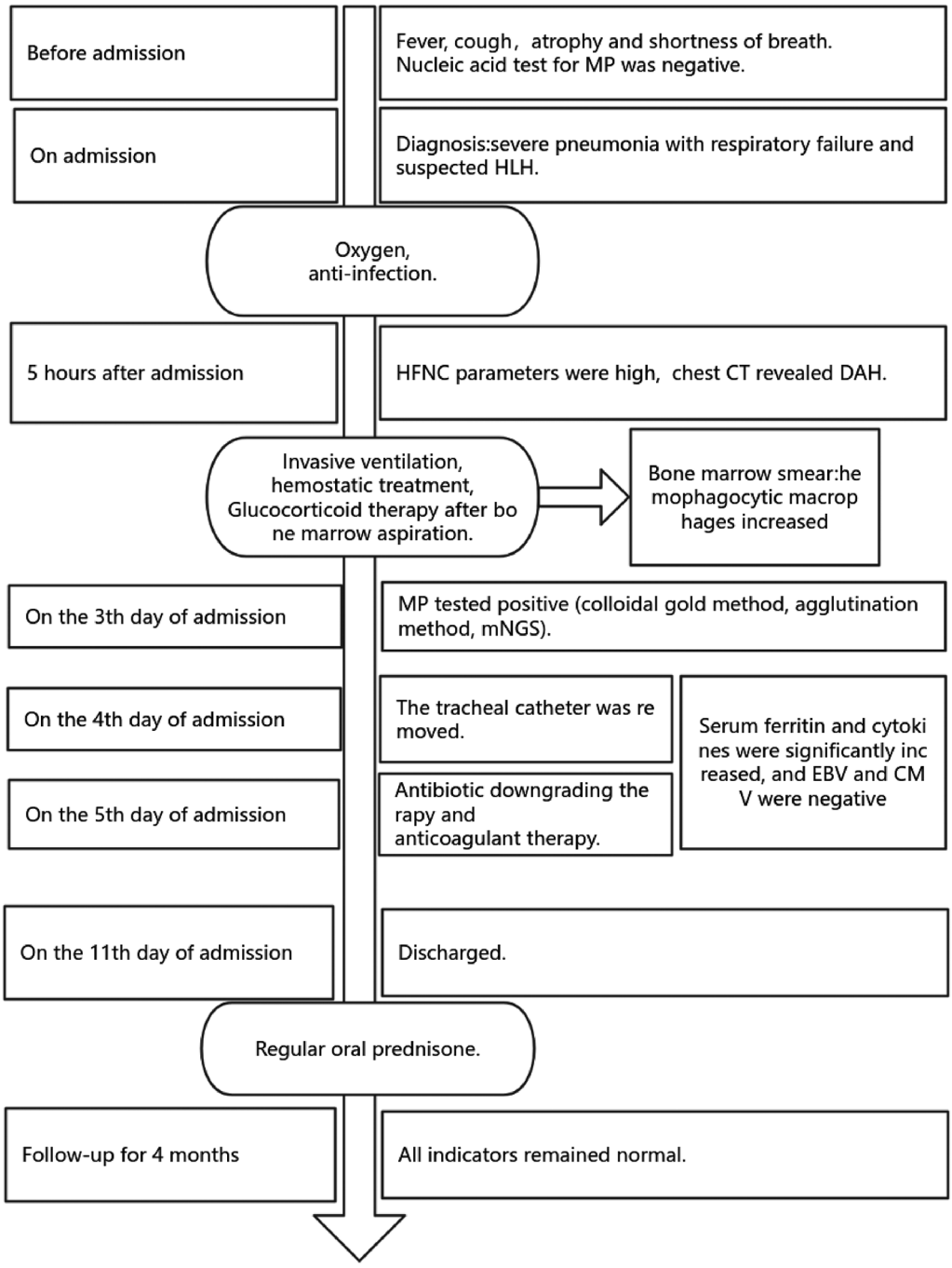


FIGURE 2
The timeline of disease progression and treatment.

was discharged after 10 days of hospitalization. The follow-up period was up to 4 months. This case may help identify atypical clinical manifestations of MP-DAH for timely recognition and management. Children with MP-DAH only showed hypoxic respiratory failure, which may be related to the developmental characteristics of children with incomplete cough reflex and respiratory muscle development and high airway resistance (half of the children had no symptoms of hemoptysis) (18). We believe that the absence of hemoptysis and anemia cannot be used as a basis for excluding DAH. The review published by Reisman et al. (18) also clearly suggests that chest X-rays in patients with DAH usually show alveolar opacity, CT may indicate the extent of the disease, and chest imaging may be used to assist in the diagnosis of DAH. Therefore, we consider that patients presenting only with hypoxic respiratory failure and significantly decreased Hb in the absence of bleeding elsewhere can be combined with chest imaging to assist the diagnosis of DAH.

TABLE 1 Published articles on pediatric cases of diffuse alveolar hemorrhage induced by *Mycoplasma pneumoniae* infection.

Author	Country, year	Male/ female	Age	Chief complaint	Underlying disease	Other complications	Etiology	Pathogen/ specimen/ method	Diagnostic method	Hospital length of stay at diagnosis	Treatment method	Hospital length of stay	In hospital mortality	Follow-up time
Xinjuan Zhang et al. (6)	China, 2022	0/1	8 years	Cough for 1week, fever for 4 days, and dyspnea for 5 h	None	ARDS	Infection	*Mycoplasma pneumoniae*/ pleural fluid and blood/mNGS	BAL	48 h	Azithromycin (3days × 2) and other antibiotics, Human blood immunoglobulin, epinephrine endotracheal instillation, glucocorticoids (1–2 mg/kg d, ivgtt, 14 days), fresh frozen plasma and fibrinogen infusions Mechanical ventilation after 48 h admission for 10days VV-ECMO after 3 days admission for 5 days	17 days	0.0%	45 days

ARDS, acute respiratory distress syndrome; mNGS, metagenomic next-generation sequencing; BAL, bronchoscopy and bronchoalveolar lavage; VV-ECMO, veno-venous extracorporeal membrane oxygenation; IPH, idiopathic pulmonary hemosiderosis; CT, computed tomography.

10.3389/fped.2024.1404872

TABLE 2 Published articles on pediatric cases of hemophagocytic lymphohistiocytosis induced by *Mycoplasma pneumoniae* infection.

Author	Country, year	Male/ female	Age	Clinical manifestation	Underlying disease	Other complications	Etiology	Pathogen/ specimen/ method	WBC (/μl)	HB (g/ dl)	PLT (/μl)	Serum C-reactive protein (mg/L)	AST (IU/L)	ALT (IU/L)	LDH (IU/L)	TG (mg/dl)	Ferritin (ng/ml)	IL-2R (U/ ml)	Fibrinogen (mg/dl)	Bone marrow aspiration	Course at diagnosis (days)	Treatment method	Hospital length of stay	In hospital mortality (%)	Cause of death	Follow-up time
Yasushi Ishida et al. (8)	Japan, 2004	1/1	11 years, 10 years	Fever (2), cough (2), and dyspnea (1)	None	None	Infection	MP/ pharyngeal swab/passive agglutination test	5,500; 3,500	13.1, 14.4	136,000–193,000	10.7, 3.8	484; 1,109	191; 866	3,327; 1,640	Undisclosed	19,620; 2,553	2,080; 2,056	Undisclosed	Hemophagocytic macrophages increased	11 and 6	CAM (1), CTM (1), EM (1), AZT (1); CFTM-PI, (1); MINO (2), prednisolone (2)	21 days, 11 days	0.0	None	10 years, 3 years
Megumi Yoshiyama et al. (1)	Japan, 2008	2/2	1–11 years	Fever (4), Hepatomegaly (2), splenomegaly (1)	Undisclosed	Consciousness disturbance, paralytic ileus	Infection	MP/serial serum samples/ particle agglutination method	3,800–8,300	7.7–13.7	57,000–131,000	0.14–10.9	Undisclosed	26–67	508–4,236	Undisclosed	1,070–36,050	3,002; >3,200 (2, none)	Undisclosed	Hemophagocytic macrophages increased	7–11	CAM (4),EM (4), MINO (4) IVIG (2), glucocorticoids (1)	Undisclosed	0.0	None	Undisclosed
Bruch LA et al. (9)	The USA, 2001	1/0	12 years	Cough, headache, photophobia, nausea, and vomiting	None	Meningoencephalitis	Infection	MP/serial serum samples/ particle agglutination method	25,700	12.9	551,000	Undisclosed	Undisclosed	Undisclosed	Undisclosed	Undisclosed	Undisclosed	Undisclosed	Undisclosed	Undisclosed	7s	Undisclosed	60 h	100.0	Brain death	None
Yuji Koike et al. (2)	Japan, 2013	0/1	7 years	Fever, cough, and malaise	None	None	Infection	MP/serum/ passive agglutination test	1,200	11.8	<10,000	6.27	48	28	921	434	2,251	Undisclosed	Undisclosed	None	4	Clarithromycin, prednisolone	11 days	0.0	None	More than 3 years
Lu Zhi-wei et al. (4)	China, 2014	2/1	1, 3, 6 years	Fever (3), cough (3), coma (1)	Undisclosed	Undisclosed	Infection	MP/BAL, pharyngeal swab/passive agglutination test and molecular diagnostic testing (3)	200–1,600	7.9–10.3	64,000–157,000	Undisclosed	Undisclosed	Undisclosed	1,170–1,285	Undisclosed	936.7–7,477	Undisclosed	130–180	Hemophagocytic macrophages increased (3)	2–30	AZT combined with other antibiotics (3), mechanical ventilation (2), IVIG (3), glucocorticoids (3), CyA + VP-16 (1) Fiber optic bronchoscope (3)	47, 20, 25 days	33.3	Intracranial hemorrhage	Undisclosed
Motoko Yasutomi, et al. (10)	Japan, 2016	1/0	3 years	Fever and cough	None	None	Infection	MP/serum/ passive agglutination test	4,800	11.9	116,000	59.4 (normal <3.2)	1,788	332	3,748	Undisclosed	7,718 (normal, 18.6–261)	2,686 (normal, 144.5–518)	143 (normal, 140–340)	Hemophagocytic macrophages increased	4	CAM, ABPC/ SBT, MEPM, and MINO, IVIG, 2 g/kg prednisolone, 2.6 mg/kg/d, 13 days	Undisclosed	Undisclosed	Undisclosed	Undisclosed
Gu Jia-li et al. (11)	China, 2020	6/5	7 months–9.5 years	Fever (11), cough (8), hepatomegaly (11)	None	Undisclosed	Infection	MP/BAL, pharyngeal swab/passive agglutination test (7) and molecular diagnostic testing (10)	Undisclosed	7.7 (median)	45,000 (median)	Undisclosed	Undisclosed	Undisclosed	1,285 (median)	4.64 (median) (9)	7,260 (median)	Undisclosed	126 (median) (6)	Hemophagocytic macrophages increased (11)	6–30	AZT combined with other antibiotics (11), mechanical ventilation (5), IVIG (10), glucocorticoids (10), CyA (6) + VP-16 (1), Fiber optic bronchoscope (7)	Undisclosed	18.2	Multiple organ failure	Undisclosed

MP, *Mycoplasma pneumoniae*; WBC, white blood cell count; HB, hemoglobin; PLT, platelets; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; TG, triglyceride; IL-2R, soluble interleukin-2 receptor; CTM, cefotiam; EM, erythromycin; AZT, azithromycin; CFTM-PI, cefteram pivoxil; IVIG, intravenous immunoglobulin; CAM, clarithromycin; ABPC/SBT, ampicillin/sulbactam; MEPM, meropenem; MINO, minocycline; BAL, bronchoscopy and bronchoalveolar lavage; CyA, cyclosporine A; VP-16, etoposide.

.

10.3389/fped.2024.1404872

At present, the etiological diagnosis of DAH is mostly based on clinical diagnosis or tissue biopsy or genetic testing. Common clinical etiologies in children include related complications or sequelae after infection, immune diseases, cardiovascular disease, and airway lesions, and are exemplary in the diagnosis of idiopathic pulmonary hemosiderosis (IPH) syndrome, etc. (5, 19). In this case, the child had definite MP infection. Despite the high sensitivity and specificity of the MP nucleic acid test (13), the nucleic acid test for MP was negative on day 3 of the course, but the antibody detection and mNGS test for MP were positive on day 6 of the course, which suggested that the early negative MP test should not exclude the pathogen. Once a pathogen infection was suspected, repeated and multiple methods of detection can be considered to search for pathogens. mNGS detection was helpful for patients with DAH to search and identify pathogens relatively comprehensively. Unfortunately, we did not perform autoantibody and other immune screening and bronchoscopy. Although the children were followed up for 4 months and glucocorticoids were discontinued for more than 3 months, no initial symptoms recurred and all indexes were normal. We considered it unlikely that this patient had an immune-related disease. We will continue to dynamically track the follow-up symptoms, signs, and indicators in this child, and consider immune-related screening after full communication and consultation with the families when necessary.

It has been reported in many countries that MP is recognized to activate B and T lymphocytes, as well as cytokines secreted by various cells, which initiates the immune cascade and causes MP-HLH (4). Of the 23 reported cases (1, 2, 4, 8–11) of MP-HLH (1 case with incomplete information), 8 cases were diagnosed after 2 weeks of the course of the disease, with a mortality rate of 37.5% (3/8 cases), and 14 cases were diagnosed within 2 weeks of the course of the disease, with a mortality rate of 7.1% (1/15 cases). Two patients died of meningoencephalitis and intracranial hemorrhage, and the other two cases both died of multiple organ failure. MP-HLH has acute onset, rapid progression, and high mortality, which may be related to late diagnosis or other serious complications (4). HLH may also be overlooked because of its clinical similarity to exacerbations such as autoimmune diseases or sepsis. For instance, patients with inflammatory bowel disease (IBD) infected with CMV, EBV, and other viruses may be considered as IBD exacerbations while HLH may be ignored, resulting in a misdiagnosis or delayed diagnosis, leading to a poor prognosis (20). In particular, the clinical features and laboratory findings of HLH often overlap with those of sepsis-related multiple organ failure, which may lead to a delayed diagnosis and treatment. The H score is helpful to distinguish HLH from sepsis-related multiple organ failure and can assist in the diagnosis of secondary HLH (21). In this case, the patient had an H score of 236, which supported the diagnosis of secondary HLH (probability 98%–99%), and treatment was initiated on the second day after admission and the patient was discharged successfully. The early identification of MP-HLH may help reduce mortality. EBV and CMV are the most common pathogens of secondary HLH in children, but diagnosis and treatment may be delayed due to omission of other pathogens. For example, the clinical manifestations of HLH induced by CMV infection were similar to those of COVID-19 infection, and HLH should be considered when fever and abnormal liver function are observed (22). The mNGS test results of the patient in this case confirmed MP infection, and ruled out the possibility of EBV, CMV, COVID-19, and other pathogen agents causing HLH. It has also been proposed (1, 2) that although MP-HLH is rare, healthcare professionals should be highly vigilant of HLH in refractory *Mycoplasma* infections with cytopenia. In this case, the child's MP was not difficult to treat, and two courses of azithromycin treatment were effective. Therefore, we consider that professionals should be vigilant for MP combined with HLH when MP infection is complicated with cytopenia, regardless of whether MP is refractory or not.

Conclusion

The child in this case had HLH on admission, and manifestations of DAH were quickly recognized. Although the MP nucleic acid test was negative, we still highly suspected MP infection by epidemiology (epidemic year, season, age, cough manifestation), empirically selected azithromycin to treat MP infection, and confirmed MP infection with a further antibody test and mNGS. Combined with a retrospective review and comprehensive analysis of several related reports, we concluded the following: (1) mNGS may become an important method for the detection of pathogens for DAH and/or HLH; (2) the clinical manifestations of DAH in children may not be typical, but only acute hypoxic respiratory failure and Hb decreased significantly without bleeding elsewhere, a combination of chest imaging can be considered to assist the diagnosis; and (3) when MP infection is complicated with cytopenia, whether MP is refractory or not, MP complicated with HLH should be considered.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The studies involving humans were approved by the Medical Ethics Committee of West China Second Hospital, Sichuan University. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

MY: Writing – original draft, Writing – review & editing. Z-QL: Writing – review & editing. YW: Writing – review & editing. L-LL: Funding acquisition, Supervision, Writing – review & editing. L-NQ: Funding acquisition, Writing – review & editing.

Funding

The authors declare financial support was received for the research, authorship, and/or publication of this article.

This work was supported by National Key Research and Development Program of China (2021YFC2701700, 2021YFC2701704) and Sichuan Province Science and Technology Support Program (2023ZYD0124).

Acknowledgments

The authors would like to thank the Pediatric Intensive Care Unit team for help with the preparation of this manuscript and acknowledge support by the National Key Research and Development Program of China and Sichuan Province Science and Technology Support Program.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Yoshiyama M, Kounami S, Nakayama K, Aoyagi N, Yoshikawa N. Clinical assessment of *Mycoplasma pneumoniae*-associated hemophagocytic lymphohistiocytosis. *Pediatr Int.* (2008) 50(4):432–5. doi: 10.1111/j.1442-200X.2008.02701.x

2. Koike Y, Aoki N. Hemophagocytic syndrome associated with *Mycoplasma pneumoniae* pneumonia. *Case Rep Pediatr.* (2013) 2013:586705. doi: 10.1155/2013/586705

3. Hibino M, Sato S, Shimizu T, Yamamoto S, Ohe M, Kondo T. Hemophagocytic lymphohistiocytosis secondary to *Mycoplasma pneumoniae* infection without pneumonia. *Intern Med.* (2014) 53(15):1679–83. doi: 10.2169/internalmedicine.53.2089

4. Lu Z, Yang J, Wang Y, He Y, Bai D, Ma H, et al. Analysis of 3 cases with *Mycoplasma pneumoniae*-associated hemophagocytic syndrome and review of literature. *Zhonghua Er Ke Za Zhi.* (2014) 52(10):792–6. doi: 10.3760/cma.j.issn.0578-1310.2014.10.016

5. Jiang T, Li QB. Diffuse alveolar hemorrhage in children. *Zhongguo Dang Dai Er Ke Za Zhi.* (2019) 21(9):949–54. doi: 10.7499/j.issn.1008-8830.2019.09.020

6. Zhang X, Yu Y. Severe pediatric mycoplasma pneumonia as the cause of diffuse alveolar hemorrhage requiring veno-venous extracorporeal membrane oxygenation: a case report. *Front Pediatr.* (2023) 10:925655. doi: 10.3389/fped.2022.925655

7. Fardet L, Galicier L, Lambotte O, Marzac C, Aumont C, Chahwan D, et al. Development and validation of the HScore, a score for the diagnosis of reactive hemophagocytic syndrome. *Arthritis Rheumatol.* (2014) 66(9):2613–20. doi: 10.1002/art.38690

8. Ishida Y, Hiroi K, Tauchi H, Oto Y, Tokuda K, Kida K. Hemophagocytic lymphohistiocytosis secondary to *Mycoplasma pneumoniae* infection. *Pediatr Int.* (2004) 46(2):174–7. doi: 10.1046/j.1442-200x.2004.01878.x

9. Bruch LA, Jefferson RJ, Pike MG, Gould SJ, Squier W. *Mycoplasma pneumoniae* infection, meningoencephalitis, and hemophagocytosis. *Pediatr Neurol.* (2001) 25(1):67–70. doi: 10.1016/s0887-8994(01)00274-0

10. Yasutomi M, Okazaki S, Hata I, Tanizawa A, Tamamura S, Kawakita M, et al. Cytokine profiles in *Mycoplasma pneumoniae* infection-associated hemophagocytic lymphohistiocytosis. *J Microbiol Immunol Infect.* (2016) 49(5):813–6. doi: 10.1016/j.jmii.2014.11.015

11. Jia-li G, Zhi-wei L, Wen-jian W, Yue-jie Z, Jing L, Yan-bing S. 11 cases of *Mycoplasma pneumoniae*-associated hemophagocytic syndrome: a case series report. *Chin J Evid Based Pediatr.* (2020) 15(3):229–32. doi: 10.3969/j.issn.1673-5501.2020.03.014

12. Parums DV. Editorial: outbreaks of post-pandemic childhood pneumonia and the re-emergence of endemic respiratory infections. *Med Sci Monit.* (2023) 29:e943312. doi: 10.12659/MSM.943312

13. Li X, Li T, Chen N, Kang P, Yang J. Changes of *Mycoplasma pneumoniae* prevalence in children before and after COVID-19 pandemic in Henan, China. *J Infect.* (2023) 86(3):256–308. doi: 10.1016/j.jinf.2022.12.030

14. Commission NH, Zhao SY, Qian SY, Chen ZM, Gao HM, Liu HM, et al. Guidelines for diagnosis and treatment of *Mycoplasma pneumoniae* pneumonia in children (2023 edition). *Infectious Diseases Information.* (2023) 36(4):291–7. doi: 10.3969/j.issn.2096-3327.2023.03.003

15. Hu J, Ye Y, Chen X, Xiong L, Xie W, Liu P. Insight into the pathogenic mechanism of *Mycoplasma pneumoniae*. *Curr Microbiol.* (2022) 80(1):14. doi: 10.1007/s00284-022-03103-0

16. Meyer Sauteur PM, Beeton ML, ESGMAC the ESGMAC MAPS study group. *Mycoplasma pneumoniae*: gone forever? *Lancet Microbe.* (2023) 4(10):e763. doi: 10.1016/S2666-5247(23)00182-9

17. Qi Y, Wang L, Qian L, Zhang X. The etiology, clinical profile, and outcome of diffuse alveolar hemorrhage in children: a ten-year single-center experience. *Transl Pediatr.* (2021) 10(11):2921–8. doi: 10.21037/tp-21-283

18. Reisman S, Chung M, Bernheim A. A review of clinical and imaging features of diffuse pulmonary hemorrhage. *AJR Am J Roentgenol.* (2021) 216(6):1500–9. doi: 10.2214/AJR.20.23399

19. de Silva C, Mukherjee A, Jat KR, Lodha R, Kabra SK. Pulmonary hemorrhage in children: etiology, clinical profile and outcome. *Indian J Pediatr.* (2019) 86(1):7–11. doi: 10.1007/s12098-018-2725-x

20. Brambilla B, Barbosa AM, Scholze CDS, Riva F, Freitas L, Balbinot RA, et al. Hemophagocytic lymphohistiocytosis and inflammatory bowel disease: case report and systematic review. *Inflamm Intest Dis.* (2020) 5(2):49–58. doi: 10.1159/000506514

21. Xu XJ, Tang YM. Dilemmas in diagnosis and management of hemophagocytic lymphohistiocytosis in children. *World J Pediatr.* (2020) 16(4):333–40. doi: 10.1007/s12519-019-00299-3

22. Soldera J, Bosi GR. Haemophagocytic lymphohistiocytosis following a COVID-19 infection: case report. *J Infect Dev Ctries.* (2023) 17(3):302–3. doi: 10.3855/jidc.16983

frontiers | Frontiers in Pediatrics

TYPE Original Research
PUBLISHED 15 August 2024
DOI 10.3389/fped.2024.1360227

OPEN ACCESS

EDITED BY
Rujipat Samransamruajkit,
Chulalongkorn University, Thailand

REVIEWED BY
Elpis Hatziagorou,
Aristotle University of Thessaloniki, Greece
Yunxiao Wu,
Capital Medical University, China

*CORRESPONDENCE
Elida Duenas-Meza
✉ eduenas@neumologica.org

RECEIVED 22 December 2023
ACCEPTED 28 June 2024
PUBLISHED 15 August 2024

CITATION
Duenas-Meza E, Proaños-Jurado NJ, Pulido-Fentanes S, Severiche-Bueno DF, Escamilla-Gil MI, Bazurto-Zapata MA, Jurado JL, Suarez MR and Giraldo-Cadavid LF (2024) Breathing patterns during sleep and their relationship with FEV1 in pediatric patients with cystic fibrosis residing at high altitude. Front. Pediatr. 12:1360227. doi: 10.3389/fped.2024.1360227

Breathing patterns during sleep and their relationship with FEV1 in pediatric patients with cystic fibrosis residing at high altitude

Elida Duenas-Meza[1,2]*, Nadia Juliana Proaños-Jurado[1,2], Sarah Pulido-Fentanes[1], Diego F. Severiche-Bueno[1,2], María Isabel Escamilla-Gil[1,2], Maria Angelica Bazurto-Zapata[1], Jenny Libeth Jurado[1], Miguel Ricardo Suarez[1,2] and Luis Fernando Giraldo-Cadavid[1,2]

[1]Departamento de investigación CINEUMO, Fundación Neumológica Colombiana, Bogotá, Colombia, [2]Facultad de Medicina, Universidad de La Sabana, Chía, Colombia

Introduction: Sleep-disordered breathing (SDB) and gas exchange disorders are common in patients with cystic fibrosis (CF). Currently, the impact of the disease on sleep patterns in patients living at high altitude and the relationship of these patterns to lung function are largely unknown. The aim of this study was to determine the frequency of SDB in children with CF aged 6–18 years and the relationship between SDB and lung function (FEV1).

Methods: This is an analytical cross-sectional study of children aged 6–18 years diagnosed with CF. Spirometry before and after bronchodilators and polysomnography with capnography were performed. Descriptive analysis of qualitative and continuous variables was performed. Spearman's correlation coefficient was used to determine the correlation between polysomnogram and lung function (FEV1).

Results: Twenty-four patients with CF were included. The mean age was 10.5 ± 3.1 years and 62.5% were male. Nine children had bronchiectasis on chest CT. The median absolute baseline FEV_1 was 1,880 (1,355–2,325) ml and 98% (83%–110%) of predicted value. No significant difference in FEV_1% was observed between subjects with obstructive sleep apnea (OSA) and those without OSA ($P = 0.56$). The prevalence of OSA was 66.7% in children younger than 13 years and 40% in children older than 13 years. The Spearman correlation coefficient between FEV_1 and percentage of total sleep time with saturation less than 90% (T90) was rho −0.52 (p-value = 0.018), and between FEV1 and percentage of total sleep time with saturation less than 85% (T85) was statistically significant with rho −0.45 (p-value = 0.041). A positive correlation was observed between FEV_1 and SpO_2 during sleep with rho 0.53 and a statistically significant p-value (0.014).

Conclusions: A high prevalence of sleep apnea was found in children with CF living at high altitude, with a negative correlation between FEV_1 and T90 and T85 oxygenation indices, and a positive correlation between FEV_1 and SpO2 during sleep.

KEYWORDS

cystic fibrosis, sleep, high altitude, obstructive sleep apnea, sleep disorder breathing (SDB)

Introduction

Cystic fibrosis (CF) is a disease with a worldwide distribution, although the incidence, carrier rate, and type of mutation vary depending on the population and ethnic group analyzed. In Latin America, CF is estimated to affect between 1 in 1,600 and 1 in 14,000 live births (1). A pilot study conducted in Bogotá that included neonatal screening for CF found an incidence of 1 in 8,297 (2). According to the National CF Registry, 64% of CF patients in Colombia live at medium altitude (1,500–2,500 m) and 32% of patients live at high altitude (2,500–3,500 m) (3).

Clinical studies in adult CF patients have focused on findings at sea level and the tolerance of patients to altitude changes during air travel, analyzing the effects of these altitude changes on lung function, arterial oxygen pressure (PaO2) and clinical manifestations (4). CF patients can develop hypoxemia and hypercapnia during exercise and sleep (5). The drop in barometric oxygen pressure with altitude and subsequent hypoxemia leads to hyperventilation to restore PaO_2, which in turn lowers PCO_2. This process is driven by changes in central and peripheral chemoreceptor sensitivity, adjustments in cerebral blood flow, changes in pulmonary artery pressure, and adjustments in the macro- and micro-architecture of sleep. In addition, exposure to high altitude has been shown to produce an increase in apnea-hypopnea index (AHI), which is explained by an increase in centrally triggered events. However, as the acclimatization process progresses, the AHI begins to decrease (6). The study of arterial gases at high altitude at our institution showed that PCO_2 and PO_2 levels are lower than those described at sea level (7). In adult obstructive sleep apnea (OSA) patients, desaturation indices were found to be worse at high altitude than those reported at sea level (8). It is important to note, however, that all these studies have been conducted in adults and not in children.

Sleep-disordered breathing (SDB) is an underdiagnosed comorbidity in children with cystic fibrosis (CF). Nocturnal hypoxemia, OSA and nocturnal hypoventilation are common in patients with lung disease. These are known to have neurocognitive, cardiovascular, and quality of life consequences (9). A high prevalence of OSA in CF patients, independent of age and lung function, has been described in a recent meta-analysis (10). However, a correlation between disease severity assessed by FEV_1 and nocturnal saturation has been described (11, 12).

To our knowledge, no studies have been conducted at high altitude to determine the effects of chronic hypoxia on the breathing patterns of children with CF during sleep. The aim of this study was to investigate the breathing patterns during sleep in children with CF living at high altitude and to correlate these patterns with lung function.

Materials and methods

Study subjects

Twenty-four children aged 6 to 18 years referred to the CF Center of the Fundación Neumológica Colombiana between January 2020 and December 2021 with a confirmed diagnosis of CF by iontophoresis and/or genetic study and who resided in a city at a high altitude (2,640 m) were included. Children who were unable to perform spirometry and children who had experienced an exacerbation of their disease within six weeks before entering the study were excluded. All participants had an informed consent form signed by a parent or guardian. Those over the age of 14 also signed an informed assent form in accordance with local regulations.

To detect a statistically significant difference in lung function (FEV1) between subjects with and without OSA, a sample size calculation was performed based on an expected mean difference of 14% with a standard deviation (SD) of 6.5%. This effect size was derived from a previous analysis of FEV1 in patients with cystic fibrosis (CF) aged 6–18 years treated at the Cystic Fibrosis Center in Fundación Neumológica Colombiana. Assuming a two-tailed alpha of 0.05, a power (beta) of 80%, a 1:1 allocation ratio and a parametric distribution, a sample size of 24 participants was determined. This sample size was sufficient to detect a significant correlation coefficient of at least 0.5 between FEV1 and AHI or oxygen desaturation index.

Study design

This is an analytical cross-sectional study of children between 6 and 18 years of age diagnosed with CF and prospectively recruited at the CF Center of Fundación Neumológica Colombiana in Bogotá, Colombia, a city located at high altitude (2,640 m). The study was approved by the Ethics Committee. Registry Number: 2019006–24507.

Polysomnogram

The polysomnogram (PSG) was performed according to the guidelines of the American Academy of Sleep Medicine (AASM) using commercially available digital equipment (Philips Respironics®; Alice 5 and LE models). It was performed at the Fundación Neumológica Colombiana Cystic Fibrosis Center by a PSG technologist with pediatric experience, in a dark and quiet room with an average room temperature of 19°C. The duration of the polysomnogram was 7–10 h. A parent or guardian was present throughout the study.

The following parameters were measured: chest wall and abdominal motion assessed by inductance plethysmography, heart rate assessed by electrocardiogram (ECG) (DII), and airflow monitored by nasal pressure cannula and oronasal thermistor. Arterial SpO2 values and heart rate during wakefulness and sleep were recorded with a high-precision pulse oximeter (Masimo; Rad 8 model) integrated into the digital PSG acquisition system with simultaneous pulse wave recording, as well as bilateral electroencephalograms (EEGs), three EEG channels (F4, C3, C4, O2), chin and anterior tibial electromyograms, and an analog output from a body position sensor.

Sleep architecture was assessed using standard techniques in accordance with AASM recommendations and guidelines (13–15). Sleep stages were classified as non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep. The proportion of time spent in each sleep stage was expressed as a percentage of total sleep time (TST). Apnea index was defined as the number of apneic episodes per hour of TST.

The following definitions were used: (1) *obstructive apnea*: absence of airflow (90% reduction in airflow signal) with continuous chest and abdominal wall movements lasting at least two breaths; (2) *central apnea* (CA): a 90% reduction in airflow signal with absence of chest or abdominal movements lasting 20 s or at least two breaths and associated with 3% oxygen desaturation; (3) *mixed apnea*: an event that meets the criteria for apnea for at least two breaths and is associated with no respiratory effort during one part of the event and the presence of inspiratory effort during another part: An event that meets the criteria for apnea for at least two breaths and is associated with no inspiratory effort during part of the event and the presence of inspiratory effort during another part, regardless of which part comes first; (4) *Hypopnea*: A 30% decrease in nasal flow for two or more breaths with a corresponding decrease in SpO2 (3%), a microarousal, or both (14, 15).

Obstructive sleep apnea (OSA) was defined from a polysomnographic perspective as follows: In children younger than 13 years: Obstructive Apnea-Hypopnea Index (OAHI): ≥ 2/h with severity classified as mild (2–4.9/h), moderate (5–9.9/h), or severe (≥10/h) (16). Children 13 years of age and older: OAHI ≥5/h with severity classified as mild (5–14.9/h), moderate (15–29.9/h), or severe (≥30/h).

PSG oxygen saturation parameters include the following:

- Oxygen desaturation index (ODI): number of desaturations ≥3% per hour of total sleep time
- T90: percentage of total sleep time with saturation less than 90%.
- T85: percentage of total sleep time with saturation less than 85%.
- Minimum saturation (nadir SpO2): minimum saturation during apneas/hypopneas
- Average saturation during respiratory events: average saturation recorded during apneas and hypopneas.
- Desaturation: SpO2 value less than 90%.

Spirometry

Spirometry was performed before and after bronchodilators according to the American Thoracic Society (ATS) guidelines. FEV1 was used as a functional parameter of severity. Spirometry was performed with a properly calibrated spirometer (Vyntus® SPIRO). All participants performed spirometry according to ATS guidelines (17).

Additional definitions include the following (18): *Normal spirometry*: Percentage values for FVC, FEV_1 and peak flow (PEF) of 100 ± 20% of predicted and for %$FEF_{25–75}$ 100 ± 35% of predicted, without response to bronchodilator *Abnormal spirometry*: Values below 80% for FVC, FEV_1, %FEV_1/FVC ratio and PEF and less than 70% for %$FEF_{25–75}$ *Bronchodilator Response*: A change of >10% relative to the predicted value for FEV1 or FVC in accordance with the ATS/ERS technical standard (19).

Statistical analysis

Qualitative variables were described as absolute and relative frequencies. For continuous variables, measures of central tendency and dispersion were used according to the assumption of normality evaluated by the Shapiro-Wilk test. Spearman's correlation coefficient was used for non-parametric samples to determine the correlation between polysomnogram parameters and lung function (FEV_1). All hypothesis systems were tested two-tailed, and a value of $p < 0.05$ was considered statistically significant. Analyses were performed using Stata 16© statistical software.

Results

A total of 24 children diagnosed with CF, living at high altitude and without exacerbations in the 6 weeks prior to recruitment were included. The mean age of the children was 10.5 ± 3.1 years, 72.2% were in the normal weight percentile and 62.5% (15/24) were male. The mean sweat chloride concentration was 103 ± 32.8 and 83.3% (20/24) were found to have digital clubbing.

Regarding the findings on chest computed tomography, 9 of 19 children had bronchiectasis and 23 of the patients had a sputum culture in the last trimester, of these 30.43% (7/23) showed colonization by *Staphylococcus aureus* and 4.34% (1/23) by *Pseudomonas aeruginosa*. The mean number of hospitalizations for pulmonary exacerbations in the previous year was 2.3 ± 2.6. It was found that 75% (18/24) of the children received pancreatic enzymes, 83% (20/24) vitamins, 70.8% (17/24) nutritional supplements and 79.2% (19/24) dornase alpha. None of the participants were on CFTR modulators (Table 1). Eight were p.F508del homozygous and 3 were compound for p.F508del mutation (Table 2).

The median absolute baseline FEV_1 was 1,880 (1,355–2,325) ml and 98% (83%–110%) of predicted value and only 4/24 had bronchodilator responsiveness ($FEV_1 > 12\%$). No significant difference in FEV_1% was observed between subjects with OSA and those without OSA ($P = 0.56$). Polysomnography was performed in 20 of 24 patients. The prevalence of obstructive sleep apnea was 66.7% in children younger than 13 years and 40% in children older than 13 years with a median total AHI of 4.2 (1.6–6.5), central AHI of 0.3 (0–1.7) and OAHI of 3.05 (1.2–5) (Table 3). Mean saturation during wakefulness was 90.2 ± 2.3, SpO2 during sleep was 89.4 ± 2.2, and desaturation index was 10.2 ± 4.6. A comparison was made between children with CF without SDB and children with SBD breathing. It was observed that patients with SDB exhibited a higher ODI (12.5 ± 4.0 vs. 6.1 ± 2.4). Regarding the echocardiographic variables, none of the patients had an intermediate or high probability of pulmonary hypertension, as the median pulmonary artery systolic pressure was 22 (20–27).

The Spearman correlation coefficient between FEV_1 and T90 was rho −0.52 (p-value = 0.018) (Figure 1), and between FEV1

TABLE 1 General characteristics of the population.

Variables	Total population *n*: 24
Sex, male	15 (62.5)
Age, years	10.5 ± 3.1
Percentiles	
Underwight	2 (8,3)
Normal wight	19 (79,2)
Overweight	2 (8,3)
Obesity	1 (4,2)
Genetic study	23 (95.8)
p.F508del Homozygous	8 (34.7)
Bronchiectasis in chest CT *n*:19	9 (50.0)
Presence of rales	2 (8.3)
Digital clubbing	20 (83.3)
Presence of wheezing	2 (8.3)
Pulse oximetry	93 ± 2.4
Sweat electrolyte technique	
Indirect technique	11 (45.8)
Gibson & Cooke technique	13 (54.1)
Sweat electrolyte value	103 ± 32.8
Sputum culture in the last trimester *n*:23	
Negative	10 (43,5)
Pseudomona aeruginosa	1 (4.34)
Staphylococcus aureus	7 (30,4)
Other microorganism	5 (21,7)
CF treatment	
Pancreatic enzymes	18 (75.0)
Vitamins	20 (83.3)
Nutritional supplement	17 (70.8)
Dornase alpha	19 (79.2)
Device for nebulized medication	24 (100)
7% Hypertonic saline solution	22 (91.6)
Inhaled antibiotic	1 (4.2)
Respiratory therapy	23 (95.8)
Clinical Outcomes	
Exacerbation in the last year	11 (45.8)
Average number of exacerbations in the last year *n*: 11	2.3 ± 2.6

BMI, body mass index; CT, computerized tomography; CF, cystic fibrosis. Values as means ± standard deviation and *n* (%).

TABLE 2 Participants' genetic study results.

Mutations in the CFTR gene	*n*:24
Homozygous p.F508del	8
Homozygous p.G542X	2
Heterozygous p.F508del and heterozygous c.489+1G>T p.(?)	1
Heterozygous p.F508del and heterozygous (c.3846G>A; p.(Trp1282Ter)	2
Homozygous c.1312A>G p.(Thr438Ala)	1
Heterozygous c.3368-2A>G p.(?) and heterozygous c.580-1G>T p.(?)	1
Heterozygous c.1000C>T p.(Arg334Trp) and heterozygous c.3484C>T p.(Arg1162Ter)	1
Homozygous p.V470M	1
Homozygous c.3484C>T p.(Arg1162Ter)	1
Negative genetic study	5
Without genetic study	1

CFTR, cystic fibrosis transmembrane conductance regulator.

TABLE 3 Results of diagnostic tests performed on study participants.

Espirometry pre & post b2	*n*:23
FEV_1 Pre ml	1,880 (1,355–2,325)
FEV_1 Post ml	1,990 (1,390–2,610)
FEV_1 (%) Pre	98 (83–110)
FEV_1 (%) Post	97 (82–120)
FEV_1 Post *n* (%)	
≤12%	19 (82,6)
>12%	4 (17,4)
FVC (%) Pre	0.83 (0.78–0.86)
FVC (%) Post	0.85 (0.80–0.90)
Echocardiogram	
PASP	22 (20–27)
TAPSE	21 (18–23)
Right ventricle acceleration	1 (4.2)
Polysomnogram *n*:20	
Total AHI	4.2 (1.6–6.5)
Central AHI	0.3 (0–1.7)
Obstructive AHI	3.05 (1.2–5)
Obstructive AHI ≥ 2	
≥ 13 years *n* = 5	2 (40,0)
≤ 12 years *n* = 15	10 (66,7)
T90	45.6 ± 37.5
T85	5.75 ± 13.9
T80	0.25 ± 0.8
SpO2 during wakefulness	90.2 ± 2.3
SpO2 during sleep	89.4 ± 2.2
Desaturation index	10.2 ± 4.6
Capnography	
≤35	7 (35.0)
≤40	3 (15.0)
≤45	9 (45.0)
≤50	1 (5.0)

FEV_1, forced expiratory volumen in first second; ml, mililiters; FVC, forced vital capacity; PASP, pulmonary artery systolic pressure; TAPSE, tricuspid annular plane systolic excursion; AHI, apnea Hypopnea index; SpO2, pulse oximetry. Values as means ± standard deviation or median (p25–p75) and *n* (%).

and T85 was statistically significant with rho −0.45 and *p*-value = 0.041 (Figure 2). A positive correlation was observed between FEV_1 and SpO_2 during sleep with rho 0.53 and a statistically significant value $p = 0.014$ (Figure 3), while between FEV_1 and $EtCO_2$ the rho was 0.44 with a *p*-value = 0.047.

Discussion

This study showed that in this group of children aged 6 to 18 years with CF living at high altitude (2,640 m), there was a high prevalence of OSA (65%) with normal lung function, regardless of disease severity as determined by $\%FEV_1$. A moderate positive correlation was found between $\%FEV_1$ and SpO_2 during sleep, and a moderate negative correlation was found between $\%FEV_1$ and T90 and T85, with a mean SpO_2 during sleep of less than 90%. This suggests that a higher $\%FEV_1$ predicts a higher SpO_2 during sleep, while a lower $\%FEV_1$ predicts a higher percentage of total sleep time with SpO_2 less than 90% and less than 85% during sleep. Additionally, we observed a higher ODI in patients with CF and SDB compared to those with CF without SDB.

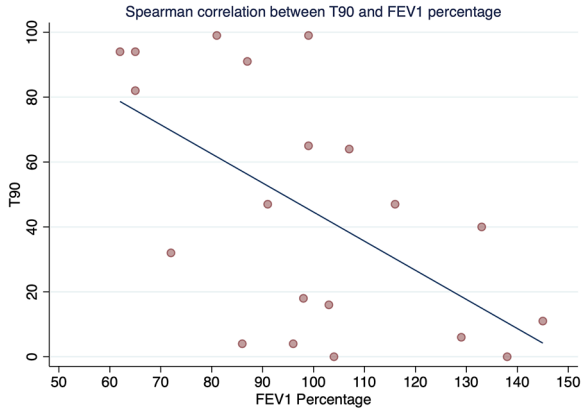


FIGURE 1
Spearman correlation between T90 and FEV1 percentage.

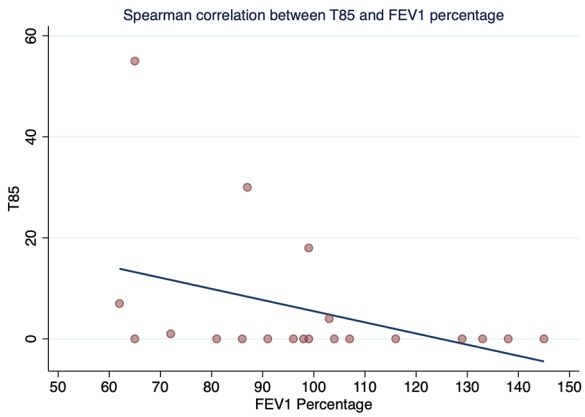


FIGURE 2
Spearman correlation between T85 and FEV1 percentage.

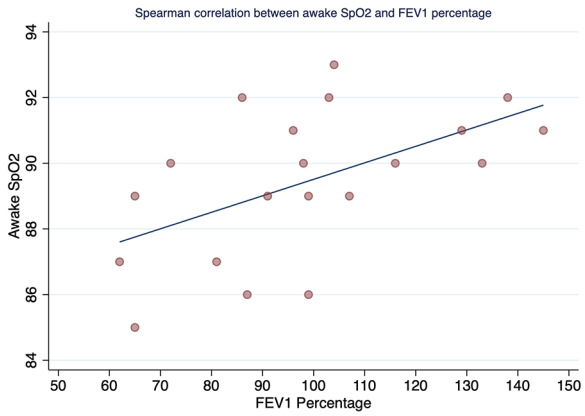


FIGURE 3
Spearman correlation between awake SpO_2 and FEV1 percentage.

The results showed a high prevalence of OSA in children with CF compared to the prevalence in healthy children, ranging from 0.7%–13%, depending on the AHI cut-off points used to diagnose OSA (20, 21). The prevalence of OSA found in this study is similar to that reported in a meta-analysis conducted in children and adolescents with CF, which included six studies in which the pooled prevalence of OSA defined as AHI >1/h was 65% (95% CI: 0.54–0.76) and with OSA defined as AHI >2/h was 51.5% (95% CI: 0.18–0.84) (10). It is important to note that the criteria for OSA in our study are consistent with those established in the literature (22). It is possible that if a lower AHI cut-off had been used (e.g., AHI >1/h), the prevalence of OSA in these children with CF living at high altitude would have been higher, but the results could potentially have been affected by a higher frequency of false-negative cases, especially in children living at high altitude.

From a functional point of view, this group of children with CF did not have advanced or severe disease, with a normal FEV_1 range, and no significant correlation was found between AHI and $\%FEV_1$. Like our study, Silva et al. reported that 87.9% of clinically stable children with normal or mildly impaired lung function had some type of sleep disorder, either respiratory or sleep architecture (23). As shown in our study and others, the severity of SDB in children with CF does not appear to be solely related to the degree of lung involvement as assessed by $\%FEV_1$ (24, 25). These findings highlight the need for early detection of SDB, before lung function deteriorates, to prevent the metabolic, cardiovascular, and neurocognitive effects of SDB (26), which increases the burden of the disease.

Although a high prevalence of OSA is recognized in children and adolescents with CF, the factors associated with its development and the role of living at high altitude are not fully understood. It is possible that nasal polyps and/or nasal mucosal inflammation, which occur in most patients with CF, may contribute to the presentation of OSA. However, we did not evaluate this aspect in our study (27). Regarding the effects of exposure to high altitude, it is likely that, as some authors have suggested in relation to children without CF, chronic adaptation to high altitude and an individual predisposition may lead to hyperventilation and a high frequency of micro-alerts, and consequently to a series of pathophysiological events culminating in obstructive apnea with even greater frequency and severity (28). However, studies in Andean populations living at high altitude have shown that chronic adaptation results in an increase in erythrocyte count with a subsequent increase in hemoglobin and a decreased hypoxic vasoconstrictor response. Regarding the increase in ventilation observed with acute exposure to high altitude, the increase in ventilation is not present in Andean natives living at high altitude and is similar to that of natives living at low altitude, both at rest and during exercise (29). However, the study of the normal values of arterial gases in our group shows lower values of PCO2 and PO2 in adults, which could indicate a degree of hyperventilation (7).

A positive correlation was found for $\%FEV_1$ (rho = 0.53, $p = 0.014$) with mean SpO_2 during sleep, and a negative correlation was found between $\%FEV_1$ and T90 and T85.

Comparison of these results with those of Uyan et al. (5) and Spicuzza et al. (30), These authors did not find a correlation between mean SpO_2 during sleep and FEV_1. In contrast, de Castro-Silva et al. (31) found a similar result to our study (correlation between mean SpO_2 and FEV1; $p < 0.001$). These inconsistent results may be due to the different methodologies used, including the evaluation of different parameters, the use of different predictive equations to assess lung function, and differences in the CF severity profiles of the participants.

In the current study, patients had an average SpO_2 during sleep of 89.4 ± 2.2 and a high ODI of 10.2 ± 4.6. Compared with results from studies conducted at sea level with similar functional characteristics (23, 31), mean SpO_2 during sleep and SpO2 during wakefulness were lower and desaturation indices were higher. Thus, our data are consistent with the results of other studies regarding oxygen desaturation during sleep in patients with CF with a higher degree of severity (12, 32, 33). It is important to note that the patients in this study showed desaturation even when the disease was mild and they were in a period of clinical stability, suggesting the impact of chronic hypoxia in CF patients living at high altitudes.

An additional finding of our study was that none of the patients had an intermediate or high probability of pulmonary hypertension based on the pulmonary artery systolic pressure (PASP) value. Estimation of PASP is based on the tricuspid regurgitant velocity (TRV) using Bernoulli's equation. However, unlike in adults where the probability of pulmonary hypertension is determined from the TRV value (34), no studies have determined the normal values of PASP in children. Since there are currently no studies that have determined the probability of pulmonary hypertension from PASP, in consensus with the research group and taking into account the parameters used in other studies and reference values extrapolated from the adult population, the upper limit of normal (ULN) for right ventricular systolic pressure was set between 30 and 35 mmHg and >40 mmHg for the TTE diagnosis of pulmonary hypertension (35–38) considering that, according to the studies in adults, a PASP of <35 mmHg implies a TRV of less than 2.8, which confers a low probability of pulmonary hypertension (34, 39, 40). This finding is consistent with the results of another study by our group in children with OSA at high altitude, where none of the children had PASP levels above ULN, regardless of OSA severity (41).

Some limitations of this study include the fact that we did not have a control group of patients living at a lower altitude, and we did not evaluate the presence of nasal polyps, which may have contributed to the high prevalence of OSA. In addition to the small number of patients. Of the 24 patients enrolled in the study, 4 did not attend the polysomnogram, thus polysomnogram analysis was performed on only 20 of the 24 children enrolled. Furthermore, we have no data on the presence of polycythemia in our population and we have no data on the presence of hyperventilation during the sleep study. The strength of this study is that it is the first study to determine the impact of chronic altitude hypoxia in children with CF living at high altitude.

In conclusion, this study found a high prevalence of sleep apnea in children with CF living at high altitude - higher than the prevalence of sleep apnea in children without CF reported in the literature, but like children and adolescents with CF living at sea level. It also found an average desaturation during sleep higher than that found in children with CF with normal lung function living at sea level. In addition, this study found a negative correlation between FEV1 and T90 and T85 oxygenation indices and a positive correlation between FEV1 and SpO2 during sleep. Furthermore, this study identified a negative correlation between FEV1 and T90 and T85 oxygenation indices and a positive correlation between FEV1 and SpO2 during sleep. Additionally, this study found that those patients with CF and SDB exhibited a higher ODI, emphasizing the importance of early detection in this group of patients.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Comite de etica en investigacion de la Fundación Neumologica Colombiana. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

ED-M: Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. NP-J: Conceptualization, Data curation, Methodology, Software, Writing – original draft, Writing – review & editing. SP-F: Investigation, Writing – original draft, Writing – review & editing. DS-B: Project administration, Supervision, Writing – original draft, Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing. ME-G: Data curation, Investigation, Supervision, Writing – review & editing. MB-Z: Conceptualization, Methodology, Writing – review & editing, Investigation. JJ: Supervision, Project administration, Writing – original draft. MS: Investigation, Writing – review & editing, Supervision. LG-C: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article.

This study received funding from "Ministerio Ciencia, Tecnología e Innovación – Minciencias" (RC 397-2020).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Silva Filho LV, Castanos C, Ruiz HH. Cystic fibrosis in Latin America-improving the awareness. *J Cystic Fibrosis*. (2016) 15:791–3. doi: 10.1016/j.jcf.2016.05.007

2. Mateus H, Amado P. *Tamizaje Neonatal Para Fibrosis Quística en una Muestra de la Ciudad de Bogotá*. Bogotá: Universidad del Rosario (2011).

3. Aristizabal C, Vasquez C. Registro Colombiano de Fibrosis Quistica 2009 Asociacion Colombiana de Neumologia Pediatrica. (2009). Available online at: https://portal.neumopediatriacolombia.com/registro-colombiano-de-fibrosis-quistica-2009/ (Accessed August 16, 2023).

4. Fischer R, Lang SM, Bruckner K, Hoyer HX, Meyer S, Griese M, et al. Lung function in adults with cystic fibrosis at altitude: impact on air travel. *Eur Respir J*. (2005) 25:718–24. doi: 10.1183/09031936.05.10087304

5. Uyan ZS, Ozdemir N, Ersu R, Akpinar I, Keskin S, Cakir E, et al. Factors that correlate with sleep oxygenation in children with cystic fibrosis. *Pediatr Pulmonol*. (2007) 42:716–22. doi: 10.1002/ppul.20643

6. Rojas-Cordova S, Torres-Fraga MG, Rodriguez-Reyes YG, Guerrero-Zuniga S, Vazquez-Garcia JC, Carrillo-Alduenda JL. Altitude and breathing during sleep in healthy persons and sleep disordered patients: a systematic review. *Sleep Sci*. (2023) 16:117–26. doi: 10.1055/s-0043-1767745

7. Gonzalez-Garcia M, Maldonado D, Barrero M, Casas A, Perez-Padilla R, Torres-Duque CA. Arterial blood gases and ventilation at rest by age and sex in an adult Andean population resident at high altitude. *Eur J Appl Physiol*. (2020) 120:2729–36. doi: 10.1007/s00421-020-04498-z

8. Bazurto Zapata MA, Duenas Meza E, Jaramillo C, Maldonado Gomez D, Torres Duque C. Sleep apnea and oxygen saturation in adults at 2640 m above sea level. *Sleep Sci*. (2014) 7:103–6. doi: 10.1016/j.slsci.2014.09.003

9. Jagpal SK, Jobanputra AM, Ahmed OH, Santiago TV, Ramagopal M. Sleep-disordered breathing in cystic fibrosis. *Pediatr Pulmonol*. (2021) 56(Suppl 1): S23–S31. doi: 10.1002/ppul.25028

10. de Sousa LP, Liberato FMG, Vendrusculo FM, Donadio MVF, Barbosa RRB. Obstructive sleep apnea in children and adolescents with cystic fibrosis and preserved lung function or mild impairment: a systematic review and meta-analysis of prevalence. *Sleep Med*. (2021) 88:36–43. doi: 10.1016/j.sleep.2021.09.017

11. Lumertz MS, Pinto LA. Sleep-disordered breathing in cystic fibrosis pediatric subjects. *Sleep Science (Sao Paulo, Brazil)*. (2019) 12:165–70. doi: 10.5935/1984-0063.20190079

12. Reiter J, Breuer O, Cohen-Cymberknoh M, Forno E, Gileles-Hillel A. Sleep in children with cystic fibrosis: more under the covers. *Pediatr Pulmonol*. (2021) 57:1944–51. doi: 10.1002/ppul.25462

13. Berry RB, Gamaldo CE, Harding SM, Brooks R, Lloyd RM, Vaughn BV, et al. AASM Scoring manual version 2.1 updates: new chapters for scoring infant sleep staging and home sleep apnea testing. *J Clin Sleep Med*. (2014) 11:1253–4. doi: 10.5664/jcsm.5176

14. Troester MM, Quan SF, Berry RB, Plante DT, Abreu AR, Alzoubaidi M, et al. *The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications, Version 2.5*. Darien, IL: American Academy of Sleep Medicine (2018).

15. Grigg-Damberger M, Gozal D, Marcus CL, Quan SF, Rosen CL, Chervin RD, et al. The visual scoring of sleep and arousal in infants and children. *J Clin Sleep Med*. (2007) 3:201–40. doi: 10.5664/jcsm.26819

16. Alonso-Álvarez M, Canet T, Cubell-Alarco M, Estivill E, Fernández-Julián E, Gozal D, et al. Consensus document on sleep apnea-hypopnea syndrome in children (full version). sociedad española de sueño. El Área de sueño de la sociedad española de neumología y cirugía torácica(SEPAR). *Arch Bronconeumol*. (2011) 47 (Suppl 5):2–18. doi: 10.1016/S0300-2896(11)70026-6

17. Kitazawa H, Jiang A, Nohra C, Ota H, Wu JKY, Ryan CM, et al. Changes in interpretation of spirometry by implementing the GLI 2012 reference equations: impact on patients tested in a hospital-based PFT lab in a large metropolitan city. *BMJ Open Respir Res*. (2022) 9:1–10. doi: 10.1136/bmjresp-2022-001389

18. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. *Eur Respir J*. (2005) 26:319–38. doi: 10.1183/09031936.05.00034805

19. Stanojevic S, Kaminsky DA, Miller MR, Thompson B, Aliverti A, Barjaktarevic I, et al. ERS/ATS technical standard on interpretive strategies for routine lung function tests. *Eur Respir J*. (2022) 60:1–32. doi: 10.1183/13993003.01499-2021

20. Bixler EO, Vgontzas AN, Lin HM, Liao D, Calhoun S, Vela-Bueno A, et al. Sleep disordered breathing in children in a general population sample: prevalence and risk factors. *Sleep*. (2009) 32:731–6. doi: 10.1093/sleep/32.6.731

21. Gulotta G, Iannella G, Vicini C, Polimeni A, Greco A, de Vincentiis M, et al. Risk factors for obstructive sleep apnea syndrome in children: state of the art. *Int J Environ Res Public Health*. (2019) 16:1–7. doi: 10.3390/ijerph16183235

22. Gozal D, Kheirandish-Gozal L. New approaches to the diagnosis of sleep-disordered breathing in children. *Sleep Med*. (2010) 11:708–13. doi: 10.1016/j.sleep.2009.12.012

23. Silva AM, Descalço A, Salgueiro M, Pereira L, Barreto C, Bandeira T, et al. Respiratory sleep disturbance in children and adolescents with cystic fibrosis. *Rev Port Pneumol*. (2016) 22:202–8. doi: 10.1016/j.rppnen.2016.02.007

24. Waters KA, Lowe A, Cooper P, Vella S, Selvadurai H. A cross-sectional analysis of daytime versus nocturnal polysomnographic respiratory parameters in cystic fibrosis during early adolescence. *J Cyst Fibros*. (2017) 16:250–7. doi: 10.1016/j.jcf.2016.09.007

25. Veronezi J, Carvalho AP, Ricachinewsky C, Hoffmann A, Kobayashi DY, Piltcher OB, et al. Sleep-disordered breathing in patients with cystic fibrosis. *J Bras Pneumol*. (2015) 41:351–7. doi: 10.1590/S1806-37132015000004468

26. Muzumdar H, Arens R. Diagnostic issues in pediatric obstructive sleep apnea. *Proc Am Thorac Soc*. (2008) 5:263–73. doi: 10.1513/pats.200707-113MG

27. Katz ES. Cystic fibrosis and sleep. *Clin Chest Med*. (2014) 35:495–504. doi: 10.1016/j.ccm.2014.06.005

28. Hughes BH, Brinton JT, Ingram DG, Halbower AC. The impact of altitude on sleep-disordered breathing in children dwelling at high altitude: a crossover study. *Sleep*. (2017) 40:1–8. doi: 10.1093/sleep/zsx120

29. Bigham AW, Lee FS. Human high-altitude adaptation: forward genetics meets the HIF pathway. *Genes Dev*. (2014) 28:2189–204. doi: 10.1101/gad.250167.114

30. Spicuzza L, Sciuto C, Leonardi S, La Rosa M. Early occurrence of obstructive sleep apnea in infants and children with cystic fibrosis. *Arch Pediatr Adolesc Med*. (2012) 166:1165–9. doi: 10.1001/archpediatrics.2012.1177

31. de Castro-Silva C, de Bruin VM, Cavalcante AG, Bittencourt LR, de Bruin PF. Nocturnal hypoxia and sleep disturbances in cystic fibrosis. *Pediatr Pulmonol*. (2009) 44:1143–50. doi: 10.1002/ppul.21122

32. Ramos RT, Santana MA, Almeida Pde C, Machado Ade S Jr, Araújo-Filho JB, Salles C. Nocturnal hypoxemia in children and adolescents with cystic fibrosis. *J Bras Pneumol*. (2013) 39:667–74. doi: 10.1590/S1806-37132013000600005

33. Tepper RS, Skatrud JB, Dempsey JA. Ventilation and oxygenation changes during sleep in cystic fibrosis. *Chest*. (1983) 84:388–93. doi: 10.1378/chest.84.4.388

34. Humbert M, Kovacs G, Hoeper MM, Badagliacca R, Berger RMF, Brida M, et al. 2022 ESC/ERS guidelines for the diagnosis and treatment of pulmonary hypertension. *Eur Heart J*. (2022) 43:3618–731. doi: 10.1093/eurheartj/ehac237

35. Maloney MA, Ward SLD, Su JA, Durazo-Arvizu RA, Breunig JM, Okpara DU, et al. Prevalence of pulmonary hypertension on echocardiogram in children with severe obstructive sleep apnea. *J Clin Sleep Med*. (2022) 18:1629–37. doi: 10.5664/jcsm.9944

36. Koestenberger M, Apitz C, Abdul-Khaliq H, Hansmann G. Transthoracic echocardiography for the evaluation of children and adolescents with suspected or confirmed pulmonary hypertension. Expert consensus statement on the diagnosis and treatment of paediatric pulmonary hypertension.

The European paediatric pulmonary vascular disease network, endorsed by ISHLT and D6PK. *Heart*. (2016) 102(Suppl 2):ii14–22. doi: 10.1136/heartjnl-2014-307200

37. Ploegstra MJ, Roofthooft MT, Douwes JM, Bartelds B, Elzenga NJ, van de Weerd D, et al. Echocardiography in pediatric pulmonary arterial hypertension: early study on assessing disease severity and predicting outcome. *Circ Cardiovasc Imaging*. (2015) 8:1–10. doi: 10.1161/CIRCIMAGING.113.000878

38. Meinel K, Koestenberger M, Sallmon H, Hansmann G, Pieles GE. Echocardiography for the assessment of pulmonary hypertension and congenital heart disease in the young. *Diagnostics (Basel)*. (2020) 11:1–25. doi: 10.3390/diagnostics11010049

39. D'Alto M, Di Maio M, Romeo E, Argiento P, Blasi E, Di Vilio A, et al. Echocardiographic probability of pulmonary hypertension: a validation study. *Eur Respir J*. (2022) 60:1–13. doi: 10.1183/13993003.02548-2021

40. Parasuraman S, Walker S, Loudon BL, Gollop ND, Wilson AM, Lowery C, et al. Assessment of pulmonary artery pressure by echocardiography-a comprehensive review. *Int J Cardiol Heart Vasc*. (2016) 12:45–51. doi: 10.1016/j.ijcha.2016.05.011

41. Duenas-Meza E, Severiche-Bueno DF, Santos Quintero C, Talani Ochoa J, Ronderos Dummit M, Stapper C, et al. Prevalence of pulmonary hypertension in children with obstructive sleep apnea living at high altitude. *Sleep Med X*. (2024) 7:100106. doi: 10.1016/j.sleepx.2024.100106

frontiers | Frontiers in Pediatrics

TYPE Case Report
PUBLISHED 12 September 2024
DOI 10.3389/fped.2024.1402545

OPEN ACCESS

EDITED BY
Rujipat Samransamruajkit,
Chulalongkorn University, Thailand

REVIEWED BY
Ayca Aykut,
Ege University, Türkiye
Yesim Oztas,
Hacettepe University, Türkiye

*CORRESPONDENCE
Xiaolei Tang
✉ tangxl9@gmail.com

RECEIVED 17 March 2024
ACCEPTED 20 August 2024
PUBLISHED 12 September 2024

CITATION
Liu Y, Wang H, Tang Y, Zhang L, Su Y, Wang Y, Xu S, Mei S, Jia C, Shen Y and Tang X (2024) Case Report: Clinical manifestations and treatment of two Chinese patients with FINCA syndrome carrying a novel variant of *NHLRC2*. Front. Pediatr. 12:1402545. doi: 10.3389/fped.2024.1402545

Case Report: Clinical manifestations and treatment of two Chinese patients with FINCA syndrome carrying a novel variant of *NHLRC2*

Yuemei Liu[1], Hongling Wang[2], Yu Tang[1], Lei Zhang[1], Yanyan Su[1], Yanqion Wang[1], Shasha Xu[1], Shiyue Mei[3], Chunyang Jia[4], Yuelin Shen[5] and Xiaolei Tang[5]*

[1]Department of Respiratory Medicine, Children's Hospital Affiliated to Zhengzhou University, Henan Children's Hospital, Zhengzhou Children's Hospital, Zhengzhou, China, [2]Neonatology Department, Maternity and Child Health Care Hospital of Rizhao, Rizhao, China, [3]Henan Key Laboratory of Pediatric Genetics and Metabolic Diseases, Children's Hospital Affiliated to Zhengzhou University, Henan Children's Hospital, Zhengzhou Children's Hospital, Zhengzhou, China, [4]Radiology Department, Children's Hospital Affiliated to Zhengzhou University, Henan Children's Hospital, Zhengzhou Children's Hospital, Zhengzhou, China, [5]Department of Respiratory Medicine, Beijing Children's Hospital, National Center for Children's Health, Capital Medical University, Beijing, China

Fibrosis, neurodegeneration, and cerebral angiomatosis (FINCA) syndrome is an autosomal recessive genetic disorder caused by mutations in NHL-repeat-containing protein 2 (*NHLRC2*) gene. This case report describes two Chinese siblings with FINCA syndrome carrying a novel frameshift variant, c.1610dupT (p.L537Ffs*17), of *NHLRC2* gene. They shared similar symptoms of interstitial lung disease (ILD) and neurodegeneration, with early onset during infancy, and shared similar chest CT findings of bilateral ground-glass opacities and consolidations. The elder brother died of infantile respiratory failure, while the younger brother showed improvement in respiratory symptoms, chest CT, and Krebs von den Lungen-6 levels after long-term systemic glucocorticoid therapy, indicating that anti-inflammatory treatment may be beneficial in the treatment of ILD caused by FINCA syndrome.

KEYWORDS

***NHLRC2*, interstitial lung disease, lung fibrosis, neurodegeneration, genetics**

Introduction

Fibrosis, neurodegeneration, and cerebral angiomatosis (FINCA) syndrome, an autosomal recessive disorder caused by a variant of the NHL-repeat-containing protein 2 (*NHLRC2*) gene, characterized by interstitial lung fibrosis, neurodegeneration, and cerebral angiomatosis (1). FINCA syndrome may cause early infant death, mainly due to respiratory failure caused by progressive interstitial lung disease (ILD). However, no

Abbreviations

FINCA, fibrosis, neurodegeneration, and cerebral angiomatosis; *NHLRC2*, NHL-repeat-containing protein 2; KL-6, Krebs von den Lungen-6; HRCT, high-resolution computed tomography; MRI, magnetic resonance imaging; GGOs, ground-glass opacities; p-ANCA, perinuclear anti-neutrophil cytoplasmic antibodies; ROS, reactive oxygen species; ILD, interstitial lung disease; WES, whole-exome sequencing; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; ANCA, anti-neutrophil cytoplasmic antibodies; DQ, development quotient; ACMG, American College of Medical Genetics and Genomics; NSIP, non-specific interstitial pneumonitis; DIP, desquamative interstitial pneumonia; DAD, diffuse alveolar damage; PAP, pulmonary alveolar proteinosis; SAVI, STING-associated vasculopathy with onset in infancy.

effective treatment has been reported currently. In this article, we report two Chinese siblings carrying a novel *NHLRC2* gene variant. The ILD responded well to long-term systemic glucocorticoid therapy in the younger brother.

Case report

These two patients were siblings. Case 1 was the elder brother, a full-term boy with a birth weight of 2.8 kg and congenital heart defects (coarctation of the aorta, ventricular septal defect, atrial septal defect, and patent ductus arteriosus). He underwent a cardiac operation at the age of 1 month and also developed neonatal jaundice. He presented with recurrent cough, wheezing, and tachypnea beginning at 2 months. He has also experienced developmental delay, recurrent diarrhea, feeding problems, and poor weight gain from the age of 2 months. During illness, he needed nasogastric feeding but was fed orally after discharge. He had two episodes of respiratory exacerbations accompanied by respiratory failure, leading to hospitalizations at the ages of 3 and 8 months. During his first hospitalization at 3 months, a physical examination revealed crackles, wheezing, retractions, funnel chest, hypotonia, and poor visual contact. His respiratory rate was 60–90 times/min, and his oxygen saturation (SpO_2) was 80%–92%, requiring oxygen supplementation. He was anemic (hemoglobin 73 g/L), and his liver function indicators were slightly elevated [alanine aminotransferase (ALT) 53 U/L, total bilirubin (TBIL) 22 μmol/L]. His white blood cell count, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), renal function, and thyroid function tests were within normal ranges. *Pneumocystis jirovecii* were found in the bronchoalveolar lavage fluid. Immunological work-up showed a mild decrease in IgG and IgA levels, while lymphocyte subtypes remained normal. A chest CT showed bilateral ground-glass opacities (GGOs) and consolidations at the age of 3 months (Figure 1A). He developed respiratory failure necessitating invasive ventilation for 6 days and received anti-infection treatment for 1 month, with multiple antibiotics including trimethoprim sulfamethoxazole for *P. jirovecii* and antifungal drugs. He also received prednisone at a daily dose of 0.75 mg/kg for 20 days. After 1 month, repeated chest CT showed little improvement. After discharge, his cough improved, but tachypnea and oxygen desaturation persisted (respiratory rate 50–60 times/min, SpO_2 94%). At the age of 8 months, he presented with a short-term fever followed by another episode of respiratory failure. Multiple pathogens were found in the aspiration of sputum obtained from the oropharynx, nasal cavity, and bronchoalveolar lavage fluid during the second hospitalization, including *Staphylococcus aureus*, *Moraxella catarrhalis*, *Candida parapsilosis*, rhinovirus, and human metapneumovirus. The repeated chest CT indicated GGOs in the same area as before and increased consolidations (Figure 1B). Unfortunately, despite anti-infection treatment, he died of respiratory failure at the age of 8 months.

Case 2 was the younger brother, a full-term boy with a birth weight of 2.85 kg, who also developed neonatal jaundice. From the age of 1 month, he developed symptoms similar to his brother, including recurrent cough, tachypnea, developmental delay, recurrent diarrhea, and poor weight gain. He was able to feed orally without swallowing disorders. A physical examination revealed crackles, retractions, hypotonia, and poor visual contact. His respiratory rate was 50–80 times/min, and his SpO_2 was 90%–92%, requiring oxygen supplementation during hospitalization. He was anemic (hemoglobin 69 g/L), and his liver function indicators were slightly elevated (ALT 103 U/L, TBIL 51.5 μmol/L). No pathogens, including *P. jirovecii*, viruses, bacteria, and fungi, were found during hospitalization. Autoantibody testing indicated positive perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCAs). Krebs von den Lungen-6 (KL-6), a biomarker for ILD activity, was elevated at 2,107 U/ml (normal range <500 U/ml). His chest CT was similar to his brother's, showing GGOs and consolidations, mainly distributed in the lower lungs (Figure 1C). At the age of 1 month, steroid treatment was started (daily dose of 2–1 mg/kg of methylprednisolone for 2 weeks, followed by oral prednisone with a tapered dosage from 0.7 to 0.2 mg/kg/day in 2 months and then maintained at 0.2 mg/kg/day for 1 year), combined with short-term antibiotics for prevention of infection. After more than 2 months of treatment (at the age of 4 months), his chest CT improved (Figure 1D), with repeated KL-6 levels decreasing to 1,417 U/ml. After 13 months of treatment (at the age of 14 months), his chest CT showed the development of cysts and improvement in GGOs and consolidations (Figure 1E). Repeated KL-6 levels decreased to 430 U/ml. His respiratory symptoms, such as cough and tachypnea, improved. His respiratory rate was 30–40 times/min, and SpO_2 was maintained at 97%–100% at the age of 14 months. He did not require oxygen supplementation at home. However, his neurological symptoms did not alleviate. At the age of 4 months, his development quotient (DQ) evaluated by the Chinese Development Scale for Children Aged 0–6 years was 39 (normal range >85), indicating moderate intellectual disability. He experienced his first seizure at 7 months with no inducement, followed by another seizure at 14 months of age, accompanied by vomiting and diarrhea. He did not receive antiepileptic therapy. At the age of 14 months, his motor development was significantly delayed compared to his peers. He could not turn over, crawl, or walk. His brain magnetic resonance imaging (MRI) showed a widening of cerebral sulci and fissures, cortical thinning, and enlargement of the frontal and temporal angles, without evidence of cerebral angiomatosis (Figures 2A–C). He continued to be fed orally at home and weighed 7 kg.

Whole-exome sequencing (WES) was performed in both of the siblings, showing the same findings of two compound heterozygous variants of *NHLRC2* gene. One variant, c.442 G>T, p.D148Y, was inherited from the father. The other, a novel frameshift variant, c.1610dupT, p.L537Ffs*17, was inherited from the mother. The results were validated by Sanger sequencing (Figures 3A–C). The p.D148Y variant is a missense mutation, which has been previously reported in the Human Gene Mutation Database (HGMD) with associated evidence of pathogenicity, and the ClinVar database classifies this variant as pathogenic. PCR Sanger sequencing validated that the probands' father is

10.3389/fped.2024.1402545

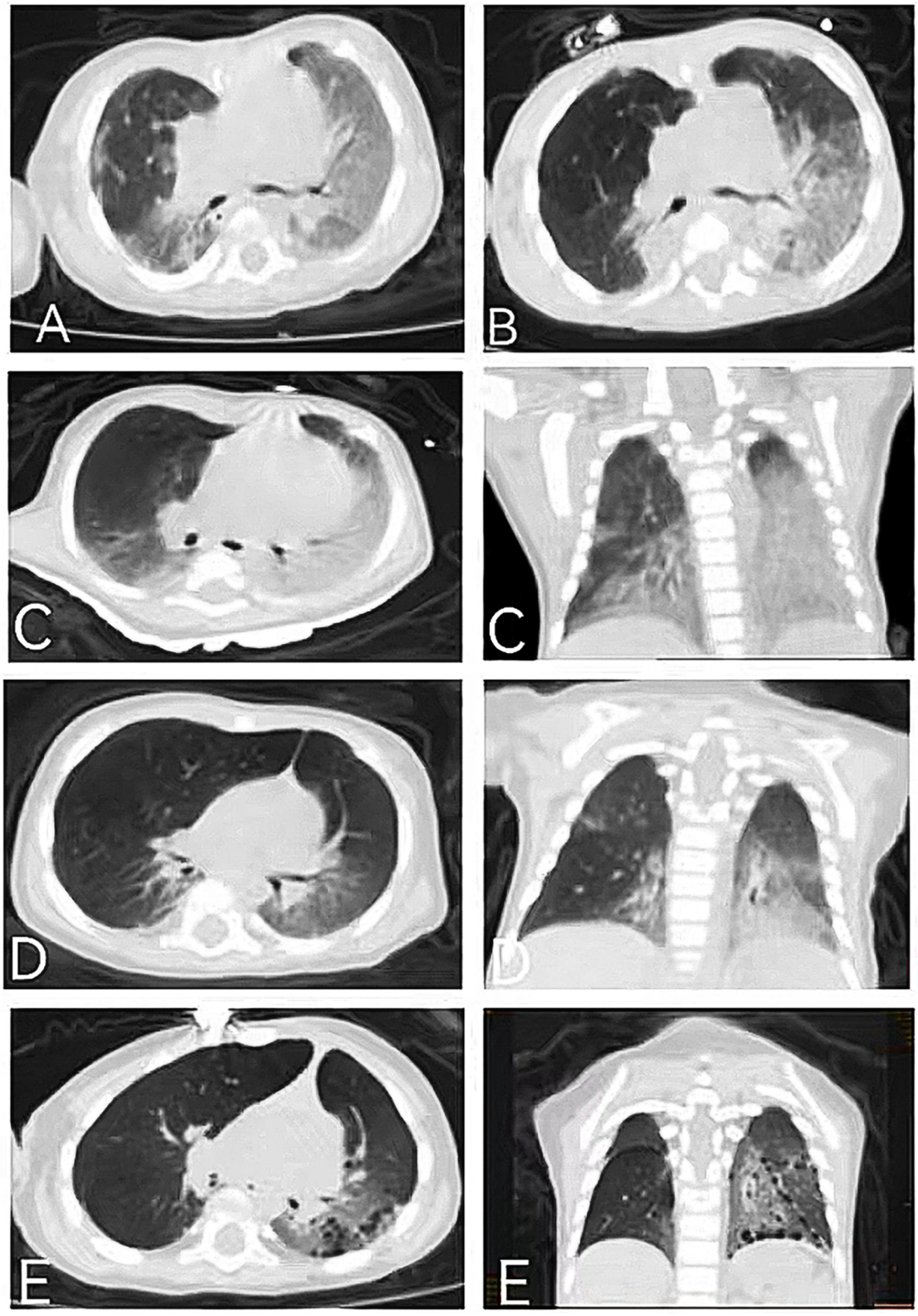


FIGURE 1
Chest CT of the elder **(A,B)** and younger **(C–E)** brothers: **(A)** chest CT of the elder brother showing bilateral GGOs, consolidations, and reticular opacities at the age of 3 months; **(B)** chest CT of the elder brother showing slightly improved GGOs but aggravated consolidations at the age of 8 months; **(C)** chest CT of the younger brother showing GGOs, consolidations, and mediastinal lung hernia at the age of 1 month; **(D)** chest CT of the younger brother showing improved GGOs and consolidations at the age of 4 months; and **(E)** chest CT of the younger brother showing the appearance of cysts and improved GGOs and consolidations at the age of 14 months.

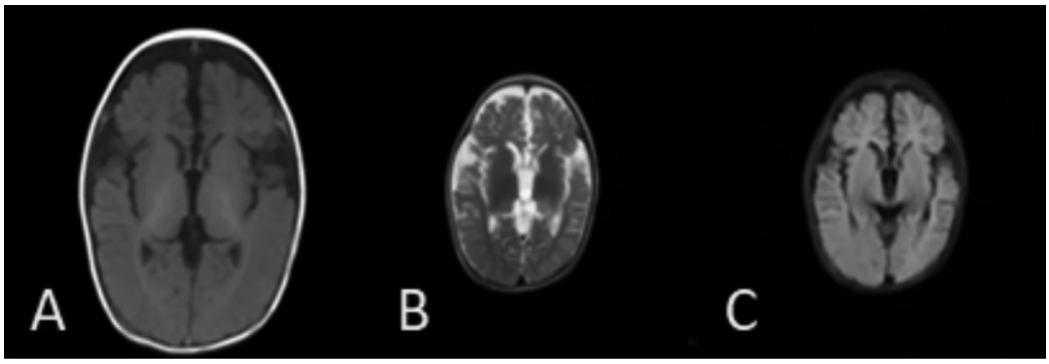


FIGURE 2
Brain MRI of the younger brother [(A) T1, (B) T2, (C) T2 FLAIR]: brain MRI of the younger brother revealing a widening of cerebral sulci and fissures, cortical thinning, and enlargement of the frontal and temporal angles at the age of 14 months.

Gene	Chromosomal location	Transcript (exon/ intron)	Nucleotide, amino acid change	RS number	MAF	ACMG pathogenicity	Proband (male)	Father (healthy)	Mother (healthy)
NHLRC2	chr10:115663401-115663402	NM_198514(exon9)	c.1610dupT p.L537fs*17	none	Not included	pathogenic	heterozygote	wild type	heterozygote
	chr10:115636390	NM_198514(exon3)	c.442G>T p.D148Y	rs201701259	0.0002	pathogenic	heterozygote	heterozygote	wild type

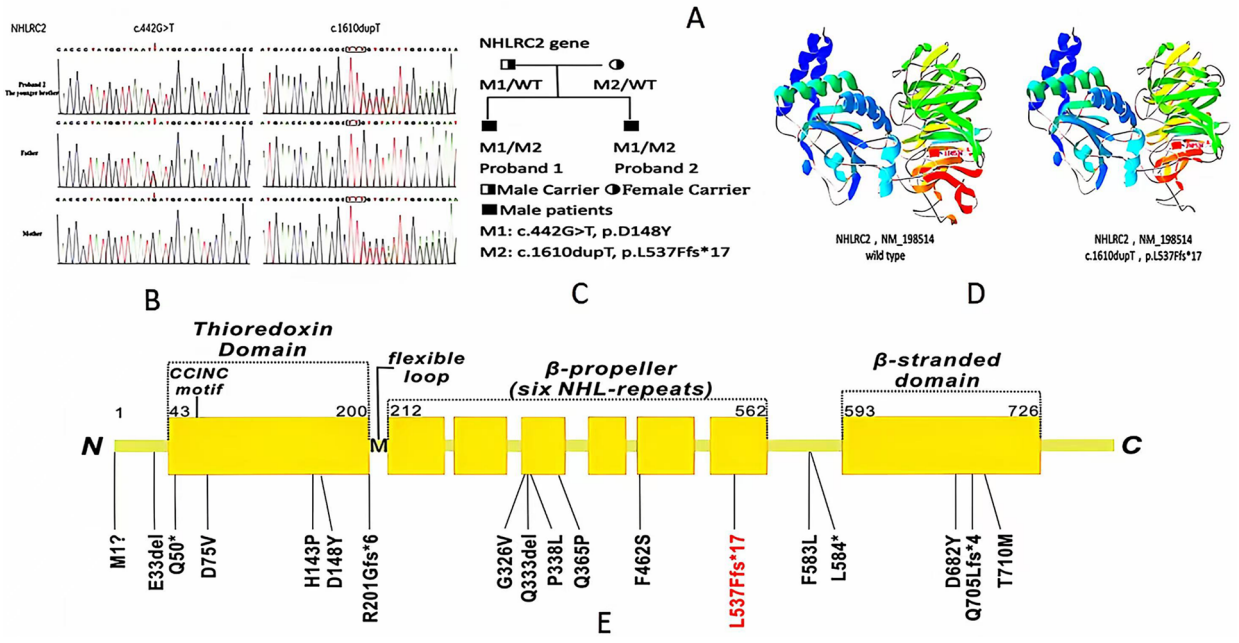


FIGURE 3
(A,B) WES information and Sanger sequencing of *NHLRC2* variants of the elder and younger brothers; (C) pedigree of the family; (D) structure of the *NHLRC2* wild-type and c.1610dupT, p.L537Ffs*17 variant; and (E) novel variant (red color) and previously reported variants of *NHLRC2* (black color) 1, 8–11.

heterozygous for this mutation, while the probands' mother does not carry the mutation. According to the standards and guidelines set by the American College of Medical Genetics and Genomics (ACMG), this variant is pathogenic. The other novel variant, p.L537Ffs*17, is a frameshift mutation leading to a truncated protein (Figure 3D), which qualifies as pathogenic very

strong (PVS1) evidence of ACMG criteria. PCR Sanger sequencing verified that the probands' father does not have this mutation, while the probands' mother is heterozygous for it, forming a compound heterozygous mutation in combination with the p.D148Y variant. The inheritance pattern is consistent with autosomal recessive inheritance, and both affected brothers in the family carry the same variants, exhibiting similar symptoms of ILD and neurological involvement, which aligns with the clinical phenotype of FINCA syndrome caused by *NHLRC2* mutations, constituting PM2 supporting evidence. This variant is exceedingly rare in the general population, with no record in normal population databases, and there are no reports in either the HGMD or ClinVar databases, which constitutes PM3 evidence. Therefore, based on ACMG criteria, the p.L537Ffs*17 variant of *NHLRC2* gene is predicted to be pathogenic. Protein structure analysis of *NHLRC2* showed that the p.L537Ffs*17 variant leads to an incomplete protein structure beyond the sixth NHL-repeat in the β-propeller domain (Figure 3D).

Discussion

FINCA syndrome is a recently discovered monogenetic disease related to NHLRC2 dysfunction. Lung fibrosis, neurodegeneration, and cerebral angiomatosis are primary manifestations of FINCA syndrome. NHLRC2 is a 79-kDa protein composed of 726 amino acids containing three domains, an N-terminal thioredoxin-like (Trx-like) domain, a six-bladed NHL-repeat-containing β-propeller domain, and a C-terminal β-stranded domain (2). Downregulation of NHLRC2 has been shown to increase the susceptibility of human colon cancer cells to reactive oxygen species (ROS)–induced apoptosis (3). NHLRC2 also plays an important role in phagocytosis by controlling actin polymerization, filopodium formation (4), and T-cell homeostasis (5). The pathogenic mechanisms leading to the clinical manifestations of ILD/lung fibrosis and neurodegeneration in FINCA syndrome caused by NHLRC2 deficiency have not yet been fully elucidated. NHLRC2 has been previously flagged in a study as a differentially expressed gene when comparing rapidly and slowly progressing idiopathic pulmonary fibrosis (IPF) patients (6). It also has been linked to decreased lung function values (7). A study conducted by Paakkola et al. suggested that NHLRC2 may induce tissue fibrosis (8). NHLRC2 was identified to interact in several cytosolic processes, including cell–cell adhesion, cell division, and intracellular protein transport using proximity-labeling mass spectrometry. A transmission electron microscopy analysis of immortalized cell cultures derived from skin biopsies of FINCA patients demonstrated multilamellar bodies and distinctly organized vimentin filaments. In addition, in two out of three cultures from patient-derived skin biopsies, cells displaying characteristics typical of myofibroblasts were identified. These findings suggested *NHLRC2* induces severe tissue fibrosis by enhancing the differentiation of fibroblasts into myofibroblasts, regulating the cytoskeleton, and affecting vimentin intermediate-size filaments, vesicle transportation, and pro-inflammatory regulators (8). In another study, Hiltunen et al. made a proteomic analysis of the *NHLRC2* FINCA mice model harboring the missense mutation p.(D148Y) of a FINCA patient. Compared to wild-type mice, FINCA mice revealed dysfunction in vesicular trafficking. According to the authors, *NHLRC2* dysfunction is associated with the accumulation of RNA-binding proteins in a FINCA mouse model, suggesting that disrupted RNA metabolism may contribute to neurodegeneration in FINCA patients (9). In future research, metabolomics and proteomics studies may play an active role in identifying biomarker signatures related to disease phenotypes and monitoring therapeutic interventions.

So far, only 17 variants from 29 patients of NHLRC2 have been identified to be associated with FINCA syndrome (1, 10–14) (Figure 3E). The p.D148Y variant is a hotspot variant found in most patients with FINCA syndrome, including our newly reported patients. The p.D148Y variant is located in the Trx-like domain, which is a characteristic of oxidoreductases and thiol–disulfide exchange (2). To investigate for a possible genotype–phenotype correlation, Sczakiel et al. observed a correlation of remaining *NHLRC2* protein levels with phenotype severity. They speculate that variants leading to severely reduced protein levels (either in a homozygous state or in a compound heterozygous state with another severe missense or frameshift/nonsense variant) are associated with an early-onset multisystem phenotype that includes pulmonary disease (14). In our study, we reported a novel variant of c.1610dupT, p.L537Ffs*17 in two patients from one family, which is the frameshift variant. It is located in the sixth NHL-repeat in the β-propeller domain, which functions as a protein–protein interaction module (15) (Figure 3E). Both siblings presented with early onset and severe respiratory and neurological symptoms, with one succumbing to respiratory failure in infancy, indicating that the p.L537Ffs*17 variant may be associated with severe phenotypes.

Among the 31 FINCA patients, including our newly reported patients, 14 patients (45.2%) were boys (1, 10–14). All FINCA patients experienced neurological symptoms including developmental delay, intellectual disability, behavior problems, movement disorder, hypotonia, dystonia, and seizures, among others (1, 10–14). Other clinical manifestations involve the respiratory system, such as ILD; gastrointestinal issues like diarrhea, hepatomegaly, feeding problems, and liver dysfunction; cardiovascular complications including congenital heart disease, cardiomegaly, pulmonary hypertension, and dilation of the ascending aorta; and other system involvements, such as anemia and renal insufficiency (1, 10–14) (as presented in Table 1). About half of the 31 FINCA patients presented with respiratory symptoms, such as tachypnea, cough, hypoxemia, and respiratory distress. Chest CT scans were performed on 11 patients, among whom 9 children were diagnosed with ILD, with the most prevalent findings being GGOs and pulmonary consolidations. It is noteworthy that fatalities predominantly occur in patients with the ILD phenotype. The overall mortality rate among 31 FINCA patients is 25.8%, whereas the mortality rate for those 9 patients with comorbid ILD is significantly higher at 77.8%. All deceased children died before the age of 3 (1, 10–14). This suggests that

Table1. Clinical manifestation and genotype of thirty-one patients with FINCA syndrome.

Individual (Family)	proband1 (F1)	proband2 (F1)	proband3[1] (F2)	proband4[1] (F2)	proband5[1] (F3)	proband6[8] (F4)	proband7[9] (F5)	proband8[9] (F5)	proband9[9] (F5)	proband10[9] (F6)	proband11[9] (F7)	proband12[9] (F7)	proband13[10] (F8)	proband14[10] (F9)	proband15[10] (F10)	proband16[11] (F11)
Sex	male	male	male	male	male	male	male	female	female	female	male	female	female	female	female	male
Genetic variants	p.D148Y/ p.L537Ffs*17	p.D148Y/ p.L537Ffs*17	p.D148Y/ p.R201Gfs*6	p.D148Y/ p.R201Gfs*6	p.D148Y/ p.R201Gfs*6	p.D148Y/ p.H143P	N/A	p.D148Y/ p.D75V	p.D148Y/ p.D75V	p.D148Y/ p.P338L	Homo p.D148Y	Homo p.D148Y	Homo p.D148Y	Homo p.D148Y	p.D148Y/ p.G326V	p.F583L/ p.T710M
Origin	Chinese	Chinese	Finland	Finland	Finland	Ukrainian	Greek	Greek	Greek	Belgian	Jordanian	Jordanian	Polish	Polish	Polish	Chinese
Duration of gestation	full term	full term	full term	full term	36+1weeks	full term	full term	full term	full term	full term	full term	full term	N/A	N/A	N/A	full term
Birth weight	2800 g	2850 g	3690 g	3280 g	2910 g	3050 g	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3700 g
Apgar score	10,10	10,10	10,10	9,9	10,10	8,8	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Age of onset	2 m	1 m	2 m	2 m	0-2 m	0 m	7 m	5 m	0-2 m	0.5m	0 m	12 m	0 m	9 m	3 m	17d
Respiratory involvements	+	+	+	+	+	+	+	+	+	+	+	–	N/A	N/A	N/A	+
ILD	+	+	+	+	+	+	+	+	–	+	N/A	N/A	N/A	N/A	N/A	N/A
[a]Neurological involvements	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
[b]Gastrointestinal involvements	+	+	+	+	+	+	+	+	+	+	N/A	N/A	–	–	+	N/A
[c]Cardiovascular involvements	+	–	+	–	–	+	–	–	–	+	–	–	–	–	–	+
Anemia	+	+	+	+	+	+	+	–	–	+	–	–	–	+	–	N/A
Kidney dysfunction	–	–	+	–	–	–	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Immune system	decreased IgG and IgA	N/A	N/A	N/A	decreased IgG	decreased IgG, decreased CD3, CD4, CD8 cells	decreased IgG and IgA	decreased IgG	–	decreased IgG	N/A	N/A	N/A	N/A	N/A	N/A
Autoantibodies	N/A	p-ANCA(+)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Age at last follow-up	8 m	14 m	21 m	13 m	14 m	29 m	22 m	17 m	10 y	4 y	14 y	7 y	6 y	9 y	12 y	27 d
Status at last follow-up	death	alive	death	death	death	death	death	death	alive	alive	alive	alive	alive	alive	alive	death

Individual (Family)	proband17[14] (F12)	Proband18[14] (F12)	proband19[14] (F13)	Proband20[14] (F14)	Proband21[14] (F15)	Proband22[14] (F16)	Proband23[14] (F17)	Proband24[14] (F17)	Proband25[14] (F18)	Proband26[14] (F19)	Proband27[14] (F20)	Proband28[14] (F21)	Proband29[14] (F21)	Proband30[14] (F22)[17]	Proband31[14] (F23)
Sex	female	male	female	female	female	Female	male	male	male	male	female	female	female	female	female
Genetic variants	Homo p.M1?	Homo p.M1?	Homo p.D148Y	p.L584*/ p.D692Y	p.Q50*/ p.D148Y	p.Q365P/ p.F462S	Homo p.M1?	Homo p.M1?	Homo p.D148Y	Homo p.E33del	p.Q333del/ p.Q705Lfs*4	Homo p.D148Y	Homo p.D148Y	Homo p.D148Y	Homo p.D148Y
Origin	Syria		Palestine	Belgian, Hungarian, English, and Scottish	German	African American, Scandanavian, Irish, English, German	Libanon		Iran	Iran	Caucasian	Iran		Pakistan	Iran
Duration of gestation	full term	N/A	full term	full term	full term	full term	full term	full term	36weeks	full term	full term	full term	N/A	full term	full term
Birth weight	3200 g	3300 g	2800 g	3033g	3300 g	3005 g	N/A	N/A	2900 g	3300 g	N/A	3200 g	3000 g	N/A	3520
Apgar score	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Age of onset	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–

(*Continued*)

10.3389/fped.2024.1402545

10.3389/fped.2024.1402545

Table1. Continued

Individual (Family)	proband17[14] (F12)	Proband18[14] (F12)	proband19[14] (F13)	Proband20[14] (F14)	Proband21[14] (F15)	Proband22[14] (F16)	Proband23[14] (F17)	Proband24[14] (F17)	Proband25[14] (F18)	Proband26[14] (F19)	Proband27[14] (F20)	Proband28[14] (F21)	Proband29[14] (F21)	Proband30[14] (F22)[17]	Proband31[14] (F23)
Respiratory involvements															
ILD	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−
[a]Neurological involvements	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
[b]Gastrointestinal involvements	−	−	−	+	+	−	−	−	−	+	−	−	−	−	+
[c]Cardiovascular involvements	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−
Anemia	+	−	−	−	+	−	−	−	−	+	−	+	−	−	−
Kidney dysfunction	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
Immune system	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Autoantibodies	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Age at last follow-up	5 y	19 y	2 y	15 y	22 m	13 y	19 y	9 y	8 y	5y	8 y	4y	10 y	7 y	6 y
Status at last follow-up	alive	alive	alive	alive	dead	alive	alive	alive	alive	alive	alive	alive	alive	alive	alive

m: months; y: years; d: days; Homo: homozygous; N/A, not available; ILD, interstitial lung disease; ANCA, antineutrophil cytoplasmic antibodies. [a]Neurological involvements: include developmental delay, intellectual disability, behavior problem, movement disorder, hypotonia, dystonia and seizures; [b]Gastrointestinal involvements: include diarrhea, hepatomegaly, feeding problem and liver dysfunction; [c]Cardiovascular involvements: include congenital heart disease, cardiomegaly, pulmonary hypertension, and dialation of ascend aorta.

ILD is a significant factor contributing to the early mortality of pediatric FINCA patients, necessitating particular attention.

Histological findings in the lung of ILD patients, based on lung biopsy or autopsy, include granuloma-like lesions surrounded by myofibroblasts, non-specific interstitial pneumonitis (NSIP), cholesterol pneumonitis, desquamative interstitial pneumonia (DIP), diffuse alveolar damage (DAD), and pulmonary alveolar proteinosis (PAP) (1, 10–12) (Table 2). These symptoms, along with chest CT and lung histological findings, are not specific to ILD and may remind us of surfactant dysfunction disorders. For example, the clinical manifestations of surfactant dysfunction disorder caused by an *NKX2-1* mutation are very similar to those of early-onset ILD and neurological involvement, which needs to be excluded as a differential diagnosis. Unlike in the *NKX2-1* mutation, FINCA patients exhibit normal thyroid function. Other diseases, such as STING-associated vasculopathy with onset in infancy (SAVI) and COPA syndrome, which may present with early-onset ILD and occasionally accompanied by neurological involvement, and FLNA mutations, which may cause ILD, skeletal dysplasia, neuronal migration abnormality, cardiovascular malformation, intellectual disability, and intestinal obstruction (16), should also be excluded as differential diagnoses.

Until now, there have been few reports on the treatment of ILD caused by FINCA syndrome. Progressive respiratory symptoms and exacerbations due to ILD were the main causes of death in the previously reported cases. Therefore, the treatment of ILD contributes significantly to the prognosis of FINCA syndrome. Systemic glucocorticoids, the first-line treatment for ILD, have been administered to four patients with FINCA syndrome before. However, the efficacy of the treatment remains uncertain (11). Two of them survived. One of them responded well to high-dose steroids. The other patient received anti-inflammatory treatment, including glucocorticoids and hydroxychloroquine, until the age of 2 years, and the patient's medical condition stabilized over the first 3 years of life (11). Two patients died despite glucocorticoid treatment, while the duration of their treatment remains unclear (11) (as presented in Table 2). In our case study, we reported a patient with FINCA syndrome undergoing long-term steroid treatment since the age of 1 month. After 13 months of treatment, the patient's respiratory symptoms improved, KL-6 levels decreased, and chest CT showed improvement, indicating that long-term glucocorticoids or other anti-inflammatory therapies may be beneficial in the treatment of FINCA-induced ILD. Although the physiological function of *NHLRC2* is not yet fully elaborated, previous studies have demonstrated that the possible pathogenic mechanism of *NHLRC2* is associated with inflammation or autoimmunity. Overexpression of *NHLRC2* has been shown to decrease the expression levels of vimentin and IL-1β, suggesting that *NHLRC2* deficiency may be involved in the mechanism of fibrogenesis by regulating inflammatory pathways (8). In addition, *NHLRC2* expression was found to increase under inflammatory conditions in an equine model of chronic asthma (17). Zinc finger and AT-hook domain containing (Zfat) protein, a transcription factor for *NHLRC2*, are essential for T-cell homeostasis (5). These findings may provide supporting evidence for the use of glucocorticoids in treating FINCA

TABLE 2 Respiratory symptoms, treatments, and prognosis of nine patients with FINCA syndrome associated with ILD.

Individual (family)	Proband1 (F1)	Proband2 (F1)	Proband3[1] (F2)	Proband4[1] (F2)	Proband5[1] (F3)	Proband6[8] (F4)	Proband7[9] (F5)	Proband8[9] (F5)	Proband10[9] (F6)
Tachypnea	+	+	+	+	+	+	+	+	+
Cough	+	+	N/A	N/A	+	N/A	N/A	N/A	N/A
Hypoxemia	+	+	–	+	+	N/A	+	+	+
Recurrent respiratory infections	+	+	+	+	+	+	+	+	+
Mechanical ventilation	+	–	N/A	N/A	N/A	+	+	+	–
Pathogens	*P. jirovecii*, *S. aureus*, *M. catarrhalis*, rhinovirus, human metapneumovirus, *C. parapsilosis*	–	–	N/A	Influenza B	Bacterial and viral	–	N/A	–
Chest HRCT	GGOs, consolidations	GGOs, consolidations, cysts, and mediastinal lung hernia	Consolidations and reticular opacities	GGOs, interstitial infiltration, atelectasis, enlarged thymus and left hilar	GGOs, interstitial septal thickening, and lobular pleural thickening	Consolidations/ atelectasis, air bronchograms, compressed trachea, and pectus excavatum	GGOs and consolidations	GGOs, consolidations, mosaic patterns, bronchiectasis, interstitial and alveolar markings, and cysts	GGOs, paraseptal and centrilobular emphysema, and cysts
Lung histology	N/A	N/A	NSIP, DAD, granuloma-like lesions surrounded by myofibroblasts	NSIP, granuloma-like lesions surrounded by myofibroblasts	NSIP, DAD, granuloma-like lesions surrounded by myofibroblasts	Fibrosis	DIP with diffuse alveolar damage	PAP	Cholesterol Pneumonitis, NSIP
Treatment for ILD	Short-term steroids and antibiotics	Long-term steroids	N/A	N/A	N/A	N/A	Steroids, catecholamines, and inhaled nitric oxide	Hydroxychloroquine and steroids	Hydroxychloroquine, pulse corticosteroids
Response to steroids	±	+	N/A	N/A	N/A	N/A	–	–	±
Age at the last follow-up	8 months	14 months	21 months	13 months	14 months	29 months	22 months	17 months	4 years
Status at the last follow-up	Death	Alive	Death	Death	Death	Death	Death	Death	Alive

GGOs, ground-glass opacities; NSIP, non-specific interstitial pneumonia; DAD, diffuse alveolar damage; DIP, desquamative interstitial pneumonia; PAP, pulmonary alveolar proteinosis; N/A, not available; ILD, interstitial lung disease; HRCT, high-resolution computed tomography.

10.3389/fped.2024.1402545

syndrome. The elevated p-ANCA found in one of our patients also indicates the involvement of an autoimmune mechanism in FINCA syndrome.

Conclusions

We report two Chinese siblings with FINCA syndrome carrying a novel variant of *NHLRC2* gene. Systemic glucocorticoids proved effective in treating ILD in one patient, indicating that anti-inflammatory therapy may be beneficial for treating FINCA-induced ILD.

Data availability statement

The datasets for this article are not publicly available due to concerns regarding participant/patient anonymity. Requests to access the datasets should be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the ethical committee of the Children's Hospital Affiliated to Zhengzhou University, Henan Children's Hospital, Zhengzhou Children's Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

Author contributions

YL: Data curation, Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. HW: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. YT: Writing – review & editing. LZ: Data curation, Writing – review & editing, Validation. YSu: Data curation, Writing – review & editing. YW: Data curation, Writing – review & editing. SX: Writing – review & editing, Data curation. SM: Validation, Writing – review & editing. CJ: Data curation, Writing – review & editing. YSh: Writing – review & editing. XT: Writing – review & editing, Writing – original draft.

Funding

The authors declare financial support was received for the research, authorship, and/or publication of this article. National Natural Science Foundation of China, Youth Science Foundation Project with approval number 82202061.

Acknowledgments

The authors thank the laboratory technologists for their collaboration.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Uusimaa J, Kaarteenaho R, Paakkola T, Tuominen H, Karjalainen MK, Nadaf J, et al. NHLRC2 variants identified in patients with fibrosis, neurodegeneration, and cerebral angiomatosis (FINCA): characterisation of a novel cerebropulmonary disease. *Acta Neuropathol.* (2018) 135(5):727–42. doi: 10.1007/s00401-018-1817-z.

2. Biterova E, Ignatyev A, Uusimaa J, Hinttala R, Ruddock LW. Structural analysis of human NHLRC2, mutations of which are associated with FINCA disease. *PLoS One.* (2018) 13(8):e0202391. doi: 10.1371/journal.pone.0202391

3. Nishi K, Iwaihara Y, Tsunoda T, Doi K, Sakata T, Shirasawa S, et al. ROS-induced cleavage of NHLRC2 by caspase-8 leads to apoptotic cell death in the HCT116 human colon cancer cell line. *Cell Death Dis.* (2017) 8:3218. doi: 10.1038/s41419-017-0006-7

4. Haney MS, Bohlen CJ, Morgens DW, Ousey JA, Barkal AA, Tsui CK, et al. Identification of phagocytosis regulators using magnetic genome-wide CRISPR screens. *Nat Genet.* (2018) 50(12):1716–27. doi: 10.1038/s41588-018-0254-1

5. Ishikura S, Tsunoda T, Nakabayashi K, Doi K, Koyanagi M, Hayashi K, et al. Molecular mechanisms of transcriptional regulation by the nuclear zinc-finger protein Zfat in T cells. *Biochim Biophys Acta.* (2016) 1859(11):1398–410. doi: 10.1016/j.bbagrm.2016.08.010

6. Boon K, Bailey NW, Yang J, Steel MP, Groshong S, Kervitsky D, et al. Molecular phenotypes distinguish patients with relatively stable from progressive idiopathic pulmonary fibrosis (IPF). *PLoS One.* (2009) 4:e5134. doi: 10.1371/journal.pone.0005134

7. Lee B-Y, Cho S, Shin DH, Kim H. Genome-wide association study of copy number variations associated with pulmonary function measures in Korea associated resource (KARE) cohorts. *Genomics.* (2011) 97:101–5. doi: 10.1016/j.ygeno.2010.11.001

8. Paakkola T, Salokas K, Miinalainen I, Lehtonen S, Manninen A, Kaakinen M, et al. Biallelic mutations in human NHLRC2 enhance myofibroblast differentiation in FINCA disease. *Hum Mol Genet.* (2018) 27(24):4288–302. doi: 10.1093/hmg/ddy298

9. Hiltunen AE, Kangas SM, Ohlmeier S, Pietilä I, Hiltunen J, Tanila H, et al. Variant in NHLRC2 leads to increased hnRNP C2 in developing neurons and the hippocampus of a mouse model of FINCA disease. *Mol Med.* (2020) 26(1):123. doi: 10.1186/s10020-020-00245-4

10. Brodsky NN, Boyarchuk O, Kovalchuk T, Hariyan T, Rice A, Ji W, et al. Novel compound heterozygous variants in NHLRC2 in a patient with FINCA syndrome. *J Hum Genet*. (2020) 65(10):911–5. doi: 10.1038/s10038-020-0776-0

11. Rapp CK, Van Dijck I, Laugwitz L, Boon M, Briassoulis G, Ilia S, et al. Expanding the phenotypic spectrum of FINCA (fibrosis, neurodegeneration, and cerebral angiomatosis) syndrome beyond infancy. *Clin Genet*. (2021) 100(4):453–61. doi: 10.1111/cge.14016

12. Badura-Stronka M, Śmigiel R, Rutkowska K, Szymańska K, Hirschfeld AS, Monkiewicz M, et al. FINCA syndrome-defining neurobehavioral phenotype in survivors into late childhood. *Mol Genet Genomic Med.* (2022) 10(4):e1899. doi: 10.1002/mgg3.1899

13. Li Y, Zhang Y, Jiang G, Wang Y, He C, Zhao X, et al. Case report: novel mutations of NDUFS6 and NHLRC2 genes potentially cause the quick postnatal death of a Chinese Hani minority neonate with mitochondrial complex I deficiency and FINCA syndrome. *Medicine (Baltimore)*. (2022) 101(27):e29239. doi: 10.1097/MD.0000000000029239

14. Sczakiel HL, Zhao M, Wollert-Wulf B, Danyel M, Ehmke N, Stoltenburg C, et al. Broadening the phenotypic and molecular spectrum of FINCA syndrome: biallelic NHLRC2 variants in 15 novel individuals. *Eur J Hum Genet*. (2023) 31(8):905–17. doi: 10.1038/s41431-023-01382-0

15. Tocchini C, Ciosk R. TRIM-NHL proteins in development and disease. *Semin Cell Dev Biol.* (2015) 47–48:52–9. doi: 10.1016/j.semcdb.2015.10.017

16. Sasaki E, Byrne AT, Phelan E, Cox DW, Reardon W. A review of filamin A mutations and associated interstitial lung disease. *Eur J Pediatr*. (2019) 178 (2):121–9. doi: 10.1007/s00431-018-3301-0

17. Lavoie J-P, Lefebvre-Lavoie J, Leclere M, Lavoie- Lamoureux A, Chamberland A, Laprise C, et al. Profiling of differentially expressed genes using suppression subtractive hybridization in an equine model of chronic asthma. *PLoS One*. (2012) 7:e29440. doi: 10.1371/journal.pone.0029440

frontiers | Frontiers in Pediatrics

TYPE Review
PUBLISHED 27 March 2025
DOI 10.3389/fped.2024.1343870

OPEN ACCESS

EDITED BY
Hongxing Dang,
Children's Hospital of Chongqing Medical University, China

REVIEWED BY
Renqiang Yu,
Jiangnan University, China
Cristina Loddo,
Azienda Ospedaliero-Universitaria Cagliari, Italy

*CORRESPONDENCE
Liang Li
li@sustech.edu.cn
Gui Yang
yanggui0017@163.com

†These authors share first authorship

RECEIVED 24 November 2023
ACCEPTED 09 September 2024
PUBLISHED 27 March 2025

CITATION
Li M, Cheng W-X, Li S, Wang J, Chen Y-R, Li L and Yang G (2025) Research progress on pathophysiologic mechanisms, clinical treatment and predictive biomarkers in bronchopulmonary dysplasia: from the perspective of oxidative stress.
Front. Pediatr. 12:1343870.
doi: 10.3389/fped.2024.1343870

Research progress on pathophysiologic mechanisms, clinical treatment and predictive biomarkers in bronchopulmonary dysplasia: from the perspective of oxidative stress

Min Li[1,2,3†], Wen-Xiang Cheng[2†], Shuo Li[4†], Jing Wang[1], Yan-Rui Chen[1], Liang Li[5*] and Gui Yang[1,6*]

[1]Department of Otolaryngology, Longgang Central Hospital, Shenzhen, China, [2]Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China, [3]Guangxi Hospital Division of the First Affiliated Hospital, Sun Yat-sen University, Nanning, China, [4]Department of Otolaryngology, Huazhong University of Science and Technology Union Shenzhen Hospital, Shenzhen, China, [5]School of Medicine, Southern University of Science and Technology, Shenzhen, China, [6]Department of Otolaryngology & Allergy Center, The Central Hospital of Shenzhen Longgang District, Shenzhen, China

With the global rise in preterm birth rates, bronchopulmonary dysplasia (BPD) continues to be a significant problem, affecting morbidity and mortality in surviving preterm infants. Preterm infants are particularly susceptible to oxidative stress induced by sudden increases in oxygen concentration, which plays a crucial role in the pathogenesis of BPD. Herein, we addressed the pathophysiologic mechanisms, clinical treatment, and predictive biomarkers of BPD from an oxidative stress perspective. We first review the importance of oxygen in preterm infants and point out that sustained exposure to hyperoxia exacerbates the susceptibility of the immature lung to free radicals. The antioxidant properties of clinical therapies for BPD in preterm infants are then summarized. Subsequently, based on lipid, protein, and DNA damage mechanisms, we obtained the most comprehensive, accurate, and representative oxidative stress biomarkers. A total of 37 research papers on oxidative stress in BPD were collected. We conclude that 8-OHdG is the most promising biomarker for early prediction of BPD pathogenesis compared to lipid and protein oxidative stress biomarkers.

KEYWORDS

bronchopulmonary dysplasia, hyperoxia exposure, oxidative stress, antioxidant treatments, predictive biomarkers

1 Introduction

Preterm birth, defined as birth before 37 weeks of gestation, is a significant global health issue for infants (1). According to the World Health Organization's report, an estimated 15 million premature births occur annually worldwide, with preterm rates varying from 5% to 18% across countries in 2022. In 2019, preterm mortality accounted for approximately 47% of all under-5 deaths globally. The increasing incidence of preterm births has attracted considerable attention, becoming a focal point in medical research (1, 2). Several factors contribute to this trend, including changes in maternal age, with both very young and older mothers being at risk, as well as multiple

pregnancies and high cesarean rates due to medical interventions. China, the second-most populous country in the world, contributes the second-highest number of preterm births, with more than 1 million births annually (3). Data from the Lancet Global Health indicates that the increasing preterm birth rate in China has accelerated since the adjustment of child policy. Two primary factors contributing to this surge include the rising maternal age at childbearing and the greater use of assisted reproductive technology, both of which elevate the multiple pregnancy rate and the proportion of late preterm births (4). Furthermore, infections caused by pathogens, chronic health conditions, inadequate prenatal care, socioeconomic disparities, and lifestyle factors such as smoking and substance abuse all play significant roles (5). Numerous clinical studies (6–8) and systematic reviews (9–12) have demonstrated the role of COVID-19 infection during pregnancy as a significant risk factor for adverse maternal and fetal outcomes, particularly preterm birth (5). These events indicate the urgent need for global attention to address the rising rates of preterm birth (13).

Premature neonates often struggle to adapt to the extrauterine environment due to their underdeveloped organs, making them vulnerable to various diseases, with BPD being particularly notable (14–16). It is noteworthy that approximately 80% of preterm infants aged 22–24 weeks and around 20% of those aged 28 weeks are affected by BPD (17). Despite advancements in perinatal medicine that have improved survival rates among premature infants, the incidence of BPD remains steady or even increases due to the rise in preterm births with underdeveloped lungs (13, 18, 19). Additionally, BPD, being a systemic condition, significantly impacts health and quality of life, leading to poor pulmonary function outcomes, an increased risk of requiring home oxygen therapy, and hospitalization for respiratory infections (20). Patients with BPD often experience airflow limitations, reduced gas transfer, and decreased lung density, which heighten their susceptibility to long-term chronic obstructive pulmonary disease (21, 22). Due to its high morbidity rates, diverse phenotypic variations, and substantial medical and economic burdens on healthcare systems, BPD has emerged as a prevalent and complex concern in perinatal medicine (17, 23–26).

Oxidative stress has long been recognized as a significant contributor to the development of numerous neonatal diseases, including BPD. Factors contributing to developing BPD-related oxidative stress include abrupt changes in postnatal oxygen tension, additional exposure to high oxygen levels due to respiratory insufficiency, deficient antioxidant mechanisms, infection, and inflammation. These factors increase oxidative burden, leading to lung injury and developmental abnormalities (27–31). Multiple studies have demonstrated elevated levels of oxidative stress biomarkers in newborns who develop BPD compared to those unaffected by the condition (32–36).

Clinical interventions for BPD encompass protective ventilation, pulmonary surfactants, steroids, caffeine, vitamin A, nitric oxide, and nutritional optimization (28). However, the efficacy and safety of some of these approaches remain controversial (31). Considering the theoretical potential of antioxidant therapy in mitigating oxidative stress and pulmonary injury in BPD (28, 31), this review aims to analyze these interventions from an antioxidant perspective to enhance their clinical applicability. Furthermore, research indicates an elevation in oxidative stress biomarkers even in the early stages of BPD, suggesting exposure to the detrimental effects of oxidative stress in immature lungs before developing BPD (37–40). Thus, early oxidative stress levels are critical for predicting BPD, aiding in disease severity assessment, and providing personalized precision therapies for affected preterm infants (27). Consequently, this review synthesizes the most accurate and representative oxidative stress biomarkers based on lipid, protein, and DNA damage mechanisms, discussing their utility in predicting BPD development.

2 The pathogenesis of BPD: oxidative stress in immature lungs

Reactive oxygen species (ROS) are highly reactive oxidative molecules or ions primarily generated by endogenous oxidative metabolism processes. Moderate ROS production is crucial in cellular signaling transduction, apoptosis, proliferation, and inflammation within biological processes. However, excessive ROS levels or compromised antioxidant defense mechanisms can lead to oxidative stress, resulting in cellular structural and functional damage, consequently contributing to various diseases (41–43).

Postnatal risk factors such as high oxygen levels, hypoxia, ventilation, infections, and inflammation dramatically increase ROS production and contribute to BPD (28, 29, 41–43). Mechanical ventilation leads to alveolar overinflation and damage and triggers the activation of inflammatory signaling pathways and pulmonary fibrosis, a critical factor in developing classical BPD (27, 44). With the introduction of antenatal steroids, exogenous surfactant therapy, and protective ventilation strategies such as reduced tidal volumes and decreased invasive ventilation, these clinical interventions have somewhat reduced the incidence and mortality rates of oxidative stress and BPD (31, 45).

Advancements in healthcare have shifted the gestational age criteria for BPD diagnosis from extremely premature infants (gestational age less than 32 weeks) to very premature infants (gestational age less than 28 weeks) (44), exposing the lungs to high oxygen levels during the late canalicular or early saccular stages (17, 46). Premature birth transitions the lungs from the low-oxygen environment of the uterus to the high-oxygen environment of indoor air, exposing them to relatively high oxygen concentrations (30). Fetuses *in utero* only need to cope with blood oxygen tensions of 25–30 mmHg and benefit from maternal antioxidant protection, thereby avoiding oxidative stress (47). In comparison, preterm infants must cope with oxygen tensions of 60–100 mmHg for an extended period with immature lungs. Furthermore, the alveolar gas exchange surface area at these stages is incompletely developed, unable to provide sufficient oxygen for metabolism (48, 49). Oxygen therapy has become a necessary standard treatment to prevent newborns from dying due to respiratory failure (13). These factors make infants prone to oxidative stress due to exposure to high oxygen concentrations, leading to the development of BPD. Notably, the endogenous

antioxidant system within immature lungs fully matures shortly before full-term delivery (41). Consequently, the excess ROS cannot be effectively eliminated, leading to ROS accumulation and oxidative stress. Excessive ROS induces apoptosis and dysfunction of alveolar epithelial type II cells responsible for synthesizing and secreting pulmonary surfactant (29), impairing lung vascular and alveolar development (28), thus contributing to the emergence of the new phenotype of BPD (50, 51).

3 Clinical treatment of BPD: an antioxidant perspective

The treatment landscape for BPD continues to evolve, yet achieving satisfactory outcomes remains elusive (17, 52, 53). Both basic researchers and clinicians are challenged by the iatrogenic injuries associated with improving the survival of preterm infants (54). Given the central role of oxidative stress in BPD pathogenesis, strategies targeting antioxidants hold promise for prevention and treatment (42, 55). Numerous studies on antioxidant therapy and reviews summarize the clinical treatment methods (56–60). However, the antioxidant aspects of the clinical treatment of BPD remain essential. Therefore, this article reviews current clinical approaches to BPD treatment from an antioxidant perspective.

We searched the PubMed database for published clinical studies covering the neonatal (birth-1 month) up to 2023. The search strategy included medical subject headings (MeSH headings) and free text terms related to antioxidants, pulmonary surfactants, vitamin A, vitamin E, vitamin D, caffeine, nutritional interventions, and BPD.

3.1 Pulmonary surfactant

Since the certified importance of pulmonary surfactants in enhancing the survival of preterm infants (61), the administration of prenatal corticosteroids to boost endogenous surfactant production before birth and the introduction of exogenous surfactants after birth have been pivotal milestones in neonatal medicine (62). These developments have altered the original definition of BPD proposed by Northway in 1967 and launched the post-surfactant era (20, 63).

Pulmonary surfactants coat the alveolar surface and are complex mixtures of phospholipids and proteins (64, 65). The alveoli of preterm infants are at the stage of the late canalicular or early saccular periods during which type II alveolar cells have not fully developed, leading to alterations in the quantity, quality, or composition of surfactant secretion (66). Additionally, surfactant proteins are influenced by hyperoxia, potentially prolonging the need for ventilator support and increasing the risk of BPD (67–69). Studies in preterm infants have demonstrated that natural surfactant treatment reduces oxidative stress parameters in tracheal aspirates from ventilated infants (25, 70–72). Firstly, exogenous surfactant therapy can improve neonatal adverse outcomes by reducing inhaled oxygen concentration and exogenous ROS formation during oxygen therapy (68, 73). Secondly, surfactant proteins inhibit inflammatory processes and enhance microbial clearance (66), which also play a vital role in reducing the production of endogenous ROS to some extent (74). Thirdly, natural surfactants contain polyunsaturated phospholipids along with enzymatic antioxidants like SOD and CAT (75–77), thereby shielding the cell membrane from the assault and damage caused by ROS. These properties also underscore the susceptibility of lungs with an insufficient antioxidant system to oxidative damage under hyperoxia (78).

Since the beginning of the last century, natural surfactants have been regarded as superior to synthetic surfactants in enhancing respiration, reducing mortality, and lowering the incidence of BPD (79, 80). Treatment with poractant alfa may offer more advantages than calfactant and other animal-derived surfactants in preventing BPD (81, 82), potentially due to its lower dipalmitoyl phosphatidylcholine value and higher activity of antioxidant components both in natural surfactants and poractants (83). Additionally, the differentiation of epithelial type II alveolar cells and the surfactant production rate may be regulated by endogenous glucocorticoids and accelerated by exogenous glucocorticoids, primarily through the regulation of gene expression associated with increased surfactant protein synthesis, which has been extensively reported in animal experiments (75, 84–87). Although antenatal corticosteroids are commonly regarded as a routine approach to promoting fetal lung maturation in preterm birth, there remains controversy surrounding their postnatal use for preventing BPD, including issues related to the timing of administration, choice of agents, and routes of administration (88, 89).

3.2 Vitamin A

Vitamin A, the best-studied non-enzyme antioxidant in BPD, plays a crucial role in regulating fetal lung development and maturation, maintaining the integrity of respiratory epithelial cells, influencing pulmonary vessel development, reducing the need for supplemental oxygen in premature infants, and ultimately decreasing premature infant mortality (25, 90). Many investigations suggest that vitamin A deficiency is prevalent in very-low-birth-weight infants from birth to term (91, 92). The deficiency in preterm infants may be related to the maternal vitamin A level or inefficient placental transmission, which leaves them malnourished (93, 94). Vitamin A deficiency is also associated with BPD, and there has been considerable evidence that supplementation can reduce the mortality of BPD and infant mortality (95–97).

In terms of sources, the two primary forms of vitamin A in our bodies are animal-derived (including retinol and its derivatives) and plant-derived carotenoids (such as α-carotene, β-carotene, and β-cryptoxanthin) (25, 98). As retinol is the main active form of vitamin A in the human body and has high absorption, the types of vitamin A used in clinical practice are retinol and its derivatives (98). Animal-derived vitamin A can act as a chain-breaking antioxidant, preventing cell damage by inhibiting the interaction of peroxyl radicals with lipids to produce hydroperoxides (99, 100). Despite the lower absorption and conversion rates compared to

retinol, it is still worth exploring whether carotenoids can reduce the incidence of BPD, primarily owing to their direct antioxidant activities in scavenging singlet oxygen and peroxide-free radicals (101, 102), as well as increasing the production of enzymatic antioxidants (103, 104).

Currently, research on the delivery route of vitamin A primarily focuses on intramuscular and oral administration. While intramuscular administration can effectively reduce BPD and infant mortality rates, it is costly and associated with painful side effects (105, 106). The efficacy of oral administration in reducing BPD incidence remains debated. Additionally, intratracheal administration shows promise due to its ability to increase retinol concentration in tissues like serum, liver, and lungs in animal models, indicating its feasibility and warranting further investigation (107–109). Although the preventive effect of vitamin A on BPD is still controversial, it has become more widely used as a result of the increasing rate of very-low-birth-weight infants (18, 110–112).

3.3 Vitamin E

Like vitamin A, vitamin E is also a crucial fat-soluble non-enzymatic antioxidant with potent anti-inflammatory and antioxidant properties, playing a significant role in embryonic lung development (113, 114). Research demonstrates that the fetus primarily obtains vitamin E from the mother through the placenta (115). Fetal levels of vitamin E are closely correlated with maternal levels during the same period and increase with gestational age (116, 117). Given our inability to synthesize this nutrient, premature and low birth weight infants commonly experience vitamin E deficiency, increasing the risk of adverse outcomes such as BPD (115, 118, 119). Moreover, premature infants have an increased demand for non-enzymatic antioxidants compared to full-term infants. Hence, additional vitamin supplementation may lower the incidence of BPD (117, 120, 121). However, research on the effectiveness of vitamin E is limited in duration, quantity, and depth when compared to vitamin A. Most studies on oral and intravenous administration of vitamin E have failed to demonstrate a reduction in BPD incidence and have even led to adverse outcomes such as infant death and necrotizing enterocolitis (122–124). Consequently, vitamin E supplementation was temporarily suspended in the late 1990s.

More recently, Ogihara and Mino discussed the research findings on BPD conducted during the pre-surfactant era in the 1980s and early 1990s (115). They suggested that these findings may not apply to modern neonatal care due to significant differences in the definition of prematurity between the pre-surfactant and post-surfactant eras (25, 55, 115). Since 2000, studies have re-confirmed the relationship between vitamin E deficiency and the severity of BPD (125, 126), although progress remains slow and the outcomes are preliminary (127–129). Since vitamin E is an essential component of pulmonary surfactant and plays a crucial role in the post-surfactant era (119, 130, 131), it is unsurprising that vitamin E possesses anti-inflammatory and antioxidant properties that can mitigate the incidence of BPD. In addition, high levels of vitamin E have been detected in breast milk, indicating its essentiality for newborns with specific nutritional needs, especially in low birth weight infants (118, 132, 133). Although various isomers of vitamin E have similar antioxidant functions in preventing lipid peroxidation (114, 134), high doses of α-tocopherol were widely used due to its availability in the human body during the pre-surfactant era (117, 135). Data on the effectiveness of specific subtypes and different routes of administration for vitamin E supplementation trials on lung health are necessary to determine whether re-challenging in modern neonatal intensive care units is worthwhile (114, 115, 130), which may improve lung development and can reduce the adverse outcomes caused by the injection and oral administration.

3.4 Vitamin D

Since 2000, there has been an increase in studies examining the relationship between vitamin D deficiency and BPD. Apart from its various immunomodulatory and anti-inflammatory functions (136), it possesses indirect antioxidant properties that may alleviate conditions induced by oxidative stress, such as diabetic retinopathy (137), endothelial dysfunction (138), skin aging (139), and mood disorders (140). The potential mechanism for reducing oxidative stress involves vitamin D enhancing the expression of SOD2, glutathione, and nuclear factor NRF2, which are responsible for antioxidant enzyme expression (137–139, 141).

The vitamin D level of early neonates is closely linked to that of their mothers and gestational age, as the fetus obtains vitamin D from the mother via the placenta (142). As a result of modern lifestyles, vitamin D deficiency is prevalent in pregnant women (143), impacting serum vitamin D levels in preterm infants and subsequently impairing lung development (144). Several clinical studies and reviews have established an association between neonatal BPD and low vitamin D levels, with a higher incidence observed in the vitamin D deficiency group (127, 144–146). A low level of 25-hydroxyvitamin D in the bloodstream is a valuable predictor for the prediction of BPD (147–149). Although early vitamin D supplementation has shown promise in significantly increasing serum levels of 25(OH)D3, reducing inflammatory responses, and decreasing the incidence of BPD in preterm infants, more extensive clinical trials with varying doses are still necessary (150–154).

Recent research has demonstrated that vitamin D administration improves alveolar structural simplification induced by hyperoxia and elucidated underlying mechanisms from the perspective of reducing inflammation (155–161). Vitamin D administration reduced the expression of proinflammatory cytokines IL-6, IFN-γ, TNF-α and IFN-γ (156–158), regulated the balance of M1 and M2 macrophages by decreasing the expression of IL-10 and Arg-1 (155), protected neonatal rats from hyperoxia-induced BPD by regulating the vitamin D-VDR signaling pathway (158), antagonized the activation of TLR4 (159), contributed to the recovery of mitochondrial morphology (156), reduced cell apoptosis (156, 159), and promoted the growth of vascular

structures (157). In addition, low doses of vitamin D improve the formation of alveolar and pulmonary vascularization in BPD by inhibiting neutrophil extracellular traps under hyperoxia, whereas higher doses may lead to more severe outcomes (157). Moreover, inhaled vitamin D is crucial for promoting surfactant phospholipid synthesis, which is vital in reducing oxidative stress caused by hyperoxia exposure (161). Overall, due to its potent antioxidant properties and the potential benefits of inhalation over oral administration in promoting neonatal lung development (137, 161), further studies on vitamin D supplementation from an antioxidant perspective are warranted to reduce the incidence of BPD.

3.5 Caffeine

Caffeine, a methylxanthine drug, has been widely used to treat apnea of prematurity for decades and is one of the few pharmacological interventions that has been shown to significantly reduce the risk of BPD in preterm infants (162). Caffeine treatment primarily enhances diaphragmatic contractility, increases minute ventilation, and stimulates the central nervous system, effectively improving respiratory function in preterm infants. These mechanisms help reduce the need for mechanical ventilation, improve lung function, and facilitate successful extubation (163). Therefore, caffeine therapy plays a crucial role in lowering the incidence of BPD. Multiple clinical trials have supported the clinical benefits of caffeine in treating and preventing BPD (163). As a result, current research on caffeine therapy mainly focuses on the timing of treatment and dosage. Although increasing evidence supports the early use of caffeine in preterm neonates, formal guidelines specifying the exact timing to start treatment have yet to be established. Clinical trials are needed to determine the optimal timing for caffeine administration and to identify the infant population that would benefit most from early caffeine therapy. Further studies are also necessary to validate and elucidate the precise impact of early caffeine treatment on complications in preterm infants.

Yan et al. found that early administration of caffeine can reduce the severity of BPD by approximately 60% (164). Similarly, Chen et al. reported that caffeine administration within the first three days of life shows promising results in preventing severe BPD and mortality in extremely preterm infants (165). Other studies have indicated that early preventive use of caffeine citrate not only significantly reduces the incidence of BPD in preterm infants but also decreases the occurrence of other complications. Ye et al. found that early preventive administration of caffeine citrate reduced the risk of later free radical disease in preterm infants, including BPD (166). Jiang et al. found that early preventive use of caffeine citrate is more effective than standard caffeine treatment in reducing the incidence of BPD in preterm infants (167). Szatkowski et al. reported that an increased proportion of early preventive caffeine use is associated with a reduced risk of BPD and brain injury in preterm infants (168). By comparing early preventive use of caffeine citrate (within 72 h after birth) and standard caffeine treatment, Elmowafi et al. found that the former reduces the duration of oxygen therapy, ventilation needs, and the incidence of mild to moderate BPD in preterm infants (169). Lamba et al. found that early high-dose caffeine therapy (10 mg/kg/day) lowers the risk of moderate to severe BPD without increasing the incidence of measured complications (170). Rauf et al. observed that early initiation of high-dose caffeine can prevent apnea and extubation failure in preterm neonates (171). Additionally, some studies suggest that early caffeine treatment may lead to complications and even increase mortality rates. Taha et al. found that early preventive use of caffeine citrate improves survival rates in preterm infants without BPD. However, it also increases the risk of fatal necrotizing enterocolitis (172). Similarly, research by Dobson (173) and Yun (174) indicates that while early oral caffeine treatment reduces the incidence of BPD, it is accompanied by an increased mortality rate.

Evidence suggests that caffeine and its methylxanthine metabolites may reduce oxidative stress by modulating inflammation-related pathways. Caffeine inhibits oxidative stress by suppressing the activation of IRE1 and PERK induced by endoplasmic reticulum stress to prevent skin senescence (175) or by activating A2AR/SIRT3/AMPK-mediated autophagy in the leptin-induced phosphorylation of STAT3 (176). Caffeine treatment has also been shown to reduce oxidative stress by enhancing the activity of antioxidant defense enzymes, mitigating DNA damage, and modulating transcription, indicating its antioxidant function to some extent (23). Recent clinical evidence indicates that caffeine's protective effect on neonatal lung health might involve reducing the expression of genes such as MMP9, TNF-α and TLR4, thus alleviating pulmonary inflammation which may be a mechanism behind the significant reduction in BPD incidence observed in the caffeine treatment group (167). Furthermore, although there is no statistically significant difference in BPD incidence between preventive and treatment groups, the preventive group showed significantly lower levels of IL-6 and IL-8 than the treatment group (177). This reduction in cytokine levels may contribute to a lower incidence of BPD.

Although clinical studies have not yet clarified how caffeine reduces the incidence of BPD through mediating redox pathways (178), a series of cellular and animal experiments indicate that caffeine has antioxidant properties. In a cellular model of BPD induced by hyperoxia, caffeine may reduce apoptosis, promote proliferation, and alleviate oxidative stress by inhibiting the A2AR/cAMP/PKA/Src/ERK1/p38MAPK signaling pathway, thus preventing lung damage (179). In the animal model of BPD, caffeine treatment significantly mitigates cell death and changes in apoptosis-related factors induced by hyperoxia (180) and protects murine lungs from oxidative damage by inhibiting the NLRP3 inflammasome and NF-κB pathways, which reduces apoptosis in type II alveolar epithelial cells (181). Additionally, caffeine treatment may protect developing lungs from injury induced by hyperoxia by alleviating endoplasmic reticulum stress (182).

Given caffeine's important role in the prevention and treatment of BPD, there is still a lack of comprehensive understanding of its molecular mechanisms, particularly regarding whether caffeine treatment can reduce lung injury by alleviating oxidative stress. Therefore, further research in this area is essential.

3.6 Nutritional interventions

Premature infants fail to regulate inflammatory immune responses, resulting in sustained lung injury and chronic pulmonary inflammation (183). However, specific functional nutrients with antioxidant properties may play a role in reducing pulmonary inflammation (184). The primary consideration here is the potential mechanism of breast milk as an antioxidant therapy, as single antioxidant therapy for BPD has not yielded the expected clinical outcomes (28). Yang et al. indicated that breast milk is the safest, most natural, and most comprehensive infant nutrition (28). It provides all the necessary calories, proteins, and lipids for newborn growth and development, along with various antioxidants such as unsaturated fatty acids, vitamins, trace elements, glutathione (GSH), SOD, glutathione peroxidase (GSH-Px), melatonin, probiotics, short-chain fatty acids, and lactoferrin, offering robust antioxidant capabilities to newborns (28, 185).

In clinical practice, breastfeeding has been shown to reduce the incidence of BPD in premature infants significantly. Compared to formula feeding, both exclusive breastfeeding and pasteurized donor human milk feeding have resulted in a lower incidence of BPD in premature infants (186, 187). A higher incidence of BPD was also found in preterm infants who received pasteurized or frozen breastfeeding compared to exclusive breastfeeding (188, 189). The possible reasons are as follows. Although carbohydrates remain relatively intact, many components in breast milk change during freezing, pasteurization, and subsequent reheating. Freezing breast milk increases the formation of lipid peroxides, which can damage cell membranes, increase oxidative stress, and potentially lead to cellular injury. Furthermore, freezing decreases the concentration of bioactive proteins, such as secretory immunoglobulin A, lactoperoxidase, and lysozyme in breast milk, which is crucial in combating oxidative stress and maintaining immune balance. Moreover, the processes may reduce the content of antioxidants in breast milk and lead to the loss of activity of immune cells and stem cells, which are vital for protecting infants from oxidative damage and promoting tissue repair (186–189).

The amount of breast milk intake in premature infants is negatively correlated with the incidence of BPD (190). For premature infants breastfed from birth to 36 weeks postmenstrual age, each 10% increase in breastfeeding was accompanied by a 9.5% reduction in the risk of developing BPD (191). Based on these studies, breast milk seems beneficial in preventing and treating BPD. Given the various antioxidants in breast milk, we believe this is a primary mechanism by which breast milk helps reduce BPD (28).

Currently, it remains uncertain whether individual components of breast milk can function independently as antioxidants due to inconsistent findings in this area (28). For instance, taking Omega-3 polyunsaturated fatty acids as an example, the risk of BPD increases with decreased DHA levels and increased LA (192). The incidence of BPD was reduced in infants whose mothers received a DHA diet compared to those who did not (184). However, some studies have found that Omega-3 polyunsaturated fatty acid interventions do not affect the incidence of BPD (193, 194). Similar results are seen with trace elements, vitamins, and lactoferrin (28, 185). Possible reasons for this inconsistency include the limited efficacy of single-ingredient antioxidants for treating BPD.

3.7 Other antioxidant treatments

Among all antioxidant enzyme replacement therapies for preclinical strategies, recombinant human SOD has shown the most promising outcomes (25). Though no positive effect on mortality and morbidity was observed at 36 weeks postmenstrual age in the treatment of intratracheal recombinant human SOD, the number of infants with respiratory sequelae or requiring pulmonary resuscitation decreased at 1-year corrected age (195). A clinical trial has also investigated the role of recombinant human SOD therapy in alleviating ROP, a form of oxygen radical disease. The incidence of ROP was significantly reduced in infants younger than 25 weeks (196). Despite these advancements, progress in recombinant human SOD therapy has been slow over the years. In animal trials, overexpression of SOD at the transcriptional level (59, 60), oral supplementation of coenzyme Q10 (57), caffeine (95), and chrysin treatment (197) could alleviate lipid oxidative stress, decrease alveolar damage, and improve lung function. Research has shown that glucosinolate and quercetin can reduce lung inflammation by regulating transcription factors or antioxidant-related proteins (56, 58). Although antioxidant treatments have shown efficacy through histopathological assessment of target organs in many animal models, translating basic research findings into clinical practice remains challenging (198, 199).

4 Oxidative stress-related biomarkers

The previous chapter described possible mechanisms for reducing oxidative stress through treating BPD in the clinic. Currently, there is a lack of studies linking oxidative damage to outcomes. However, there is a strong need to identify validated biomarkers of oxidative stress, which would provide a theoretical basis for clinicians to develop preventive and immediate adjustments to therapeutic strategies, improve the prognosis of preterm infants, and reduce the burden of this condition on the preterm infant. Although biomarkers of oxidative stress have been identified in different tissues or body fluids, many of these biomarkers do not correlate well with BPD, do not reflect the state of oxidative stress, or lack specificity. Therefore, the focus should be on understanding which markers can be practically applied in the clinic to predict the occurrence of BPD.

Overall, oxidative stress can be categorized into four broad categories: “antioxidant defenses”, “lipid peroxidation”, “nucleic acid oxidative damage”, and “oxidative damage to proteins”. The antioxidant defense system consists of enzymatic and non-enzymatic categories. The former mainly include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), thioredoxin reductase (TRX), peroxiredoxin (PRX), and

glutathione S-transferase (GST), while the latter mainly includes metal-binding proteins (MBP), ascorbate (AA), Vitamin E, Vitamin C, uric acid, and GSH (200).

4.1 Oxidative stress-induced lipid peroxidation and corresponding biomarkers

Structural lipids, also known as membrane lipids, are essential components of cells, such as the plasma membrane, Golgi apparatus, and endoplasmic reticulum. They are composed of more than 70% phospholipids and 10%–20% cholesterol (Ch). Each phospholipid consists of two esterified fatty acyl chains, one saturated (sn-1 chain) and the other unsaturated (sn-2 chain). The unsaturated chain includes linoleic acid (LA, C18:2, omega6), arachidonic acid (AA, C20:4, omega-6), conjugated linoleic acid (CLA, C18:2, omega6), eicosapentaenoic acid (EPA, C20:5, omega-3), docosahexaenoic acid (DHA, C22:6, omega-3), and alpha-linolenic acid (ALA, C18:3, w-6). Among these, LA, AA, DHA, and Ch are the most abundant unsaturated fatty acids in mammalian cell membranes, and their double bonds are susceptible to oxidation under oxidative stress, leading to lipid peroxidation (201).

Lipid peroxidation, driven by a free radical chain reaction, consists of three reaction phases: initiation, propagation, and termination (202, 203). In the initial phase, hydrogen atoms in the methylene groups of the unsaturated fatty acids side chains are captured by pro-oxidants such as hydroxyl radicals ($OH^{\cdot}$) to produce lipid radicals ($L^{\cdot}$). During the propagation phase, $L^{\cdot}$ reacts with O2 to form lipid peroxyl radicals ($LOO^{\cdot}$). $LOO^{\cdot}$ can further extract hydrogen atoms from neighboring PUFA residues to generate new $L^{\cdot}$ and lipid peroxyl radicals (LOOH), and the resulting new $L^{\cdot}$ drives lipid peroxidation similarly. In the termination phase, the $LOO^{\cdot}$ species reacts with hydrogen atoms from the antioxidant to generate lipid hydroxide (LOH) or combines with another $LOO^{\cdot}$ to form non-radical product, as shown in Figure 1.

Unsaturated fatty acids with more than three double bonds generate intracyclic peroxide intermediate isomers (LOOH), which are subsequently reduced to prostaglandin-like compounds (LOH) and their isomers. This process involves the formation of F2-IsoPs from AA, F3-IsoPs from EPA, and F4-neuroprostanes (NPs) from DHA. These primary products are further metabolized by Hock rearrangement or β-breakage reactions to form toxic reactive carbonyl species (RCS), including malondialdehyde (MDA), 4-hydroxynonenal (4-HNE), formaldehyde (FA), acrolein, methylglyoxal (MGO) (202–204). The RCS readily triggers irreversible modification and cross-linking with proteins, nucleic acids, and other biological macromolecules, resulting in physical dysfunction, as shown in Figure 2.

In contrast, fatty acids containing fewer double bonds are less susceptible to oxidation than substances containing polyunsaturated fatty acids. Linoleates (containing two double bonds), the body's most abundant polyunsaturated fatty acids, and Ch (monounsaturated lipids) generate oxidized products only through free radical chain reactions. LA generates hydroperoxyoctadecadienoic acid (HPODE) and its isomers, and these hydroperoxides are subsequently reduced to hydroxyoctadecadienoic acid (HODE) and its isomers (204, 205). Ch generates 7α- and 7β-hydroperoxycholesterol (7α-OOHCh and 7β-OOHCh) as well as 5α, 6α- and 5β, 6β-epoxycholesterol. The first two are subsequently reduced to 7α- and 7β-hydroxycholesterol (7α-OHCh, 7β-OHCh), and the latter can be produced as Cholestane-3β,5α,6β-Triol (C-Triol) in the presence of hydrolases (206). However, 7α-OHCh can be generated by enzymatic reactions, and thus, 7β-OHCh is considered the primary product of Ch under free radical mediation, as shown in Figure 2.

Lipid hydroxides may serve as more suitable biomarkers than hydroperoxides, and trans-hydroxides are formed only due to free radical-mediated peroxidation (200, 202). Considering the abundance of unsaturated fatty acids, it is generally accepted that F2-IsoPs, HODE, and 7β-OHCh are the primary oxidative stress markers (highlighted in red), as shown in Figure 2. In addition, with increasing oxygen tension, isofurans (IsoFs) and neurofurans (NFs) can be formed by peroxidation of AA and DHA, respectively (204). The formation mechanism of IsoFs and NFs compounds is similar to that of F2-IsoPs, as shown in Figure 2.

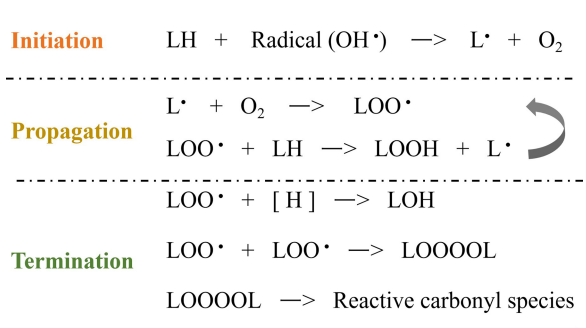


FIGURE 1
Schematic representation of the free radical-driven oxidative stress process for lipid.

4.2 Oxidative stress-induced protein oxidation and corresponding biomarkers

Free radicals attack protein molecules and mainly affect amino acid side chains, which causes them to undergo oxidative modifications. These modifications include oxidation of sulfur-containing amino acids, oxidation of aromatic amino acids, carbonylation, sugar oxidation, and nitration formation (207).

Although all amino acids can be modified by ROS, sulfur-containing amino acids (methionine, cysteine) are particularly susceptible to oxidative reactions due to the higher sensitivity of the sulfur group. Cysteine thiol undergoes two oxidation pathways (207). One pathway involves the reversible formation of a disulfide bond with another thiol, regulated by several intracellular enzymes. The second pathway involves the gradual

10.3389/fped.2024.1343870

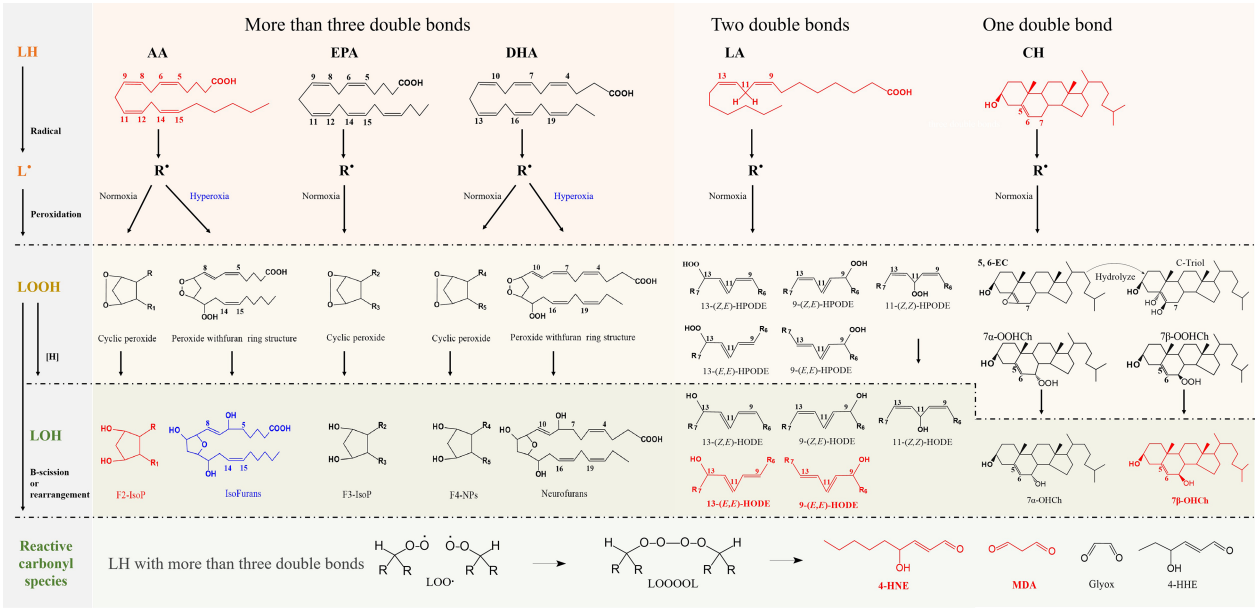


FIGURE 2
Schematic representation of the free radical-driven oxidative stress process for lipid. The processes in generating biomarkers for lipid peroxidation and the abbreviations of representative biomarkers are highlighted in red.

oxidation of thiol, resulting in the reversible formation of sulfenic acid (-SOH) and sulfinic acid (-SO_2H) or the irreversible formation of sulfonic acid (-SO_3H) (208). The selenol of selenocysteine can be regarded as a specialized thiol as it catalyzes thiol/disulfide exchange reactions (209). Like thiols, selenols can undergo oxidation to diselenides, selenoxides, selenenic acids, seleninic acids, and selenonic acids (210, 211). Selenium's higher nucleophilicity and electrophilicity enable it to efficiently cycle between its reduced state (selenols) and oxidized state (diselenides), making it an effective redox regulator (212). Notably, five glutathione peroxidases (GPx), three thioredoxin reductases (TrxR), and methionine sulfoxide reductase 2 (MsrB) are selenium enzymes involved in redox reactions (213).

Methionine is highly susceptible to ROS oxidation, a reaction prevalent in almost all living organisms (214). Under more intense experimental conditions, such as higher concentrations of N-bromosuccinimide, methionine sulfoxide can undergo irreversible oxidation to methionine sulfone (215). However, relatively few studies have investigated the physiological conditions of methionine sulfone. In aging mice, methionine sulfoxide and methionine sulfone have been identified as the most abundant amino acid oxidation modifications. The levels of methionine sulfone in histones and the cytoplasm of aging mice are significantly higher than those in young mice (216). In contrast to the well-known susceptibility of cysteine to oxidation, methionine oxidation has been largely overlooked (217). This oversight can be attributed to its hydrophobic and relatively weak nucleophilicity in the thioether group of methionine (218). Additionally, the reversible catalysis of methionine sulfoxide by methionine sulfoxide reductases and its identification as methionine in traditional Edman sequencing procedures further contributes to the neglect of methionine oxidation (219). The levels of methionine sulfoxide indicate the overall cellular redox status, making it a promising clinical biomarker (207, 215).

The oxidation of other amino acids requires more stringent conditions than that of sulfur-containing amino acids, and aromatic amino acids are secondarily susceptible to oxidation because their molecular structure contains multiple conjugated double bonds, and their high electron density properties make them susceptible to oxidation, generating various stable oxidative modification products (220). Tyrosine generates an intermediate tyrosyl radical followed by dihydroxyphenylalanine or bis-tyrosine. Tryptophan is oxidized to hydroxytryptophan by hydroxyl radicals, followed by oxygen cleavage of hydroxytryptophan to form N-formyl kynurenine. Hydroxyl radicals oxidize phenylalanine and histidine to form ortho-tyrosine and 2-oxohistidine, respectively (207).

The oxidative modification of amino acids formed by the combined action of glycosylation and oxidation, known as glycoxidation, is an irreversible process (221). Reducing sugars first react non-enzymatically with free amino groups in amino acids or proteins (usually lysine or arginine) to form compounds with sugar groups called basal glycation products. The basal glycation products are susceptible to oxidative stress, which triggers oxidation and condensation reactions. These reactions lead to further structural changes in the basal glycation products to form more complex advanced glycation end products (AGEs). The most abundant AGEs in the body are carboxymethyl lysine and pentosidine, produced from lysine and formed by cross-linking between lysine and arginine residues, respectively (207).

Nitrification modifications are also formed under conditions of oxidative stress. Nitric oxide reacts with superoxide anion to form

the reactive nitrogen species—peroxynitrite, which subsequently undergoes nitration with phenolic groups in the tyrosine molecule, irreversibly adding nitro substituents to the amino acid side chain of tyrosine to form 3-nitrotyrosine (207, 222).

The process of introducing reactive carbonyl groups such as aldehydes, ketones, and lactams into the amino acid side chains of proteins is called "protein carbonylation" (223). Protein carbonylation is usually defined as an irreversible post-translational modification that causes conformational changes in the polypeptide chain and results in loss of protein function. Carbonylation is relatively challenging to induce compared to other oxidative modifications and is considered a significant marker of oxidative damage to proteins. The protein carbonylation generation pathways in which ROS are directly involved fall into two categories. ROS can directly attack lysine, arginine, proline, and threonine side chains, introducing carbonyl groups and generating α-aminoadipic acid semialdehydes (AAS) derived from lysine and γ-glutamic acid semialdehydes derived from arginine and proline. In addition, the active carbonyls formed by ROS-attacking lipids can also be added to nucleophilic amino acids (i.e., cysteine, histidine, and lysine) by Michael addition or by generation of Schiff bases (207, 224).

These modifications also irreversibly cause proteins to produce cross-links, altering the composition and folding of proteins and affecting their function as receptors, enzymes, carriers, or structural proteins (207). Advanced oxidized protein products (AOPPs) are cross-linked protein-containing products containing dityrosine and carbonyl groups formed by the reaction of plasma proteins with oxidants and are also considered markers of oxidant-mediated protein damage (225).

4.3 Oxidative stress-induced nucleic acid oxidation and corresponding biomarkers

Due to the reactivity of nitrogen and oxygen atoms in nucleic bases, nucleic acids are highly susceptible to damage induced by oxidative stress. While all four bases are impacted by ROS, guanine (G) exhibits the lowest redox potential relative to the other bases (G: −3.0 V, A: −2.71 V, C: −2.56 V, and T: −2.32 V) (226). As a result, guanine nucleotides and deoxyguanine nucleotides are more prone to oxidation, resulting in the formation of 8-oxoGuo and 8-oxo-dG, respectively. Furthermore, 8-oxoGuo and 8-oxodG can be released into the bloodstream and excreted in urine, allowing their detection in human serum/plasma and urine samples. They serve as well-established biomarkers for oxidative damage to DNA and RNA (227, 228).

However, the oversight of RNA oxidative damage primarily stems from the relatively short half-life of RNA, and the initial research focused on DNA oxidation, leading previous studies to concentrate predominantly on DNA. In fact, RNA molecules are more susceptible to the influence of reactive oxygen species (ROS) than DNA. This susceptibility is mainly due to ribonucleotides being more abundant than deoxyribonucleotides, and RNA lacks protective and repair mechanisms, making its bases more prone to oxidation. Although previous research has linked 8-oxoGuo to various diseases, including Alzheimer's disease (229, 230), Parkinson's disease (231), type 2 diabetes (232), obesity (233), atherosclerosis (227), heart failure (234), and tumors (235, 236), studies on RNA oxidative stress products related to BPD, are yet to be reported. Hence, this section primarily discusses DNA oxidation.

In the free nucleotide pool, guanine is oxidized to 8-oxo-dGTP and involved in DNA; in the DNA molecule, guanine is oxidized to 8-oxo-7,8-dihydroguanine (8-oxoG). The 8-oxo-dGTP in the nucleotide pool is first hydrolyzed to 8-oxodGMP by MutT homolog 1 (MTH1), followed by 8-oxo-2′-deoxyguanosine (8-oxodG or 8-OHdG) formation by 5′-Nucleotidase (237, 238). The base excision repair (BER) system recognizes and eliminates 8-oxoG from DNA strands. When the BER system fails to recognize larger or more complex damages, nucleotide excision repair (NER) is initiated to excise 8-oxodG-containing DNA fragments (237). In addition, cells with the most severe DNA damage (necrosis or apoptosis) will also release 8-oxodG-containing DNA fragments. The excision of 8-oxoG by the BER system also generates apurinic (AP) sites, which are highly reactive and susceptible to 3′ phosphate bond breaks, resulting in single-strand breaks. In the presence of oxygen, 8-oxoG readily produces a synconformation and pairs with adenine (226). Although DNA repair enzymes continuously monitor and repair chromosomes, 8-oxoG readily accumulates and induces deleterious mutations through free radical overload, causing guanine to thymine mutations and cytosine to adenine transversions during DNA replication, as shown in Figure 3. Thus, the major types of DNA damage following oxidation by ROS are oxidative base modifications (8-oxoG), AP sites, single-strand breaks, and mutations (G: C-T: A) (226). Whereas 8-oxoG, 8-oxodG, and 8-oxodG-containing DNA fragments are released into the bloodstream and are taken up by the kidneys and excreted into the urine, 8-oxoG and 8-oxodG are the most critical types of DNA damage, which have been extensively studied in tissues and body fluids (239).

5 Review of oxidative stress-related biomarkers for early prediction of BPD

We thoroughly searched the PubMed database for published clinical studies encompassing the neonatal (birth-1 month) up to 2023. Our search strategy incorporated medical subject headings (MeSH headings) and free-text terms associated with "BPD", "lipid peroxidation", "protein oxidative damage", "nucleic acid oxidative damage", and representative biomarkers. Our analysis was centered on identifying biomarkers applicable in clinical settings to forecast the progression of BPD. In total, we reviewed and summarized 37 clinical studies, which are detailed in Supplementary Table S1.

The publication dates of the articles ranged from 1988 to 2023 and involved 15 countries. Predominantly, studies were conducted in the USA ($n = 11$) and Finland ($n = 4$). The study subjects included premature and full-term infants, with sample sizes ranging from 19 to 253. The most common sample types used in

10.3389/fped.2024.1343870

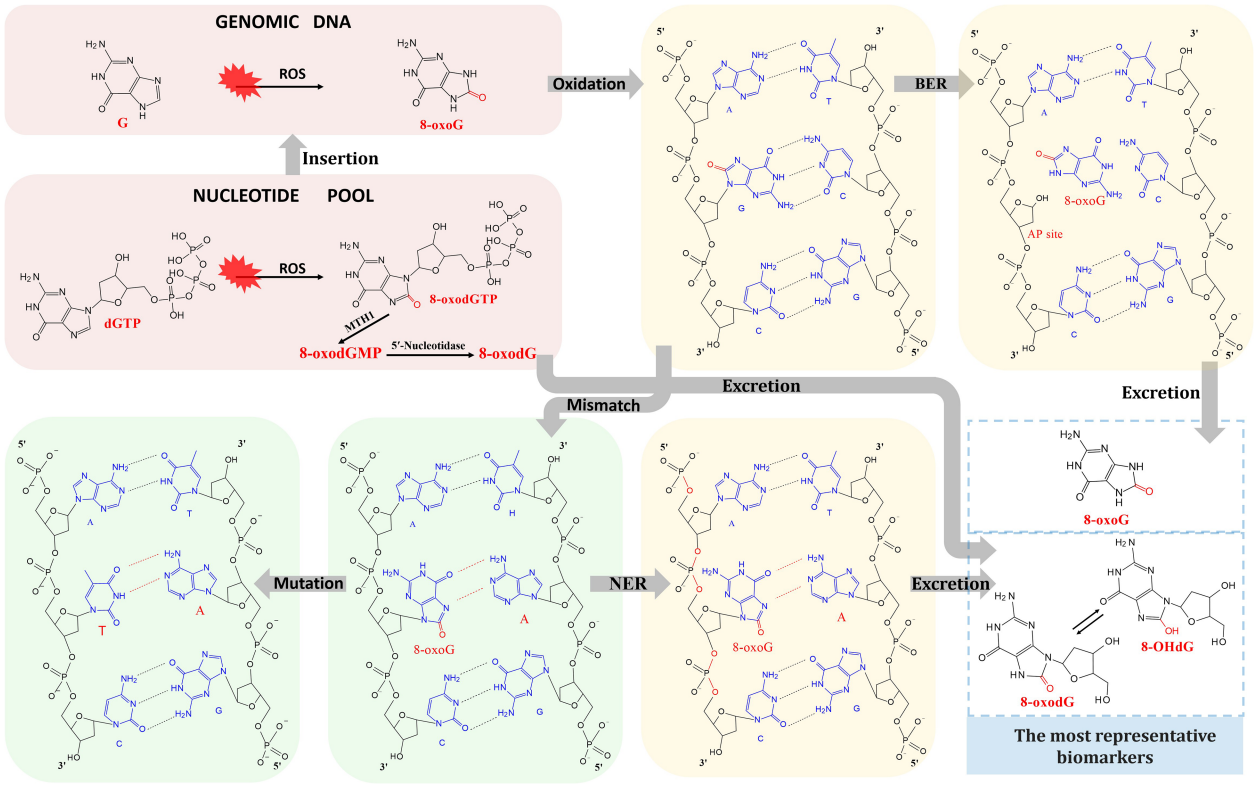


FIGURE 3
Mechanisms of DNA oxidation and the formation of 8-oxodG and 8-oHdG.

the research were urine-derived (30.77%), blood-derived (42.31%), and respiratory-derived (26.92%) samples, including BALF, exhaled breath condensate (EBC), tracheal aspirates (TA), erythrocytes, cord, serum, blood, plasma, and urine. Among these, urine (30.77%), plasma (17.31%), and TA (17.31%) were the most frequently studied sample types, as shown in Figure 4A.

Our analysis reveals that biomarkers of lipid peroxidation were the most investigated assays, followed by antioxidant systems, protein oxidation, and nucleic acid oxidation. Notably, F2-isoPs ($n = 9$), MDA ($n = 7$), and 8-OHdG ($n = 7$) emerged as the most studied biomarkers, as illustrated in Figure 4B. We also elucidated the correlations between the antioxidant system, lipid peroxidation, protein oxidative damage, nucleic acid oxidative damage, and the development of BPD in Supplementary Table S1. Additionally, the accuracy of biomarkers associated with the antioxidant system, lipid peroxidation, and oxidative damage to proteins is depicted in Figure 4B. However, despite F2-isoPs and MDA being the two most extensively studied biomarkers of oxidative stress in these articles, only 33.3% and 12.5% of articles reported their predictive capability for BPD, respectively.

The darkest color blocks in Figure 4B indicate that the biomarkers can predict the development of BPD; darker color blocks indicate that the biomarkers show differences between premature infants and control groups or between different treatment groups but cannot predict the development of BPD; the lightest color blocks indicate that the biomarkers cannot predict the development of BPD.

Among the nine studies investigating F2-isoPs levels and their association with BPD, only two suggested that elevated plasma F2-isoPs levels can predict adverse outcomes in preterm infants (240, 241). One study indicated a correlation between increased F2-isoPs levels and a more minor gestational age (36). However, the remaining six reports concluded that F2-isoPs levels cannot predict the development of BPD in preterm infants (40, 242–246). Notably, two studies also examined the levels of other lipid peroxidation products, such as isofurans. They indicated that isofurans may exhibit more robust predictive capabilities, potentially emerging as the optimal biomarker for lipid peroxidation (40, 245). Considering the mechanism of lipid peroxidation, IsoFs may be more suitable than F2-isoPs as a lipid peroxidation metabolite for predicting BPD due to their specificity as a biomarker generated in the presence of increased oxygen concentration. Nonetheless, a limited number of studies investigating isofuran levels and their correlation with BPD emphasize the need for further research in this area (40, 245).

Seven studies have assessed MDA levels as a secondary product of lipid peroxidation. Among them, three reported that MDA levels could not predict BPD (135, 247, 248) and the other three showed that higher MDA concentrations are associated with gestational age (33, 35) or lower body weights (32). Only Weinberger et al. reported a correlation between elevated urinary MDA measurements and the risk for oxidative respiratory distress (249).

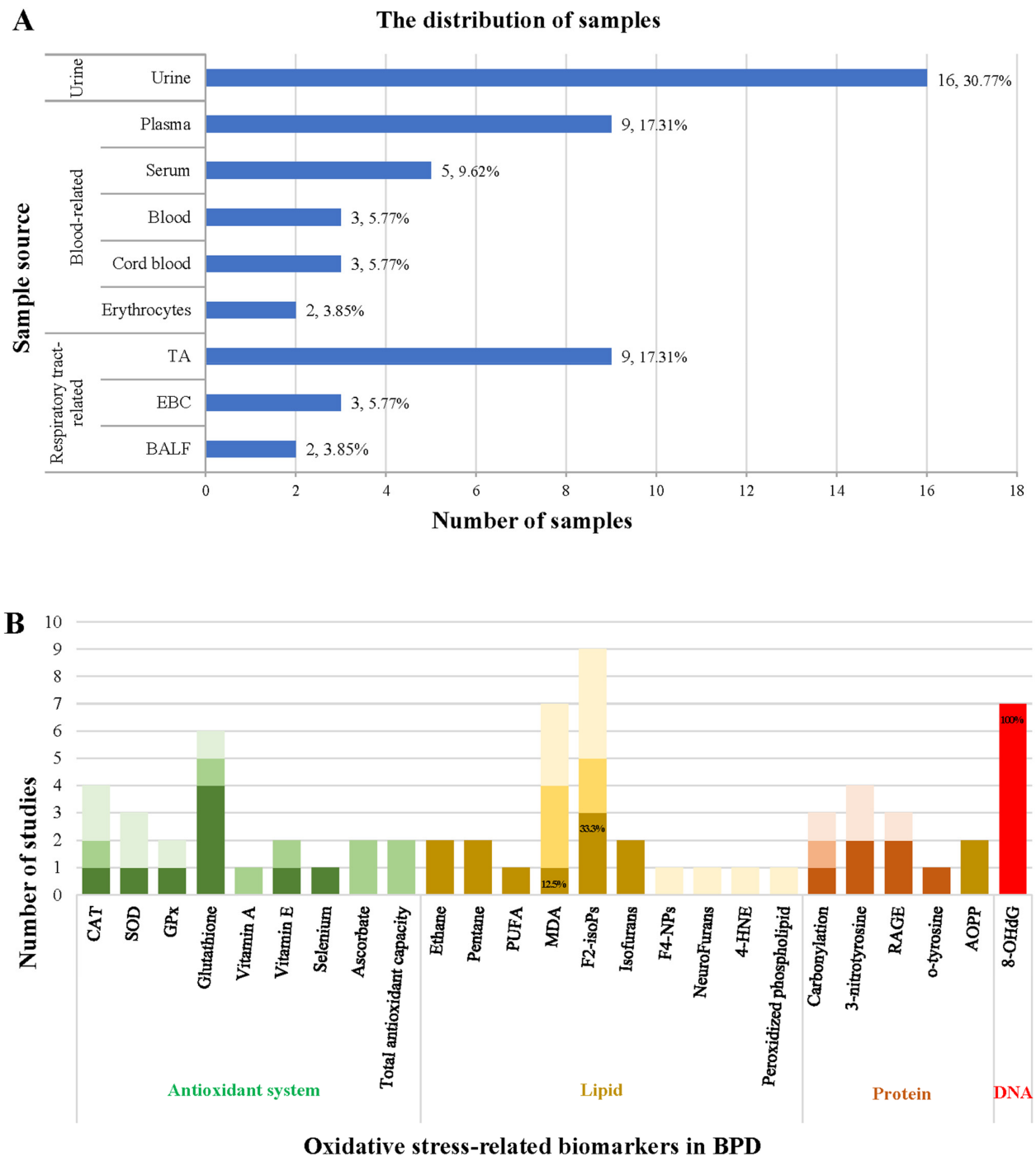


FIGURE 4
Summary of the source of samples (A) and the correlation of oxidative stress-related biomarkers with BPD (B).

In contrast, elevated levels of 8-OHdG have been consistently associated with the clinical outcome of BPD across studies, suggesting that 8-OHdG could serve as a reliable biomarker for predicting BPD (37–40, 245, 250–252). Joung et al. demonstrated that the 8-OHdG values in "classic" BPD on the third day were higher than those of "atypical" BPD. Furthermore, 8-OHdG levels on the seventh day were an independent risk factor for developing moderate/severe BPD (250). Hsiao et al. reported higher 8-OHdG levels in serum and tracheal aspirates (TA) in the BPD group on the 1st day after birth ($p < 0.05$) and persistently 8-OHdG levels increased in TA fluid on the 28th day of life in the BPD group ($p < 0.05$) compared to the non-BPD group (37). Moreover, Hsiao et al. also suggested that urine 8-OHdG concentrations from days 14 to 28 may be practical non-invasive predictors of BPD development in preterm infants (39).

In a prospective cohort study, Vento et al. found that extremely preterm neonates receiving antenatal steroids had decreased 8-OHdG levels accompanied by increased antioxidant enzyme activity, lower ortho-tyrosine levels, and a lower incidence of BPD compared to those not receiving steroids (252). Tokuriki et al. demonstrated that urinary levels of 8-OHdG during the early postnatal period correlated with the subsequent development of BPD. In contrast, urinary levels of advanced oxidative protein products (AOPP) and Nϵ-(hexanoyl) lysine showed no such correlation (251), suggesting that 8-OHdG may be preferable to protein oxidation products as a predictor of oxidative stress biomarkers for BPD.

DNA oxidative stress biomarkers may offer greater accuracy in predicting the development of BPD for several reasons. (i) Location: Lipids and proteins in the cytoplasm or cell membrane are usually more susceptible to OS, significantly exacerbated by oxygen therapy (83, 201). In contrast, DNA, located in the nucleus and protected by the nuclear membrane, is less vulnerable to the effects of ROS. (ii) Biochemical properties: Lipids and proteins contain a large number of easily oxidizable groups, such as unsaturated fatty acids (201, 207, 253) and amino acids (207, 220), making them more prone to oxidation under hyperoxia. (iii) Metabolic activity: Lipids and proteins participate in metabolic processes that generate ROS and other oxidative substances, leading to oxidative damage. In contrast, DNA does not directly engage in metabolic reactions, reducing its exposure to oxidation (201). (iv) Biological importance: DNA integrity is crucial for proper cellular function and overall health, as it stores and transmits genetic information. Cells have evolved defense mechanisms, including enzymes and antioxidant systems, to repair and protect DNA from oxidative damage, whereas lipids and proteins lack comparable protective mechanisms (254). (v) Reflecting oxidative damage directly: DNA molecules have a more straightforward structure than lipids and proteins, making them more directly reflective of oxidative damage (201). (vi) Wide range of applications: 8-OHdG emerges as a pivotal biomarker with promising applications in disease research, clinical diagnostics, and environmental health assessments (255–257). However, its lack of tissue and diagnostic specificity poses challenges, necessitating consideration of other relevant factors when interpreting experimental results to accurately assess the extent of oxidative damage (243).

Based on our comprehensive review of clinical studies, it is evident that the relationship between many reported biomarkers and the outcome of BPD is ambiguous. Only 8-OHdG shows the most promise as an oxidative damage biomarker for predicting BPD and complications related to prematurity, and the relevant information is described in Table 1. However, its integration into clinical practice has been hindered by several factors, including the need for standardized methods and reference ranges and the absence of validation through prospective trials (258). Selecting sample sources and assays is crucial in obtaining convenient and reliable biomarkers for early BPD prognostic prediction in clinical settings. Five of the seven studies on 8-OHdG utilized urine samples (39, 40, 250–252), while the remaining two collected serum and tracheal aspirates (37, 38). The collection and processing of urine samples are relatively straightforward, usually involving minimal pre-processing steps. In contrast, collecting blood and BALF may compromise the integrity of the skin and mucosa, thereby raising the risk of pain and infection in premature infants (39). Consequently, urine samples are convenient for large-scale research and clinical monitoring purposes. Urinary 8-OHdG is generally regarded as a reflection of systemic oxidative stress, thereby serving as an indicator for assessing overall oxidative stress status (256). Rigorous analytical methods and quality control measures are imperative to ensure a reliable assessment of 8-OHdG levels, providing researchers and clinicians with a basis for optimizing neonatal care. Five of the seven studies investigating 8-OHdG utilized ELISA assay kits for measurement (37–39, 250, 251), while the remaining two employed mass spectrometry (40, 252). Although ELISA assays have been calibrated for measurements and proven helpful in assessing the impact on BPD development and progression, there remains a necessity for more sensitive, specific, and clinically validated laboratory detection methods. Techniques like HPLC-MS and LC-MS/MS hold promise in obtaining urinary 8-OHdG biomarkers from urine samples, thereby enhancing the accuracy and reliability of BPD prediction (256, 259).

Since the levels of oxidative stress-induced biomarkers can reflect the severity of BPD, their levels can guide clinical management decisions for premature infants, thereby reducing oxidative stress-related diseases and providing valuable insights into the treatment of oxidative stress-related neonatal diseases (260). Currently, various strategies have been proposed for treating neonatal BPD, including protective ventilation, surfactant therapy, corticosteroids, caffeine, vitamin A, nitric oxide, and nutritional interventions (261). Therefore, assessing the relationship between these strategies and oxidative stress levels or BPD incidence is critical. Ten studies have described the changes in oxidative stress biomarkers following drug administration (252, 262), inhaled nitric oxide therapy (34, 263), exposure to different oxygen concentrations (40, 245, 246), and nutritional interventions (243, 248, 264), and their relationship with the outcome of BPD.

Reports suggest that using corticosteroids improves the oxidative-reductive balance, resulting in increased antioxidant enzyme activity, decreased 8-OHdG levels, lower ortho-tyrosine, and a reduced incidence of BPD (252). Although no significant differences in clinical outcomes were observed between control and beclomethasone-treated infants, bronchoalveolar lining fluid analysis revealed evidence of phospholipid peroxidation in control infants compared to beclomethasone-treated infants on day 2 of life (262).

There were no significant differences in concentrations of 3-nitrotyrosine and carbonylation between control and inhaled nitric oxide-treated infants (34, 263).

A study conducted in Spain in 2009 showed that urinary markers of oxidative stress were significantly elevated in infants receiving 90% oxygen compared to those receiving 30% oxygen in the first week after birth. Additionally, GSSG levels on day three and urinary isofuran, o-tyrosine, and 8-oxodG levels on day seven significantly correlated with chronic lung disease development. However, the groups had no differences in urinary F2-isoPs levels (40). Furthermore, another study from Spain in 2015 reported no differences in oxidative stress biomarkers, mortality, or major perinatal morbidities between infants receiving 30% oxygen and those receiving 60%–65% oxygen. Nonetheless, isofurans detected

TABLE 1 Summary of studies reporting on 8-oHdG in BPD.

Country of publication	Publication date	Purpose of study	Subjects	Sample type	Sample collection time	Test indicators	8-OHdG concentrations	Cite
Korea	2011	Compare urinary inflammatory and oxidative stress markers between BPD groups	60 Preterm infants <30 weeks gestation or <1,250 g (24 "atypical" BPD and 36 'classic' BPD)	Urine	Days 1, 3 and 7 of life	Enzyme-linked immunosorbent assay (JalCA, Fukuroi, Shizuoka, Japan)	No/mild BPD group (1.6 ng/mg) vs. moderate/severe group (2.8 ng/mg) on Day 7 after birth, $p = 0.002$.	(250)
Japan	2015	To evaluate carboxyhemoglobin (CO-Hb) levels as a biomarker for predicting BPD development and severity	25 Preterm infants <33 weeks gestation and/or <1,500 g (16 No-or-mild BPD and 9 Moderate-to-severe BPD)	Urine	Postnatal days 5–8 and 26–29	Enzyme-linked immunosorbent assay (JalCA, Fukuroi, Shizuoka, Japan)	The moderate-to-severe BPD group [18.8 (13.1–86.6) ng/mg] vs. the no-or-mild BPD group [11.9 (3.6–26.6) ng/mg] on Day 5–8 after birth, $p < 0.05$.	(251)
China	2017	Compare changes between IL-6 and oxidative stress marker with 8-OHdG in VLBW preterm infants following development of BPD.	80 VLBW preterm infants (26 BPD and 54 Non-BPD)	Serum	Day 1 and Day 28 after birth	Enzyme-linked immunosorbent assay (JalCA, Fukuroi, Shizuoka, Japan)	The moderate-to-severe BPD group [19.6 (9.8–176.8) ng/ml] vs. the non-BPD group [18.8 (5.9–50.6) ng/ml] on Day 1 after birth, $p < 0.05$; the moderate-to-severe BPD group [39.5 (11.3–115.4) ng/ml] vs. the non-BPD group [17.3 (3.8–51.6) ng/ml] on Day 28 after birth, $p < 0.05$.	(37)
China	2021	Examine Hsp-70 and 8-OHdG from TA in VLBW preterm infants to predict BPD	109 VLBW preterm infants (32 BPD and 77 Non-BPD)	TA	Day 1 and Day 28	Enzyme-linked immunosorbent assay (JalCA, Fukuroi, Shizuoka, Japan)	The BPD group (20.9 ± 8.9 ng/mg) vs. the non-BPD group (14.8 ± 10.4 ng/mg) on Day 1 after birth, $p < 0.05$; the BPD group (42.0 ± 28.5 ng/mg) vs. the non-BPD group (14.1 ± 10.6 ng/mg) on Day 28 after birth, $p < 0.05$.	(38)
China	2022	Predict BPD in preterm infants using urinary 8-OHdG and NT-proBNP	165 Preterm infants <33 weeks gestation or <1,500 g (70 BPD and 95 Non-BPD)	Urine	Days 7, 14, 21 and 28 after birth	Enzyme-linked immunosorbent assay (Uscn Life Science Inc., Wuhan, P.R. China)	The BPD group (19.34 ± 2.24 ng/mg) vs. the non-BPD group (17.63 ± 1.59 ng/mg) on Day 7 after birth, $p < 0.05$; the BPD group (26.48 ± 4.92 ng/mg) vs. the non-BPD group (20.24 ± 2.93 ng/mg) on Day 14 after birth, $p < 0.05$; the BPD group (27.55 ± 3.66 ng/mg) vs. the non-BPD group (20.86 ± 3.28 ng/mg) on Day 21 after birth, $p < 0.05$; the BPD group (23.95 ± 4.06 ng/mg) vs. the non-BPD group (17.21 ± 2.75 ng/mg) on Day 28 after birth, $p < 0.05$.	(39)
Spain	2009	To study the association between antenatal steroids and antioxidant activity, and their impact on postnatal oxidative stress	57 Preterm infants <28 weeks gestation (37 receiving antenatal steroids and 20 not receiving antenatal steroids)	Urine	At birth	Mass spectrometry (8OHdG/1dG ratio)	Group receiving antenatal steroids (6.73 ± 2.18) vs. group not receiving antenatal steroids (9.53 ± 3.83) at birth, $p < 0.01$	(252)
Spain	2009	Reduce adverse pulmonary outcomes, oxidative stress, and inflammation in infants of 24–28 weeks of gestation	78 Preterm infants of 24–28 weeks gestation (37 infants receiving 30% oxygen and 41 infants receiving 90% oxygen)	Urine	Days 1 and 7 of life	Mass spectrometry (8OHdG/2dG ratio)	Group receiving 30% oxygen (about 12.5) vs. group receiving 90% oxygen (about 19) on Day 1 after birth, $p < 0.01$; group receiving 30% oxygen (about 24) vs. group receiving 90% oxygen (about 32) on Day 7 after birth, $p < 0.01$.	(40)

10.3389/fped.2024.1343870

in the first 4 days after birth were correlated with the later development of BPD compared to F2-isoPs, F4-NPs, and NeuroFurans (245). However, a recent study from India indicated that in premature infants receiving room air vs. 100% oxygen therapy within 4 h after birth, F2-isoPs levels showed no significant difference in mortality or BPD (246).

Unfortunately, nutritional supplementation does not reduce the incidence of BPD significantly. A study from Canada in 2010 suggested that while the LIP + MVP TPN modality may help protect against the oxidant load associated with oxygen supplementation, its effectiveness in reducing the incidence of BPD remains unclear (243). Another study from Turkey in 2019 demonstrated that total antioxidant capacity was higher in the SMOFlipid group compared with the ClinOleic group on day 7. Although BPD was lower in the SMOFlipid group than in the ClinOleic group, this finding was non-significant (264). Similarly, another study from Turkey in 2019 indicated that FMOS and OO/SO lipid emulsions had similar effects on lipid peroxidation on the 28th day of life and short-term morbidities such as BPD (248).

6 Conclusions

We have been grappling with BPD since Northway first outlined it in 1967, spanning over 60 years. BPD stands out as the most prevalent chronic lung disease affecting premature infants, with a solid correlation to significant morbidity and mortality. The pathogenesis of BPD is complex, primarily characterized by increased exposure to oxidative stress and immature antioxidant systems, ultimately resulting in abnormal pulmonary and vascular growth. Oxidative stress triggers oxidative damage to lipids, proteins, and nucleic acids. Numerous studies have shown elevated levels of oxidative stress markers in newborns who develop BPD. Thus, monitoring these markers in premature infants helps predict the severity of BPD and evaluate the effectiveness of therapeutic interventions.

With advancements in medical care, various approaches, including pulmonary surfactants, vitamin A, vitamin E, vitamin D, caffeine, and nutritional interventions, have been utilized in the clinical management of BPD. However, the efficacy and safety of some of these methods remain contentious and necessitate further evaluation. Considering the pivotal role of oxidative stress in BPD pathogenesis, interpreting clinical treatment strategies from an antioxidant perspective is justifiable.

Furthermore, we analyzed 37 clinical studies on oxidative stress markers to investigate the relationship between lipid peroxidation, protein oxidation, oxidative DNA damage, and the outcome of BPD in newborns. 8-OHdG is the most informative among these biomarkers, elucidating the disease severity and enabling personalized precision treatment for affected infants.

Overall, our review contributes to a deeper understanding of BPD pathogenesis stemming from continuous hyperoxia exposure during treatment. It sheds light on the mechanisms underlying the antioxidant aspects of clinical BPD management. As pathogenesis is not fully understood, it is promising to explore new evidence related to oxidative stress and the pathogenesis of BPD from the perspective of microbiomics, metabolomics, and proteomics (265).

Author contributions

ML: Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing. W-XC: Writing – original draft, Writing – review & editing. SL: Conceptualization, Writing – original draft. JW: Writing – review & editing. Y-RC: Writing – review & editing. LL: Conceptualization, Writing – review & editing. GY: Conceptualization, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This research was funded by China Postdoctoral Science Foundation, grant number 2022M712183.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2024.1343870/full#supplementary-material

References

1. Chawanpaiboon S, Vogel JP, Moller AB, Lumbiganon P, Petzold M, Hogan D, et al. Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. *Lancet Glob Health*. (2019) 7(1):e37–46. doi: 10.1016/S2214-109X(18)30451-0

2. Cao G, Liu J, Liu M. Global, regional, and national incidence and mortality of neonatal preterm birth, 1990–2019. *JAMA Pediatr*. (2022) 176(8):787–96. doi: 10.1001/jamapediatrics.2022.1622

3. Zhang J, Sun K, Zhang Y. The rising preterm birth rate in China: a cause for concern. *Lancet Glob Health*. (2021) 9(9):e1179–80. doi: 10.1016/S2214-109X(21)00337-5

4. Deng K, Liang J, Mu Y, Liu Z, Wang Y, Li M, et al. Preterm births in China between 2012 and 2018: an observational study of more than 9 million women. *Lancet Glob Health*. (2021) 9(9):e1226–41. doi: 10.1016/S2214-109X(21)00298-9

5. Kumar VHS, Prasath A, Blanco C, Kenney PO, Ostwald CM, Meyer TS, et al. Respiratory failure in an extremely premature neonate with COVID-19. *Children (Basel)*. (2021) 8(6):477. doi: 10.3390/children8060477

6. Piersigilli F, Carkeek K, Hocq C, van Grambezen B, Hubinont C, Chatzis O, et al. COVID-19 in a 26-week preterm neonate. *Lancet Child Adolesc Health*. (2020) 4 (6):476–8. doi: 10.1016/S2352-4642(20)30140-1

7. Marchand G, Patil AS, Masoud AT, Ware K, King A, Ruther S, et al. Systematic review and meta-analysis of COVID-19 maternal and neonatal clinical features and pregnancy outcomes up to June 3, 2021. *AJOG Glob Rep*. (2022) 2(1):100049. doi: 10.1016/j.xagr.2021.100049

8. Ng DC, Chin L, Choo PPL, Paramasivam U. COVID-19 in a premature infant. *BMJ Case Rep*. (2021) 14(5):e243783. doi: 10.1136/bcr-2021-243783

9. de Medeiros KS, Sarmento ACA, Costa APF, Macedo LTA, da Silva LAS, de Freitas CL, et al. Consequences and implications of the coronavirus disease (COVID-19) on pregnancy and newborns: a comprehensive systematic review and meta-analysis. *Int J Gynaecol Obstet*. (2022) 156(3):394–405. doi: 10.1002/ijgo.14015

10. Gosdin L, Wallace B, Lanzieri TM, Olsen EO, Lewis EL, Chang DJ, et al. Six-month outcomes of infants born to people with sars-cov-2 in pregnancy. *Pediatrics*. (2022) 150(6):e2022059009. doi: 10.1542/peds.2022-059009

11. Maslin K, McKeon-Carter R, Hosking J, Stockley L, Southby C, Shawe J, et al. Preterm births in South-West England before and during the COVID-19 pandemic: an audit of retrospective data. *Eur J Pediatr*. (2022) 181(2):859–63. doi: 10.1007/s00431-021-04265-y

12. Perez-Lopez FR, Saviron-Cornudella R, Chedraui P, Lopez-Baena MT, Perez-Roncero G, Sanz-Arenal A, et al. Obstetric and perinatal outcomes of pregnancies with COVID 19: a systematic review and meta-analysis. *J Matern Fetal Neonatal Med*. (2022) 35(25):9742–58. doi: 10.1080/14767058.2022.2051008

13. Gilfillan M, Bhandari A, Bhandari V. Diagnosis and management of bronchopulmonary dysplasia. *Br Med J*. (2021) 375:n1974. doi: 10.1136/bmj.n1974

14. Socha-Banasiak A, Pawlowska M, Czkwianianc E, Pierzynowska K. From intrauterine to extrauterine life-the role of endogenous and exogenous factors in the regulation of the intestinal microbiota community and gut maturation in early life. *Front Nutr*. (2021) 8:696966. doi: 10.3389/fnut.2021.696966

15. Flake AW. A supportive physiologic environment for the extreme premature infant: improving life outside the womb. *J Pediatr Surg*. (2022) 57(2):167–71. doi: 10.1016/j.jpedsurg.2021.10.025

16. Salimi U, Dummula K, Tucker MH, Dela Cruz CS, Sampath V. Postnatal sepsis and bronchopulmonary dysplasia in premature infants: mechanistic insights into "new bpd". *Am J Respir Cell Mol Biol*. (2022) 66(2):137–45. doi: 10.1165/rcmb.2021-0353PS

17. Thebaud B, Goss KN, Laughon M, Whitsett JA, Abman SH, Steinhorn RH, et al. Bronchopulmonary dysplasia. *Nat Rev Dis Primers*. (2019) 5(1):78. doi: 10.1038/s41572-019-0127-7

18. Abiramalatha T, Ramaswamy VV, Bandyopadhyay T, Somanath SH, Shaik NB, Pullattayil AK, et al. Interventions to prevent bronchopulmonary dysplasia in preterm neonates: an umbrella review of systematic reviews and meta-analyses. *JAMA Pediatr*. (2022) 176(5):502–16. doi: 10.1001/jamapediatrics.2021.6619

19. Muehlbacher T, Bassler D, Bryant MB. Evidence for the management of bronchopulmonary dysplasia in very preterm infants. *Children (Basel)*. (2021) 8 (4):298. doi: 10.3390/children8040298

20. Haggie S, Robinson P, Selvadurai H, Fitzgerald DA. Bronchopulmonary dysplasia: a review of the pulmonary sequelae in the post-surfactant era. *J Paediatr Child Health*. (2020) 56(5):680–9. doi: 10.1111/jpc.14878

21. William DL, Sarath R, Jeanie C. Bronchopulmonary dysplasia and expiratory airflow at 8 years in children born extremely preterm in the post-surfactant era. *Thorax*. (2023) 78(5):484. doi: 10.1136/thoraxjnl-2022-218792

22. Lowe J, Kotecha SJ, Kotecha S. Long term effects following extreme prematurity: respiratory problems. In: Boyle EM, Cusack J, editors. *Emerging Topics and Controversies in Neonatology*. Cham: Springer International Publishing (2020). p. 351–66.

23. Yuan Y, Yang Y, Lei X, Dong W. Caffeine and bronchopulmonary dysplasia: clinical benefits and the mechanisms involved. *Pediatr Pulmonol*. (2022) 57 (6):1392–400. doi: 10.1002/ppul.25898

24. Shinwell ES, Portnov I, Meerpohl JJ, Karen T, Bassler D. Inhaled corticosteroids for bronchopulmonary dysplasia: a meta-analysis. *Pediatrics*. (2016) 138(6): e20162511. doi: 10.1542/peds.2016-2511

25. Behnke J, Dippel CM, Choi Y, Rekers L, Schmidt A, Lauer T, et al. Oxygen toxicity to the immature lung-part II: the unmet clinical need for causal therapy. *Int J Mol Sci*. (2021) 22(19):10694. doi: 10.3390/ijms221910694

26. Santema HY, Stolk J, Los M, Stoel BC, Tsonaka R, Merth IT. Prediction of lung function and lung density of young adults who had bronchopulmonary dysplasia. *ERJ Open Res*. (2020) 6(4):00157–2020. doi: 10.1183/23120541.00157-2020

27. Kimble A, Robbins ME, Perez M. Pathogenesis of bronchopulmonary dysplasia: role of oxidative stress from "omics" studies. *Antioxidants (Basel)*. (2022) 11(12):2380. doi: 10.3390/antiox11122380

28. Yang X, Jiang S, Deng X, Luo Z, Chen A, Yu R. Effects of antioxidants in human milk on bronchopulmonary dysplasia prevention and treatment: a review. *Front Nutr*. (2022) 9:924036. doi: 10.3389/fnut.2022.924036

29. Deng X, Bao Z, Yang X, Mei Y, Zhou Q, Chen A, et al. Molecular mechanisms of cell death in bronchopulmonary dysplasia. *Apoptosis*. (2023) 28(1-2):39–54. doi: 10.1007/s10495-022-01791-4

30. Martini S, Aceti A, Della Gatta AN, Beghetti I, Marsico C, Pilu G, et al. Antenatal and postnatal sequelae of oxidative stress in preterm infants: a narrative review targeting pathophysiological mechanisms. *Antioxidants (Basel)*. (2023) 12(2):422. doi: 10.3390/antiox12020422

31. Ferrante G, Montante C, Notarbartolo V, Giuffre M. Antioxidants: role the in prevention and treatment of bronchopulmonary dysplasia. *Paediatr Respir Rev*. (2022) 42:53–8. doi: 10.1016/j.prrv.2022.01.003

32. Buss IH, Darlow BA, Winterbourn CC. Elevated protein carbonyls and lipid peroxidation products correlating with myeloperoxidase in tracheal aspirates from premature infants. *Pediatr Res*. (2000) 47(5):640–5. doi: 10.1203/00006450-200005000-00014

33. Collard KJ, Godeck S, Holley JE, Quinn MW. Pulmonary antioxidant concentrations and oxidative damage in ventilated premature babies. *Arch Dis Child Fetal Neonatal Ed*. (2004) 89(5):F412–6. doi: 10.1136/adc.2002.016717

34. Ballard PL, Truog WE, Merrill JD, Gow A, Posencheg M, Golombek SG, et al. Plasma biomarkers of oxidative stress: relationship to lung disease and inhaled nitric oxide therapy in premature infants. *Pediatrics*. (2008) 121(3):555–61. doi: 10.1542/peds.2007-2479

35. Abdel Ghany EA, Alsharany W, Ali AA, Youness ER, Hussein JS. Anti-Oxidant profiles and markers of oxidative stress in preterm neonates. *Paediatr Int Child Health*. (2016) 36(2):134–40. doi: 10.1179/2046905515Y.0000000017

36. Urs R, Ni Chin R, Hemy N, Wilson AC, Pillow JJ, Hall GL, et al. Elevated leukotriene B4 and 8-isoprostane in exhaled breath condensate from preterm-born infants. *BMC Pediatr*. (2023) 23(1):386. doi: 10.1186/s12887-023-04210-y

37. Hsiao CC, Chang JC, Tsao LY, Yang RC, Chen HN, Lee CH, et al. Correlates of elevated interleukin-6 and 8-hydroxy-2'-deoxyguanosine levels in tracheal aspirates from very low birth weight infants who develop bronchopulmonary dysplasia. *Pediatr Neonatol*. (2017) 58(1):63–9. doi: 10.1016/j.pedneo.2016.01.004

38. Hsiao CC, Lee CH, Yang RC, Chen JY, Su TC, Chang YJ, et al. Heat shock protein-70 levels are associated with a state of oxidative damage in the development of bronchopulmonary dysplasia. *Front Pediatr*. (2021) 9:616452. doi: 10.3389/fped.2021.616452

39. Cui X, Fu J. Urinary biomarkers for the early prediction of bronchopulmonary dysplasia in preterm infants: a pilot study. *Front Pediatr*. (2022) 10:959513. doi: 10.3389/fped.2022.959513

40. Vento M, Moro M, Escrig R, Arruza L, Villar G, Izquierdo I, et al. Preterm resuscitation with low oxygen causes less oxidative stress, inflammation, and chronic lung disease. *Pediatrics*. (2009) 124(3):e439–49. doi: 10.1542/peds.2009-0434

41. Sies H, Jones DP. Reactive oxygen species (ros) as pleiotropic physiological signalling agents. *Nat Rev Mol Cell Biol*. (2020) 21(7):363–83. doi: 10.1038/s41580-020-0230-3

42. Schieber M, Chandel NS. Ros function in redox signaling and oxidative stress. *Curr Biol*. (2014) 24(10):R453–62. doi: 10.1016/j.cub.2014.03.034

43. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev*. (2002) 82(1):47–95. doi: 10.1152/physrev.00018.2001

44. Voynow JA. "New" bronchopulmonary dysplasia and chronic lung disease. *Paediatr Respir Rev*. (2017) 24:17–8. doi: 10.1016/j.prrv.2017.06.006

45. Schulzke SM, Stoecklin B. Update on ventilatory management of extremely preterm infants-a neonatal intensive care unit perspective. *Paediatr Anaesth*. (2022) 32(2):363–71. doi: 10.1111/pan.14369

46. Bancalari E, Jain D. Bronchopulmonary dysplasia: 50 years after the original description. *Neonatology*. (2019) 115(4):384–91. doi: 10.1159/000497422

47. Matta BF, Lam AM, Mayberg TS. The influence of arterial oxygenation on cerebral venous oxygen saturation during hyperventilation. *Can J Anaesth*. (1994) 41(11):1041–6. doi: 10.1007/BF03015651

48. Burri PH. Fetal and postnatal development of the lung. *Annu Rev Physiol*. (1984) 46:617–28. doi: 10.1146/annurev.ph.46.030184.003153

49. Schittny JC. Development of the lung. *Cell Tissue Res*. (2017) 367(3):427–44. doi: 10.1007/s00441-016-2545-0

50. Lara-Canton I, Solaz A, Parra-Llorca A, Garcia-Robles A, Millan I, Torres-Cuevas I, et al. Oxygen supplementation during preterm stabilization and the relevance of the first 5 min after birth. *Front Pediatr*. (2020) 8:12. doi: 10.3389/fped.2020.00012

51. Wang J, Dong W. Oxidative stress and bronchopulmonary dysplasia. *Gene*. (2018) 678:177–83. doi: 10.1016/j.gene.2018.08.031

52. Bancalari E, Claure N. Importance and challenges associated with oxygen control in premature infants. *J Pediatr*. (2022) 247:8–9. doi: 10.1016/j.jpeds.2022.05.042

53. Slaughter JL, Stenger MR, Reagan PB. Variation in the use of diuretic therapy for infants with bronchopulmonary dysplasia. *Pediatrics*. (2013) 131(4):716–23. doi: 10.1542/peds.2012-1835

54. Twilhaar ES, Wade RM, de Kieviet JF, van Goudoever JB, van Elburg RM, Oosterlaan J. Cognitive outcomes of children born extremely or very preterm since the 1990s and associated risk factors: a meta-analysis and meta-regression. *JAMA Pediatr*. (2018) 172(4):361–7. doi: 10.1001/jamapediatrics.2017.5323

55. Perez M, Robbins ME, Revhaug C, Saugstad OD. Oxygen radical disease in the newborn, revisited: oxidative stress and disease in the newborn period. *Free Radic Biol Med*. (2019) 142:61–72. doi: 10.1016/j.freeradbiomed.2019.03.035

56. Li Q, Wall SB, Ren C, Velten M, Hill CL, Locy ML, et al. Thioredoxin reductase inhibition attenuates neonatal hyperoxic lung injury and enhances nuclear factor E2-related factor 2 activation. *Am J Respir Cell Mol Biol*. (2016) 55(3):419–28. doi: 10.1165/rcmb.2015-0228OC

57. D'Agrosa C, Cai CL, Siddiqui F, Deslouches K, Wadowski S, Aranda JV, et al. Comparison of coenzyme Q10 or fish oil for prevention of intermittent hypoxia-induced oxidative injury in neonatal rat lungs. *Respir Res*. (2021) 22(1):196. doi: 10.1186/s12931-021-01786-w

58. Wu Q, Chong L, Shao Y, Chen S, Li C. Lipoxin A4 reduces hyperoxia-induced lung injury in neonatal rats through Pink1 signaling pathway. *Int Immunopharmacol*. (2019) 73:414–23. doi: 10.1016/j.intimp.2019.05.046

59. Ahmed MN, Codipilly C, Hogg N, Auten RL. The protective effect of overexpression of extracellular superoxide dismutase on nitric oxide bioavailability in the lung after exposure to hyperoxia stress. *Exp Lung Res*. (2011) 37(1):10–7. doi: 10.3109/01902148.2010.497893

60. Ilizarov AM, Koo HC, Kazzaz JA, Mantell LL, Li Y, Bhapat R, et al. Overexpression of manganese superoxide dismutase protects lung epithelial cells against oxidant injury. *Am J Respir Cell Mol Biol*. (2001) 24(4):436–41. doi: 10.1165/ajrcmb.24.4.4240

61. Bonadies L, Zaramella P, Porzionato A, Perilongo G, Muraca M, Baraldi E. Present and future of bronchopulmonary dysplasia. *J Clin Med*. (2020) 9(5):1539. doi: 10.3390/jcm9051539

62. Speer CP, Sweet DG, Halliday HL. Surfactant therapy: past, present and future. *Early Hum Dev*. (2013) 89 Suppl 1:S22–4. doi: 10.1016/S0378-3782(13)70008-2

63. Northway WH J, Rosan RC, Porter DY. Pulmonary disease following respirator therapy of hyaline-membrane disease. Bronchopulmonary dysplasia. *N Engl J Med*. (1967) 276(7):357–68. doi: 10.1056/NEJM196702162760701

64. Morgan TE. Pulmonary surfactant. *N Engl J Med*. (1971) 284(21):1185–93. doi: 10.1056/NEJM197105272842105

65. Goerke J. Pulmonary surfactant: functions and molecular composition. *Biochim Biophys Acta*. (1998) 1408(2–3):79–89. doi: 10.1016/s0925-4439(98)00060-x

66. Milad N, Morissette MC. Revisiting the role of pulmonary surfactant in chronic inflammatory lung diseases and environmental exposure. *Eur Respir Rev*. (2021) 30 (162):210077. doi: 10.1183/16000617.0077-2021

67. Jin Y, Peng LQ, Zhao AL. Hyperoxia induces the apoptosis of alveolar epithelial cells and changes of pulmonary surfactant proteins. *Eur Rev Med Pharmacol Sci*. (2018) 22(2):492–7. doi: 10.26355/eurrev_201801_14200

68. Dargaville PA, Kamlin COF, Orsini F, Wang X, De Paoli AG, Kanmaz Kutman HG, et al. Effect of minimally invasive surfactant therapy vs sham treatment on death or bronchopulmonary dysplasia in preterm infants with respiratory distress syndrome: the optimist-a randomized clinical trial. *JAMA*. (2021) 326(24):2478–87. doi: 10.1001/jama.2021.21892

69. Zenri H, Rodriquez-Capote K, McCaig L, Yao LJ, Brackenbury A, Possmayer F, et al. Hyperoxia exposure impairs surfactant function and metabolism. *Crit Care Med*. (2004) 32(5):1155–60. doi: 10.1097/01.ccm.0000126264.00551.c8

70. Boda D, Nemeth I, Pinter S. Surface tension, glutathione content and redox ratio of the tracheal aspirate fluid of premature infants with IRDS. *Biol Neonate*. (1998) 74 (4):281–8. doi: 10.1159/000014035

71. Dumpa V, Bhandari V. Surfactant, steroids and non-invasive ventilation in the prevention of BPD. *Semin Perinatol*. (2018) 42(7):444–52. doi: 10.1053/j.semperi.2018.09.006

72. Verder H, Bohlin K, Kamper J, Lindwall R, Jonsson B. Nasal CPAP and surfactant for treatment of respiratory distress syndrome and prevention of bronchopulmonary dysplasia. *Acta Paediatr*. (2009) 98(9):1400–8. doi: 10.1111/j.1651-2227.2009.01413.x

73. Griese M. Pulmonary surfactant in health and human lung diseases: state of the art. *Eur Respir J*. (1999) 13(6):1455–76. doi: 10.1183/09031936.99.13614779

74. Caruso G, Fresta CG, Costantino A, Lazzarino G, Amorini AM, Lazzarino G, et al. Lung surfactant decreases biochemical alterations and oxidative stress induced by a sub-toxic concentration of carbon nanoparticles in alveolar epithelial and microglial cells. *Int J Mol Sci*. (2021) 22(5):2694. doi: 10.3390/ijms22052694

75. Rooney SA. Lung surfactant. *Environ Health Perspect*. (1984) 55::205–26. doi: 10.1289/ehp.8455205

76. Fridovich I, Freeman B. Antioxidant defenses in the lung. *Annu Rev Physiol*. (1986) 48:693–702. doi: 10.1146/annurev.ph.48.030186.003401

77. Forman HJ, Zhang H. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. *Nat Rev Drug Discov*. (2021) 20(9):689–709. doi: 10.1038/s41573-021-00233-1

78. Yang WS, Stockwell BR. Ferroptosis: death by lipid peroxidation. *Trends Cell Biol*. (2016) 26(3):165–76. doi: 10.1016/j.tcb.2015.10.014

79. Halliday HL. Natural vs synthetic surfactants in neonatal respiratory distress syndrome. *Drugs*. (1996) 51(2):226–37. doi: 10.2165/00003495-199651020-00004

80. Halliday HL. Controversies: synthetic or natural surfactant. The case for natural surfactant. *J Perinat Med*. (1996) 24(5):417–26. doi: 10.1515/jpme.1996.24.5.417

81. Lemyre B, Fusch C, Schmolzer GM, Rouvinez Bouali N, Reddy D, Barrowman N, et al. Poractant alfa versus bovine lipid extract surfactant for infants 24+0 to 31+6 weeks gestational age: a randomized controlled trial. *PLoS One*. (2017) 12(5):e0175922. doi: 10.1371/journal.pone.0175922

82. Malloy CA, Nicoski P, Muraskas JK. A randomized trial comparing beractant and poractant treatment in neonatal respiratory distress syndrome. *Acta Paediatr*. (2005) 94(6):779–84. doi: 10.1111/j.1651-2227.2005.tb01984.x

83. Veldhuizen EJ, Haagsman HP. Role of pulmonary surfactant components in surface film formation and dynamics. *Biochim Biophys Acta*. (2000) 1467(2):255–70. doi: 10.1016/s0005-2736(00)00256-x

84. Whitsett JA, Weaver TE, Clark JC, Sawtell N, Glasser SW, Korfhagen TR, et al. Glucocorticoid enhances surfactant proteolipid Phe and pVal synthesis and RNA in fetal lung. *J Biol Chem*. (1987) 262(32):15618–23. doi: 10.1016/S0021-9258(18)47771-X

85. Gerber AN. Glucocorticoids and the lung. *Adv Exp Med Biol*. (2015) 872:279–98. doi: 10.1007/978-1-4939-2895-8_12

86. Bry K, Lappalainen U, Hallman M. Cytokines and production of surfactant components. *Semin Perinatol*. (1996) 20(3):194–205. doi: 10.1016/s0146-0005(96)80048-6

87. Tillis CC, Huang HW, Bi W, Pan S, Bruce SR, Alcorn JL. Glucocorticoid regulation of human pulmonary surfactant protein-B (Sp-B) mrna stability is independent of activated glucocorticoid receptor. *Am J Physiol Lung Cell Mol Physiol*. (2011) 300(6):L940–50. doi: 10.1152/ajplung.00420.2010

88. Kemp MW, Newnham JP, Challis JG, Jobe AH, Stock SJ. The clinical use of corticosteroids in pregnancy. *Hum Reprod Update*. (2016) 22(2):240–59. doi: 10.1093/humupd/dmv047

89. Sotiriadis A, McGoldrick E, Makrydimas G, Papatheodorou S, Ioannidis JP, Stewart F, et al. Antenatal corticosteroids prior to planned caesarean at term for improving neonatal outcomes. *Cochrane Database Syst Rev*. (2021) 12(12):CD006614. doi: 10.1002/14651858.CD006614.pub4

90. Wiseman EM, Bar-El Dadon S, Reifen R. The vicious cycle of vitamin a deficiency: a review. *Crit Rev Food Sci Nutr*. (2017) 57(17):3703–14. doi: 10.1080/10408398.2016.1160362

91. Huang L, Zhu D, Pang G. The effects of early vitamin a supplementation on the prevention and treatment of bronchopulmonary dysplasia in premature infants: a systematic review and meta-analysis. *Transl Pediatr*. (2021) 10(12):3218–29. doi: 10.21037/tp-21-496

92. Timoneda J, Rodriguez-Fernandez L, Zaragoza R, Marin MP, Cabezuelo MT, Torres L, et al. Vitamin a deficiency and the lung. *Nutrients*. (2018) 10(9):1132. doi: 10.3390/nu10091132

93. Inder TE, Carr AC, Winterbourn CC, Austin NC, Darlow BA. Vitamin a and E status in very low birth weight infants: development of an improved parenteral delivery system. *J Pediatr*. (1995) 126(1):128–31. doi: 10.1016/s0022-3476(95)70515-5

94. Haider BA, Sharma R, Bhutta ZA. Neonatal vitamin a supplementation for the prevention of mortality and morbidity in term neonates in low and middle income countries. *Cochrane Database Syst Rev*. (2017) 2(2):CD006980. doi: 10.1002/14651858.CD006980.pub3

95. Endesfelder S, Strauss E, Scheuer T, Schmitz T, Buhrer C. Antioxidative effects of caffeine in a hyperoxia-based rat model of bronchopulmonary dysplasia. *Respir Res*. (2019) 20(1):88. doi: 10.1186/s12931-019-1063-5

96. Blaner WS. Vitamin a signaling and homeostasis in obesity, diabetes, and metabolic disorders. *Pharmacol Ther*. (2019) 197:153–78. doi: 10.1016/j.pharmthera.2019.01.006

97. Friel JK, Diehl-Jones WL, Suh M, Tsopmo A, Shirwadkar VP. Impact of iron and vitamin C-containing supplements on preterm human milk: in vitro. *Free Radic Biol Med*. (2007) 42(10):1591–8. doi: 10.1016/j.freeradbiomed.2007.02.022

98. Carazo A, Macakova K, Matousova K, Krcmova LK, Protti M, Mladenka P. Vitamin a update: forms, sources, kinetics, detection, function, deficiency, therapeutic use and toxicity. *Nutrients*. (2021) 13(5):1703. doi: 10.3390/nu13051703

99. Sotler R, Poljsak B, Dahmane R, Jukic T, Pavan Jukic D, Rotim C, et al. Prooxidant activities of antioxidants and their impact on health. *Acta Clin Croat*. (2019) 58(4):726–36. doi: 10.20471/acc.2019.58.04.20

100. Palace VP, Khaper N, Qin Q, Singal PK. Antioxidant potentials of vitamin a and carotenoids and their relevance to heart disease. *Free Radic Biol Med*. (1999) 26(5–6):746–61. doi: 10.1016/s0891-5849(98)00266-4

101. Kabir MT, Rahman MH, Shah M, Jamiruddin MR, Basak D, Al-Harrasi A, et al. Therapeutic promise of carotenoids as antioxidants and anti-inflammatory agents in neurodegenerative disorders. *Biomed Pharmacother*. (2022) 146:112610. doi: 10.1016/j.biopha.2021.112610

102. Islam F, Muni M, Mitra S, Emran TB, Chandran D, Das R, et al. Recent advances in respiratory diseases: dietary carotenoids as choice of therapeutics. *Biomed Pharmacother*. (2022) 155:113786. doi: 10.1016/j.biopha.2022.113786

103. Lu LW, Gao Y, Quek SY, Foster M, Eason CT, Liu M, et al. The landscape of potential health benefits of carotenoids as natural supportive therapeutics in protecting against coronavirus infection. *Biomed Pharmacother*. (2022) 154:113625. doi: 10.1016/j.biopha.2022.113625

104. Kaulmann A, Bohn T. Carotenoids, inflammation, and oxidative stress–implications of cellular signaling pathways and relation to chronic disease prevention. *Nutr Res*. (2014) 34(11):907–29. doi: 10.1016/j.nutres.2014.07.010

105. Poets CF, Lorenz L. Prevention of bronchopulmonary dysplasia in extremely low gestational age neonates: current evidence. *Arch Dis Child Fetal Neonatal Ed*. (2018) 103(3):F285–91. doi: 10.1136/archdischild-2017-314264

106. Giridhar S, Kumar J, Attri SV, Dutta S, Kumar P. Intramuscular followed by oral vitamin a supplementation in neonates with birth weight from 750 to 1250 g: a randomized controlled trial. *Indian J Clin Biochem*. (2020) 35(2):197–204. doi: 10.1007/s12291-018-0807-1

107. Wahl HB, Hutten MC, Monz D, Tutdibi E, Ophelders D, Nikiforou M, et al. Vitamin a supplementation by endotracheal application of a nano-encapsulated preparation is feasible in ventilated preterm lambs. *J Aerosol Med Pulm Drug Deliv*. (2018) 31(6):323–30. doi: 10.1089/jamp.2017.1438

108. Biesalski HK. Effects of intra-tracheal application of vitamin a on concentrations of retinol derivatives in plasma, lungs and selected tissues of rats. *Int J Vitam Nutr Res*. (1996) 66(2):106–12.

109. Singh AJ, Bronshtein V, Khashu M, Lee K, Potts JE, Friel J, et al. Vitamin a is systemically bioavailable after intratracheal administration with surfactant in an animal model of newborn respiratory distress. *Pediatr Res*. (2010) 67(6):619–23. doi: 10.1203/PDR.0b013e3181da8fe8

110. Mactier H, Mokaya MM, Farrell L, Edwards CA. Vitamin a provision for preterm infants: are we meeting current guidelines? *Arch Dis Child Fetal Neonatal Ed*. (2011) 96(4):F286–9. doi: 10.1136/adc.2010.190017

111. Agostoni C, Buonocore G, Carnielli VP, De Curtis M, Darmaun D, Decsi T, et al. Enteral nutrient supply for preterm infants: commentary from the European society of paediatric gastroenterology, hepatology and nutrition committee on nutrition. *J Pediatr Gastroenterol Nutr*. (2010) 50(1):85–91. doi: 10.1097/MPG.0b013e3181adaee0

112. Rakshasbhuvankar AA, Simmer K, Patole SK, Stoecklin B, Nathan EA, Clarke MW, et al. Enteral vitamin a for reducing severity of bronchopulmonary dysplasia: a randomized trial. *Pediatrics*. (2021) 147(1):e2020009985. doi: 10.1542/peds.2020-009985

113. Blaner WS, Shmarakov IO, Traber MG. Vitamin a and vitamin E: will the real antioxidant please stand up? *Annu Rev Nutr*. (2021) 41:105–31. doi: 10.1146/annurev-nutr-082018-124228

114. Stone CA J, McEvoy CT, Aschner JL, Kirk A, Rosas-Salazar C, Cook-Mills JM, et al. Update on vitamin E and its potential role in preventing or treating bronchopulmonary dysplasia. *Neonatology*. (2018) 113(4):366–78. doi: 10.1159/000487388

115. Ogihara T, Mino M. Vitamin E and preterm infants. *Free Radic Biol Med*. (2022) 180:13–32. doi: 10.1016/j.freeradbiomed.2021.11.037

116. da Silva A, de Sousa Reboucas A, Mendonca BMA, Silva D, Dimenstein R, Ribeiro K. Relationship between the dietary intake, Serum, and breast milk concentrations of vitamin a and vitamin E in a cohort of women over the course of lactation. *Matern Child Nutr*. (2019) 15(3):e12772. doi: 10.1111/mcn.12772

117. Bortolotti A, Traina GL, Barzago MM, Celardo A, Bonati M. Placental transfer and tissue distribution of vitamin E in pregnant rabbits. *Biopharm Drug Dispos*. (1990) 11(8):679–88. doi: 10.1002/bdd.2510110804

118. Xi Y, Wang X, Liu K, Zhang H, Ren X, Zhao A, et al. Vitamin E concentration in breast milk in different periods of lactation: meta-analysis. *Front Nutr*. (2022) 9:1050011. doi: 10.3389/fnut.2022.1050011

119. Kolleck I, Sinha P, Rustow B. Vitamin E as an antioxidant of the lung: mechanisms of vitamin E delivery to alveolar type ii cells. *Am J Respir Crit Care Med*. (2002) 166(12 Pt 2):S62–6. doi: 10.1164/rccm.2206019

120. Ehrenkranz RA, Bonta BW, Ablow RC, Warshaw JB. Amelioration of bronchopulmonary dysplasia after vitamin E administration. A preliminary report. *N Engl J Med*. (1978) 299(11):564–9. doi: 10.1056/NEJM197809142991102

121. Coleman M, Thompson TR. A possible role of vitamin E in the prevention or amelioration of bronchopulmonary dysplasia. *Am J Pediatr Hematol Oncol*. (1979) 1 (2):175–8.

122. Mino M. Use and safety of elevated dosages of vitamin E in infants and children. *Int J Vitam Nutr Res Suppl*. (1989) 30:69–80.

123. Watts JL, Milner R, Zipursky A, Paes B, Ling E, Gill G, et al. Failure of supplementation with vitamin E to prevent bronchopulmonary dysplasia in infants less than 1,500 g birth weight. *Eur Respir J*. (1991) 4(2):188–90. doi: 10.1183/09031936.93.04020188

124. Bell EF, Filer LJ Jr. The role of vitamin E in the nutrition of premature infants. *Am J Clin Nutr*. (1981) 34(3):414–22. doi: 10.1093/ajcn/34.3.414

125. Zasada M, Suski M, Bokiniec R, Szwarc-Duma M, Borszewska-Kornacka MK, Madej J, et al. Comparative two time-point proteome analysis of the plasma from preterm infants with and without bronchopulmonary dysplasia. *Ital J Pediatr*. (2019) 45(1):112. doi: 10.1186/s13052-019-0676-0

126. Stone CA Jr., Cook-Mills J, Gebretsadik T, Rosas-Salazar C, Turi K, Brunwasser SM, et al. Delineation of the individual effects of vitamin E isoforms on early life incident wheezing. *J Pediatr*. (2019) 206:156–63.e3. doi: 10.1016/j.jpeds.2018.10.045

127. Ge H, Liu W, Li H, Zhang M, Zhang M, Liu C, et al. The association of vitamin D and vitamin E levels at birth with bronchopulmonary dysplasia in preterm infants. *Pediatr Pulmonol*. (2021) 56(7):2108–13. doi: 10.1002/ppul.25414

128. Deger I, Ertugrul S, Yilmaz ST, Ozbey ZK, Yolbas I, Kaplan I. The relationship between vitamin a and vitamin E levels and neonatal morbidities. *Eur Rev Med Pharmacol Sci*. (2022) 26(6):1963–9. doi: 10.26355/eurrev_202203_28344

129. Amin SB, Laroia N, Sinkin RA, Kendig JW. Effect of dexamethasone therapy on Serum vitamin E concentrations in premature infants with bronchopulmonary dysplasia. *J Perinatol*. (2003) 23(7):552–5. doi: 10.1038/sj.jp.7210984

130. Guthmann F, Kolleck I, Schachtrup C, Schlame M, Spener F, Rustow B. Vitamin E deficiency reduces surfactant lipid biosynthesis in alveolar type II cells. *Free Radic Biol Med*. (2003) 34(6):663–73. doi: 10.1016/s0891-5849(02)01376-x

131. Guthmann F, Haupt R, Schlame M, Stevens PA, Rustow B. Alveolar surfactant subfractions differ in their lipid composition. *Int J Biochem Cell Biol*. (1995) 27 (10):1021–6. doi: 10.1016/1357-2725(95)00078-4

132. Nguyen MTT, Kim J, Lee H, Won S, Kim Y, Jung JA, et al. A comparison of vitamin and lutein concentrations in breast milk from four Asian countries. *Nutrients*. (2020) 12(6):1794. doi: 10.3390/nu12061794

133. Calvo-Lerma J, Selma-Royo M, Hervas D, Yang B, Intonen L, Gonzalez S, et al. Breast milk lipidome is associated with maternal diet and Infants' growth. *Front Nutr*. (2022) 9:854786. doi: 10.3389/fnut.2022.854786

134. Saito Y. Diverse cytoprotective actions of vitamin E isoforms- role as peroxyl radical scavengers and complementary functions with selenoproteins. *Free Radic Biol Med*. (2021) 175:121–9. doi: 10.1016/j.freeradbiomed.2021.08.234

135. Falciglia HS, Johnson JR, Sullivan J, Hall CF, Miller JD, Riechmann GC, et al. Role of antioxidant nutrients and lipid peroxidation in premature infants with respiratory distress syndrome and bronchopulmonary dysplasia. *Am J Perinatol*. (2003) 20(2):97–107. doi: 10.1055/s-2003-38315

136. Murdaca G, Tonacci A, Negrini S, Greco M, Borro M, Puppo F, et al. Emerging role of vitamin D in autoimmune diseases: an update on evidence and therapeutic implications. *Autoimmun Rev*. (2019) 18(9):102350. doi: 10.1016/j.autrev.2019.102350

137. Valle MS, Russo C, Malaguarnera L. Protective role of vitamin D against oxidative stress in diabetic retinopathy. *Diabetes Metab Res Rev*. (2021) 37(8):e3447. doi: 10.1002/dmrr.3447

138. Kim DH, Meza CA, Clarke H, Kim JS, Hickner RC. Vitamin D and endothelial function. *Nutrients*. (2020) 12(2):575. doi: 10.3390/nu12020575

139. Bocheva G, Slominski RM, Slominski AT. The impact of vitamin D on skin aging. *Int J Mol Sci*. (2021) 22(16):9097. doi: 10.3390/ijms22169097

140. Akpinar S, Karadag MG. Is vitamin D important in anxiety or depression? What is the truth? *Curr Nutr Rep*. (2022) 11(4):675–81. doi: 10.1007/s13668-022-00441-0

141. Szymczak-Pajor I, Drzewoski J, Sliwinska A. The molecular mechanisms by which vitamin D prevents insulin resistance and associated disorders. *Int J Mol Sci*. (2020) 21(18):6644. doi: 10.3390/ijms21186644

142. Cetinkaya M, Cekmez F, Erener-Ercan T, Buyukkale G, Demirhan A, Aydemir G, et al. Maternal/neonatal vitamin D deficiency: a risk factor for bronchopulmonary dysplasia in preterms? *J Perinatol*. (2015) 35(10):813–7. doi: 10.1038/jp.2015.88

143. Kanatani KT, Nakayama T, Adachi Y, Hamazaki K, Onishi K, Konishi Y, et al. High frequency of vitamin D deficiency in current pregnant Japanese women associated with UV avoidance and hypo-vitamin D diet. *PLoS One*. (2019) 14(3): e0213264. doi: 10.1371/journal.pone.0213264

144. Lu T, Liang B, Jia Y, Cai J, Wang D, Liu M, et al. Relationship between bronchopulmonary dysplasia, long-term lung function, and vitamin D level at birth in preterm infants. *Transl Pediatr*. (2021) 10(11):3075–81. doi: 10.21037/tp-21-494

145. Park HW, Lim G, Park YM, Chang M, Son JS, Lee R. Association between vitamin D level and bronchopulmonary dysplasia: a systematic review and meta-analysis. *PLoS One*. (2020) 15(7):e0235332. doi: 10.1371/journal.pone.0235332

146. Mao X, Qiu J, Zhao L, Xu J, Yin J, Yang Y, et al. Vitamin D and il-10 deficiency in preterm neonates with bronchopulmonary dysplasia. *Front Pediatr*. (2018) 6:246. doi: 10.3389/fped.2018.00246

147. Papalia H, Samonini A, Buffat C, Gras E, des Robert C, Landrier JF, et al. Low vitamin D levels at birth and early respiratory outcome in infants with gestational age less than 29 weeks. *Front Pediatr*. (2021) 9:790839. doi: 10.3389/fped.2021.790839

148. Zhang X, Luo K, He X, Chen P. Association of vitamin D status at birth with pulmonary disease morbidity in very preterm infants. *Pediatr Pulmonol*. (2021) 56 (5):1215–20. doi: 10.1002/ppul.25233

149. Yu H, Fu J, Feng Y. Utility of umbilical cord blood 25-hydroxyvitamin D levels for predicting bronchopulmonary dysplasia in preterm infants with very low and extremely low birth weight. *Front Pediatr*. (2022) 10:956952. doi: 10.3389/fped.2022.956952

150. Byun SY, Bae MH, Lee NR, Han YM, Park KH. Association between vitamin D deficiency at one month of age and bronchopulmonary dysplasia. *Medicine (Baltimore)*. (2021) 100(48):e27966. doi: 10.1097/MD.0000000000027966

151. Ge H, Qiao Y, Ge J, Li J, Hu K, Chen X, et al. Effects of early vitamin D supplementation on the prevention of bronchopulmonary dysplasia in preterm infants. *Pediatr Pulmonol*. (2022) 57(4):1015–21. doi: 10.1002/ppul.25813

152. Yang Y, Li Z, Yan G, Jie Q, Rui C. Effect of different doses of vitamin D supplementation on preterm infants—an updated meta-analysis. *J Matern Fetal Neonatal Med*. (2018) 31(22):3065–74. doi: 10.1080/14767058.2017.1363731

153. Aristizabal N, Holder MP, Durham L, Ashraf AP, Taylor S, Salas AA. Safety and efficacy of early vitamin D supplementation in critically ill extremely preterm infants: an ancillary study of a randomized trial. *J Acad Nutr Diet*. (2023) 123 (1):87–94. doi: 10.1016/j.jand.2022.06.012

154. Fort P, Salas AA, Nicola T, Craig CM, Carlo WA, Ambalavanan N. A comparison of 3 vitamin D dosing regimens in extremely preterm infants: a randomized controlled trial. *J Pediatr*. (2016) 174:132–8.e1. doi: 10.1016/j.jpeds.2016.03.028

155. Zhen H, Hu H, Rong G, Huang X, Tan C, Yu X. Vita or VitD ameliorates bronchopulmonary dysplasia by regulating the balance between M1 and M2 macrophages. *Biomed Pharmacother*. (2021) 141:111836. doi: 10.1016/j.biopha.2021.111836

156. Hu J, Wu Z, Wang H, Geng H, Huo J, Zhu X, et al. Vitamin D ameliorates apoptosis and inflammation by targeting the mitochondrial and Mek1/2-Erk1/2 pathways in hyperoxia-induced bronchopulmonary dysplasia. *J Inflamm Res*. (2022) 15:4891–906. doi: 10.2147/JIR.S371906

157. Chen C, Weng H, Zhang X, Wang S, Lu C, Jin H, et al. Low-dose vitamin D protects hyperoxia-induced bronchopulmonary dysplasia by inhibiting neutrophil extracellular traps. *Front Pediatr*. (2020) 8:335. doi: 10.3389/fped.2020.00335

158. Wang Y, Jiang L. Role of vitamin D-vitamin D receptor signaling on hyperoxia-induced bronchopulmonary dysplasia in neonatal rats. *Pediatr Pulmonol*. (2021) 56 (7):2335–44. doi: 10.1002/ppul.25418

159. Yao L, Shi Y, Zhao X, Hou A, Xing Y, Fu J, et al. Vitamin D attenuates hyperoxia-induced lung injury through downregulation of toll-like receptor 4. *Int J Mol Med*. (2017) 39(6):1403–8. doi: 10.3892/ijmm.2017.2961

160. Liu C, Chen Z, Li W, Huang L, Zhang Y. Vitamin D enhances alveolar development in antenatal lipopolysaccharide-treated rats through the suppression of interferon-gamma production. *Front Immunol*. (2017) 8:1923. doi: 10.3389/fimmu.2017.01923

161. Taylor SK, Sakurai R, Sakurai T, Rehan VK. Inhaled vitamin D: a novel strategy to enhance neonatal lung maturation. *Lung*. (2016) 194(6):931–43. doi: 10.1007/s00408-016-9939-3

162. Moschino L, Zivanovic S, Hartley C, Trevisanuto D, Baraldi E, Roehr CC. Caffeine in preterm infants: where are we in 2020? *ERJ Open Res*. (2020) 6(1):00330–2019. doi: 10.1183/23120541.00330-2019

163. Principi N, Di Pietro GM, Esposito S. Bronchopulmonary dysplasia: clinical aspects and preventive and therapeutic strategies. *J Transl Med*. (2018) 16(1):36. doi: 10.1186/s12967-018-1417-7

164. Yan B, Li Y, Sun M, Meng Y, Li X. Variables related to bronchopulmonary dysplasia severity: a six-year retrospective study. *J Matern Fetal Neonatal Med*. (2023) 36(2):2248335. doi: 10.1080/14767058.2023.2248335

165. Chen X, Yuan L, Jiang S, Gu X, Lei X, Hu L, et al. Synergistic effects of achieving perinatal interventions on bronchopulmonary dysplasia in preterm infants. *Eur J Pediatr*. (2024) 183(4):1711–21. doi: 10.1007/s00431-023-05355-9

166. Ye H, Bai L, Yang M, Yang X, Zheng M, Zhong X, et al. A two-center retrospective study: association of early caffeine administration and oxygen radical diseases in neonatology in Chinese preterm neonates. *Front Pediatr*. (2023) 11:1158286. doi: 10.3389/fped.2023.1158286

167. Jiang Q, Wu X. Effect of early preventive use of caffeine citrate on prevention together with treatment of BPD within premature infants and its influence on inflammatory factors. *Biotechnol Genet Eng Rev*. (2023) 40(3):2730–44. doi: 10.1080/02648725.2023.2202517

168. Szatkowski L, Fateh S, Abramson J, Kwok TC, Sharkey D, Budge H, et al. Observational cohort study of use of caffeine in preterm infants and association between early caffeine use and neonatal outcomes. *Arch Dis Child Fetal Neonatal Ed*. (2023) 108(5):505–10. doi: 10.1136/archdischild-2022-324919

169. Elmowafi M, Mohsen N, Nour I, Nasef N. Prophylactic versus therapeutic caffeine for apnea of prematurity: a randomized controlled trial. *J Matern Fetal Neonatal Med*. (2022) 35(25):6053–61. doi: 10.1080/14767058.2021.1904873

170. Lamba V, Winners O, Fort P. Early high-dose caffeine improves respiratory outcomes in preterm infants. *Children (Basel)*. (2021) 8(6):501. doi: 10.3390/children8060501

171. Rauf S, Shah S, Bibi Z, Munir R, Jiskani H, Ahmad S, et al. Association of caffeine daily dose with respiratory outcomes in preterm neonates: a retrospective cohort study. *Inquiry*. (2024) 61:469580241248098. doi: 10.1177/00469580241248098

172. Taha D, Kirkby S, Nawab U, Dysart KC, Genen L, Greenspan JS, et al. Early caffeine therapy for prevention of bronchopulmonary dysplasia in preterm infants. *J Matern Fetal Neonatal Med*. (2014) 27(16):1698–702. doi: 10.3109/14767058.2014.885941

173. Dobson NR, Patel RM, Smith PB, Kuehn DR, Clark J, Vyas-Read S, et al. Trends in caffeine use and association between clinical outcomes and timing of therapy in very low birth weight infants. *J Pediatr*. (2014) 164(5):992–8.e3. doi: 10.1016/j.jpeds.2013.12.025

174. Yun WZ, Kassab YW, Yao LM, Khairuddin N, Ming LC, Hadi MA. Effectiveness and safety of early versus late caffeine therapy in managing apnoea of prematurity among preterm infants: a retrospective cohort study. *Int J Clin Pharm*. (2022) 44(5):1140–8. doi: 10.1007/s11096-022-01437-0

175. Li YF, Ouyang SH, Tu LF, Wang X, Yuan WL, Wang GE, et al. Caffeine protects skin from oxidative stress-induced senescence through the activation of autophagy. *Theranostics*. (2018) 8(20):5713–30. doi: 10.7150/thno.28778

176. Hosoi T, Toyoda K, Nakatsu K, Ozawa K. Caffeine attenuated ER stress-induced leptin resistance in neurons. *Neurosci Lett*. (2014) 569:23–6. doi: 10.1016/j.neulet.2014.03.053

177. Kou C, Han D, Li Z, Wu W, Liu Z, Zhang Y, et al. Influence of prevention of caffeine citrate on cytokine profile and bronchopulmonary dysplasia in preterm infants with apnea. *Minerva Pediatr*. (2020) 72(2):95–100. doi: 10.23736/S0026-4946.19.05428-8

178. Tian C, Li D, Fu J. Molecular mechanism of caffeine in preventing bronchopulmonary dysplasia in premature infants. *Front Pediatr*. (2022) 10:902437. doi: 10.3389/fped.2022.902437

179. Wang X, Lv S, Sun J, Zhang M, Zhang L, Sun Y, et al. Caffeine reduces oxidative stress to protect against hyperoxia-induced lung injury via the adenosine A2a receptor/cAMP/PKA/Src/ERK1/2/p38MAPK pathway. *Redox Rep*. (2022) 27 (1):270–8. doi: 10.1080/13510002.2022.2143114

180. Endesfelder S, Strauss E, Bendix I, Schmitz T, Buhrer C. Prevention of oxygen-induced inflammatory lung injury by caffeine in neonatal rats. *Oxid Med Cell Longev*. (2020) 2020:3840124. doi: 10.1155/2020/3840124

181. Chen S, Wu Q, Zhong D, Li C, Du L. Caffeine prevents hyperoxia-induced lung injury in neonatal mice through Nlrp3 inflammasome and nf-kappab pathway. *Respir Res*. (2020) 21(1):140. doi: 10.1186/s12931-020-01403-2

182. Teng RJ, Jing X, Michalkiewicz T, Afolayan AJ, Wu TJ, Konduri GG. Attenuation of endoplasmic reticulum stress by caffeine ameliorates hyperoxia-induced lung injury. *Am J Physiol Lung Cell Mol Physiol*. (2017) 312(5):L586–98. doi: 10.1152/ajplung.00405.2016

183. Shahzad T, Radajewski S, Chao CM, Bellusci S, Ehrhardt H. Pathogenesis of bronchopulmonary dysplasia: when inflammation meets organ development. *Mol Cell Pediatr*. (2016) 3(1):23. doi: 10.1186/s40348-016-0051-9

184. Manley BJ, Makrides M, Collins CT, McPhee AJ, Gibson RA, Ryan P, et al. High-Dose docosahexaenoic acid supplementation of preterm infants: respiratory and allergy outcomes. *Pediatrics*. (2011) 128(1):e71–7. doi: 10.1542/peds.2010-2405

185. Rocha G, Guimaraes H, Pereira-da-Silva L. The role of nutrition in the prevention and management of bronchopulmonary dysplasia: a literature review and clinical approach. *Int J Environ Res Public Health*. (2021) 18(12):6245. doi: 10.3390/ijerph18126245

186. Hanford J, Mannebach K, Ohler A, Patten M, Pardalos J. Rates of comorbidities in very low birth weight infants fed an exclusive human milk diet versus a bovine supplemented diet. *Breastfeed Med*. (2021) 16(10):814–20. doi: 10.1089/bfm.2020.0345

187. Lund AM, Domellof M, Pivodic A, Hellstrom A, Stoltz Sjostrom E, Hansen-Pupp I. Mother's own milk and its relationship to growth and morbidity in a population-based cohort of extremely preterm infants. *J Pediatr Gastroenterol Nutr*. (2022) 74(2):292–300. doi: 10.1097/MPG.0000000000003352

188. Sun H, Cao Y, Han S, Cheng R, Liu L, Liu J, et al. A randomized controlled trial protocol comparing the feeds of fresh versus frozen mother's own milk for preterm infants in the nicu. *Trials*. (2020) 21(1):170. doi: 10.1186/s13063-019-3981-4

189. Uberos J, Sanchez-Ruiz I, Fernandez-Marin E, Ruiz-Lopez A, Cubero-Millan I, Campos-Martinez A. Breast-Feeding as protective factor against bronchopulmonary dysplasia in preterm infants. *Br J Nutr*. (2024) 131(8):1405–12. doi: 10.1017/S0007114523002982

190. Xu Y, Yu Z, Li Q, Zhou J, Yin X, Ma Y, et al. Dose-dependent effect of human milk on bronchopulmonary dysplasia in very low birth weight infants. *BMC Pediatr*. (2020) 20(1):522. doi: 10.1186/s12887-020-02394-1

191. Patel AL, Johnson TJ, Robin B, Bigger HR, Buchanan A, Christian E, et al. Influence of own mother's milk on bronchopulmonary dysplasia and costs. *Arch Dis Child Fetal Neonatal Ed*. (2017) 102(3):F256–61. doi: 10.1136/archdischild-2016-310898

192. Wendel K, Aas MF, Gunnarsdottir G, Rossholt ME, Bratlie M, Nordvik T, et al. Effect of arachidonic and docosahexaenoic acid supplementation on respiratory outcomes and neonatal morbidities in preterm infants. *Clin Nutr*. (2023) 42 (1):22–8. doi: 10.1016/j.clnu.2022.11.012

193. Marc I, Boutin A, Pronovost E, Perez Herrera NM, Guillot M, Bergeron F, et al. Association between enteral supplementation with high-dose docosahexaenoic acid and risk of bronchopulmonary dysplasia in preterm infants: a systematic review and meta-analysis. *JAMA Netw Open*. (2023) 6(3):e233934. doi: 10.1001/jamanetworkopen.2023.3934

194. Sullivan TR, Gould JF, Bednarz JM, McPhee AJ, Gibson R, Anderson PJ, et al. Mediation analysis to untangle opposing associations of high-dose docosahexaenoic acid with iq and bronchopulmonary dysplasia in children born preterm. *JAMA Netw Open*. (2023) 6(6):e2317870. doi: 10.1001/jamanetworkopen.2023.17870

195. Davis JM, Parad RB, Michele T, Allred E, Price A, Rosenfeld W, et al. Pulmonary outcome at 1 year corrected age in premature infants treated at birth with recombinant human CuZn superoxide dismutase. *Pediatrics*. (2003) 111 (3):469–76. doi: 10.1542/peds.111.3.469

196. Parad RB, Allred EN, Rosenfeld WN, Davis JM. Reduction of retinopathy of prematurity in extremely low gestational age newborns treated with recombinant human Cu/Zn superoxide dismutase. *Neonatology*. (2012) 102(2):139–44. doi: 10.1159/000336639

197. Ozdemir R, Gokce IK, Taslidere AC, Tanbek K, Gul CC, Sandal S, et al. Does chrysin prevent severe lung damage in hyperoxia-induced lung injury model? *Int Immunopharmacol*. (2021) 99:108033. doi: 10.1016/j.intimp.2021.108033

198. Hsia CC, Hyde DM, Ochs M, Weibel ER, Structure AEJTFoQAoL. An official research policy statement of the American thoracic society/European respiratory society: standards for quantitative assessment of lung structure. *Am J Respir Crit Care Med*. (2010) 181(4):394–418. doi: 10.1164/rccm.200809-1522ST

199. Sodhi C, Richardson W, Gribar S, Hackam DJ. The development of animal models for the study of necrotizing enterocolitis. *Dis Model Mech*. (2008) 1(2–3):94–8. doi: 10.1242/dmm.000315

200. Demirci-Cekic S, Ozkan G, Avan AN, Uzunboy S, Capanoglu E, Apak R. Biomarkers of oxidative stress and antioxidant defense. *J Pharm Biomed Anal*. (2022) 209:114477. doi: 10.1016/j.jpba.2021.114477

201. Harayama T, Riezman H. Understanding the diversity of membrane lipid composition. *Nat Rev Mol Cell Biol*. (2018) 19(5):281–96. doi: 10.1038/nrm.2017.138

202. Li J, Pan L, Pan W, Li N, Tang B. Recent progress of oxidative stress associated biomarker detection. *Chem Commun (Camb)*. (2023) 59(48):7361–74. doi: 10.1039/d3cc00878a

203. Wolk M, Prabutzki P, Fedorova M. Analytical toolbox to unlock the diversity of oxidized lipids. *Acc Chem Res*. (2023) 56(7):835–45. doi: 10.1021/acs.accounts.2c00842

204. Weinberger B, Hirsch D, Yin K, Spur BW. Chapter 21—lipid mediators and lung function. In: Parent RA, editor. *Comparative Biology of the Normal Lung*. 2nd ed. San Diego: Academic Press (2015). p. 403–21.

205. Ito J, Shimizu N, Kobayashi E, Hanzawa Y, Otoki Y, Kato S, et al. A novel chiral stationary phase LC-MS/MS method to evaluate oxidation mechanisms of edible oils. *Sci Rep*. (2017) 7(1):10026. doi: 10.1038/s41598-017-10536-2

206. Eckl PM, Bresgen N. Genotoxicity of lipid oxidation compounds. *Free Radic Biol Med*. (2017) 111:244–52. doi: 10.1016/j.freeradbiomed.2017.02.002

207. Kehm R, Baldensperger T, Raupbach J, Hohn A. Protein oxidation—formation mechanisms, detection and relevance as biomarkers in human diseases. *Redox Biol*. (2021) 42:101901. doi: 10.1016/j.redox.2021.101901

208. Tu BP, Weissman JS. Oxidative protein folding in eukaryotes: mechanisms and consequences. *J Cell Biol*. (2004) 164(3):341–6. doi: 10.1083/jcb.200311055

209. Hondal RJ, Marino SM, Gladyshev VN. Selenocysteine in thiol/disulfide-like exchange reactions. *Antioxid Redox Signal*. (2013) 18(13):1675–89. doi: 10.1089/ars.2012.5013

210. Palla T, Mirzahosseini A, Noszal B. Properties of selenolate-diselenide redox equilibria in view of their thiolate-disulfide counterparts. *Antioxidants (Basel)*. (2023) 12(4):822. doi: 10.3390/antiox12040822

211. Orian L, Flohe L. Selenium-catalyzed reduction of hydroperoxides in chemistry and biology. *Antioxidants (Basel)*. (2021) 10(10):1560. doi: 10.3390/antiox10101560

212. Guo L, Yang W, Huang Q, Qiang J, Hart JR, Wang W, et al. Selenocysteine-specific mass spectrometry reveals tissue-distinct selenoproteomes and candidate selenoproteins. *Cell Chem Biol*. (2018) 25(11):1380–8.e4. doi: 10.1016/j.chembiol.2018.08.006

213. Steinbrenner H, Speckmann B, Klotz LO. Selenoproteins: antioxidant selenoenzymes and beyond. *Arch Biochem Biophys*. (2016) 595:113–9. doi: 10.1016/j.abb.2015.06.024

214. Hoshi T, Heinemann S. Regulation of cell function by methionine oxidation and reduction. *J Physiol*. (2001) 531(Pt 1):1–11. doi: 10.1111/j.1469-7793.2001.0001j.x

215. Suzuki S, Kodera Y, Saito T, Fujimoto K, Momozono A, Hayashi A, et al. Methionine sulfoxides in serum proteins as potential clinical biomarkers of oxidative stress. *Sci Rep*. (2016) 6:38299. doi: 10.1038/srep38299

216. Baldensperger T, Eggen M, Kappen J, Winterhalter PR, Pfirrmann T, Glomb MA. Comprehensive analysis of posttranslational protein modifications in aging of subcellular compartments. *Sci Rep*. (2020) 10(1):7596. doi: 10.1038/s41598-020-64265-0

217. Lim JM, Kim G, Levine RL. Methionine in proteins: it's not just for protein initiation anymore. *Neurochem Res*. (2019) 44(1):247–57. doi: 10.1007/s11064-017-2460-0

218. Kim J, Li BX, Huang RY, Qiao JX, Ewing WR, MacMillan DWC. Site-selective functionalization of methionine residues via photoredox catalysis. *J Am Chem Soc*. (2020) 142(51):21260–6. doi: 10.1021/jacs.0c09926

219. Guan Z, Yates NA, Bakhtiar R. Detection and characterization of methionine oxidation in peptides by collision-induced dissociation and electron capture dissociation. *J Am Soc Mass Spectrom*. (2003) 14(6):605–13. doi: 10.1016/S1044-0305(03)00201-0

220. Stadtman ER, Levine RL. Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. *Amino Acids*. (2003) 25(3–4):207–18. doi: 10.1007/s00726-003-0011-2

221. Reddy VP, Aryal P, Darkwah EK. Advanced glycation end products in health and disease. *Microorganisms*. (2022) 10(9):1848. doi: 10.3390/microorganisms10091848

222. Campolo N, Issoglio FM, Estrin DA, Bartesaghi S, Radi R. 3-Nitrotyrosine and related derivatives in proteins: precursors, radical intermediates and impact in function. *Essays Biochem*. (2020) 64(1):111–33. doi: 10.1042/EBC20190052

223. Akagawa M. Protein carbonylation: molecular mechanisms, biological implications, and analytical approaches. *Free Radic Res*. (2021) 55(4):307–20. doi: 10.1080/10715762.2020.1851027

224. Hawkins CL, Davies MJ. Detection, identification, and quantification of oxidative protein modifications. *J Biol Chem*. (2019) 294(51):19683–708. doi: 10.1074/jbc.REV119.006217

225. Zhao Y, Zhang L, Ouyang X, Jiang Z, Xie Z, Fan L, et al. Advanced oxidation protein products play critical roles in liver diseases. *Eur J Clin Invest*. (2019) 49(6):e13098. doi: 10.1111/eci.13098

226. Hahm JY, Park J, Jang ES, Chi SW. 8-Oxoguanine: from oxidative damage to epigenetic and epitranscriptional modification. *Exp Mol Med*. (2022) 54(10):1626–42. doi: 10.1038/s12276-022-00822-z

227. Zou Y, Ma X, Tang Y, Lin L, Yu J, Zhong J, et al. A robust LC-MS/MS method to measure 8-oxoguo, 8-oxodg, and nmn in human serum and urine. *Anal Biochem*. (2023) 660:114970. doi: 10.1016/j.ab.2022.114970

228. Chao MR, Evans MD, Hu CW, Ji Y, Moller P, Rossner P, et al. Biomarkers of nucleic acid oxidation—a summary state-of-the-art. *Redox Biol*. (2021) 42:101872. doi: 10.1016/j.redox.2021.101872

229. Cioffi F, Adam RHI, Bansal R, Broersen K. A review of oxidative stress products and related genes in early Alzheimer's disease. *J Alzheimers Dis*. (2021) 83 (3):977–1001. doi: 10.3233/JAD-210497

230. Nunomura A, Hofer T, Moreira PI, Castellani RJ, Smith MA, Perry G. RNA oxidation in Alzheimer disease and related neurodegenerative disorders. *Acta Neuropathol*. (2009) 118(1):151–66. doi: 10.1007/s00401-009-0508-1

231. Gmitterova K, Gawinecka J, Heinemann U, Valkovic P, Zerr I. DNA versus rna oxidation in Parkinson's disease: which is more important? *Neurosci Lett*. (2018) 662:22–8. doi: 10.1016/j.neulet.2017.09.048

232. Legaard GE, Feineis CS, Johansen MY, Hansen KB, Vaag AA, Larsen EL, et al. Effects of an exercise-based lifestyle intervention on systemic markers of oxidative stress and advanced glycation endproducts in persons with type 2 diabetes: secondary analysis of a randomised clinical trial. *Free Radic Biol Med*. (2022) 188:328–36. doi: 10.1016/j.freeradbiomed.2022.06.013

233. Jors A, Lund MAV, Jespersen T, Hansen T, Poulsen HE, Holm JC. Urinary markers of nucleic acid oxidation increase with age, obesity and insulin resistance in Danish children and adolescents. *Free Radic Biol Med*. (2020) 155:81–6. doi: 10.1016/j.freeradbiomed.2020.05.009

234. Liu T, Cai JP, Zhang LQ, Sun N, Cui J, Wang H, et al. The mechanism of RNA oxidation involved in the development of heart failure. *Free Radic Res*. (2019) 53 (8):910–21. doi: 10.1080/10715762.2019.1646424

235. Shih YM, Chang YJ, Cooke MS, Pan CH, Hu CH, Chao MR, et al. Alkylating and oxidative stresses in smoking and non-smoking patients with COPD: implications for lung carcinogenesis. *Free Radic Biol Med*. (2021) 164:99–106. doi: 10.1016/j.freeradbiomed.2020.12.442

236. Hong X, Hu Y, Yuan Z, Fang Z, Zhang X, Yuan Y, et al. Oxidatively damaged nucleic acid: linking diabetes and cancer. *Antioxid Redox Signal*. (2022) 37(16–18):1153–67. doi: 10.1089/ars.2022.0096

237. Ock CY, Kim EH, Choi DJ, Lee HJ, Hahm KB, Chung MH. 8-hydroxydeoxyguanosine: not mere biomarker for oxidative stress, but remedy for

oxidative stress-implicated gastrointestinal diseases. *World J Gastroenterol*. (2012) 18 (4):302–8. doi: 10.3748/wjg.v18.i4.302

238. Mundt JM, Hah SS, Sumbad RA, Schramm V, Henderson PT. Incorporation of extracellular 8-oxodg into DNA and RNA requires purine nucleoside phosphorylase in mcf-7 cells. *Nucleic Acids Res*. (2008) 36(1):228–36. doi: 10.1093/nar/gkm1032

239. Stoddard S, Riggleman A, Carpenter A, Baranova A. The detection of 8-oxo-7,8-dihydro-2'-deoxyguanosine in circulating cell-free DNA: a step towards longitudinal monitoring of health. *Adv Exp Med Biol*. (2020) 1241:125–38. doi: 10.1007/978-3-030-41283-8_8

240. Ahola T, Fellman V, Kjellmer I, Raivio KO, Lapatto R. Plasma 8-isoprostane is increased in preterm infants who develop bronchopulmonary dysplasia or periventricular leukomalacia. *Pediatr Res*. (2004) 56(1):88–93. doi: 10.1203/01.PDR.0000130478.05324.9D

241. Matthews MA, Aschner JL, Stark AR, Moore PE, Slaughter JC, Steele S, et al. Increasing F2-isoprostanes in the first month after birth predicts poor respiratory and neurodevelopmental outcomes in very preterm infants. *J Perinatol*. (2016) 36 (9):779–83. doi: 10.1038/jp.2016.74

242. Reuter SD, O'Donovan DJ, Hegemier SE, Smith EO, Heird WC, Fernandes CJ. Urinary F2-isoprostanes are poor prognostic indicators for the development of bronchopulmonary dysplasia. *J Perinatol*. (2007) 27(5):303–6. doi: 10.1038/sj.jp.7211684

243. Chessex P, Watson C, Kaczala GW, Rouleau T, Lavoie ME, Friel J, et al. Determinants of oxidant stress in extremely low birth weight premature infants. *Free Radic Biol Med*. (2010) 49(9):1380–6. doi: 10.1016/j.freeradbiomed.2010.07.018

244. Vera KB, Moore D, Flack E, Liske M, Summar M. Significant differences in markers of oxidant injury between idiopathic and bronchopulmonary-dysplasia-associated pulmonary hypertension in children. *Pulm Med*. (2012) 2012:301475. doi: 10.1155/2012/301475

245. Kuligowski J, Aguar M, Rook D, Lliso I, Torres-Cuevas I, Escobar J, et al. Urinary lipid peroxidation byproducts: are they relevant for predicting neonatal morbidity in preterm infants? *Antioxid Redox Signal*. (2015) 23(2):178–84. doi: 10.1089/ars.2015.6262

246. Liyakat NA, Kumar P, Sundaram V. Room air versus 100% oxygen for delivery room resuscitation of preterm neonates in low resource settings: a randomised, blinded, controlled trial. *J Paediatr Child Health*. (2023) 59(6):794–801. doi: 10.1111/jpc.16391

247. Bhunwal S, Mukhopadhyay K, Bhattacharya S, Dey P, Dhaliwal LK. Bronchopulmonary dysplasia in preterm neonates in a level iii neonatal unit in India. *Indian Pediatr*. (2018) 55(3):211–5. doi: 10.1007/s13312-018-1319-z

248. Yildizdas HY, Poyraz B, Atli G, Sertdemir Y, Mert K, Ozlu F, et al. Effects of two different lipid emulsions on antioxidant Status, lipid peroxidation and parenteral nutrition- related cholestasis in premature babies, a randomized-controlled study. *Pediatr Neonatol*. (2019) 60(4):359–67. doi: 10.1016/j.pedneo.2018.07.012

249. Weinberger B, Anwar M, Henien S, Sosnovsky A, Hiatt M, Jochnowitz N, et al. Association of lipid peroxidation with antenatal betamethasone and oxygen radial disorders in preterm infants. *Biol Neonate*. (2004) 85(2):121–7. doi: 10.1159/000074968

250. Joung KE, Kim HS, Lee J, Shim GH, Choi CW, Kim EK, et al. Correlation of urinary inflammatory and oxidative stress markers in very low birth weight infants with subsequent development of bronchopulmonary dysplasia. *Free Radic Res*. (2011) 45(9):1024–32. doi: 10.3109/10715762.2011.588229

251. Tokuriki S, Okuno T, Ohta G, Ohshima Y. Carboxyhemoglobin formation in preterm infants is related to the subsequent development of bronchopulmonary dysplasia. *Dis Markers*. (2015) 2015:620921. doi: 10.1155/2015/620921

252. Vento M, Aguar M, Escobar J, Arduini A, Escrig R, Brugada M, et al. Antenatal steroids and antioxidant enzyme activity in preterm infants: influence of gender and timing. *Antioxid Redox Signal*. (2009) 11(12):2945–55. doi: 10.1089/ars.2009.2671

253. Ahmed N, Thornalley PJ. Quantitative screening of protein biomarkers of early glycation, advanced glycation, oxidation and nitrosation in cellular and extracellular proteins by tandem mass spectrometry multiple reaction monitoring. *Biochem Soc Trans*. (2003) 31(Pt 6):1417–22. doi: 10.1042/bst0311417

254. Chatterjee N, Walker GC. Mechanisms of DNA damage, repair, and mutagenesis. *Environ Mol Mutagen*. (2017) 58(5):235–63. doi: 10.1002/em.22087

255. Rafiee A, Ospina MB, Pitt TM, Quemerais B. Oxidative stress and DNA damage resulting from welding fumes exposure among professional welders: a systematic review and meta-analysis. *Environ Res*. (2022) 214(Pt 4):114152. doi: 10.1016/j.envres.2022.114152

256. Goriuc A, Cojocaru KA, Luchian I, Ursu RG, Butnaru O, Foia L. Using 8-hydroxy-2'-deoxiguanosine (8-ohdg) as a reliable biomarker for assessing periodontal disease associated with diabetes. *Int J Mol Sci*. (2024) 25(3):1425. doi: 10.3390/ijms25031425

257. Wang D, Liang Q, Tai D, Wang Y, Hao H, Liu Z, et al. Association of urinary arsenic with the oxidative DNA damage marker 8-hydroxy-2 deoxyguanosine: a meta-analysis. *Sci Total Environ*. (2023) 904:166600. doi: 10.1016/j.scitotenv.2023.166600

258. Bigagli E, Lodovici M. Circulating oxidative stress biomarkers in clinical studies on type 2 diabetes and its complications. *Oxid Med Cell Longev*. (2019) 2019:5953685. doi: 10.1155/2019/5953685

259. Blahova L, Janos T, Mustieles V, Rodriguez-Carrillo A, Fernandez MF, Blaha L. Rapid extraction and analysis of oxidative stress and DNA damage biomarker 8-hydroxy-2'-deoxyguanosine (8-ohdg) in urine: application to a study with pregnant women. *Int J Hyg Environ Health*. (2023) 250:114175. doi: 10.1016/j.ijheh.2023.114175

260. Poulsen HE, Weimann A, Henriksen T, Kjaer LK, Larsen EL, Carlsson ER, et al. Oxidatively generated modifications to nucleic acids in vivo: measurement in urine and plasma. *Free Radic Biol Med*. (2019) 145:336–41. doi: 10.1016/j.freeradbiomed.2019.10.001

261. Zayegh AM, Davis PG. Bpd treatments: the never-ending smorgasbord. *Semin Fetal Neonatal Med*. (2021) 26(2):101223. doi: 10.1016/j.siny.2021.101223

262. Zimmerman JJ, Gabbert D, Shivpuri C, Kayata S, Ciesielski W, Miller J, et al. Meter-dosed, inhaled beclomethasone attenuates bronchoalveolar oxyradical inflammation in premature infants at risk for bronchopulmonary dysplasia. *Am J Perinatol*. (1998) 15(10):567–76. doi: 10.1055/s-2007-994062

263. Laube M, Amann E, Uhlig U, Yang Y, Fuchs HW, Zemlin M, et al. Inflammatory mediators in tracheal aspirates of preterm infants participating in a randomized trial of inhaled nitric oxide. *PLoS One*. (2017) 12(1):e0169352. doi: 10.1371/journal.pone.0169352

264. Ozkan H, Koksal N, Dorum BA, Kocael F, Ozarda Y, Bozyigit C, et al. New-generation fish oil and olive oil lipid for prevention of oxidative damage in preterm infants: single center clinical trial at university hospital in Turkey. *Pediatr Int*. (2019) 61(4):388–92. doi: 10.1111/ped.13798

265. Capasso L, Vento G, Loddo C, Tirone C, Iavarone F, Raimondi F, et al. Oxidative stress and bronchopulmonary dysplasia: evidences from microbiomics, metabolomics, and proteomics. *Front Pediatr*. (2019) 7:30. doi: 10.3389/fped.2019.00030

frontiers | Frontiers in Pediatrics

TYPE Original Research
PUBLISHED 25 July 2025
DOI 10.3389/fped.2025.1388951

OPEN ACCESS

EDITED BY
Rujipat Samransamruajkit,
Chulalongkorn University, Thailand

REVIEWED BY
Yingtang Shi,
University of Virginia, United States
Zorica Momcilo Zivkovic,
University Hospital Center Dr Dragiša Mišović, Serbia
Yu Xiuhua,
First Affiliated Hospital of Jilin University, China

*CORRESPONDENCE
Sha Liu
✉ lsls2628@126.com

RECEIVED 20 February 2024
ACCEPTED 14 July 2025
PUBLISHED 25 July 2025

CITATION
Qin J, Liu F, Wang T, Fu Z, Lin Y, Wang X, Zhao J and Liu S (2025) Correlation between tidal breathing pulmonary function, exhaled nitric oxide and airway hyperresponsiveness in children aged 0–3 years with suspected asthma.
Front. Pediatr. 13:1388951.
doi: 10.3389/fped.2025.1388951

Correlation between tidal breathing pulmonary function, exhaled nitric oxide and airway hyperresponsiveness in children aged 0–3 years with suspected asthma

Jiangjiao Qin[1,2], Fangjun Liu[1,2], Ting Wang[2,3], Zhou Fu[2,3], Ying Lin[1,2], Xia Wang[1,2], Jing Zhao[1,2] and Sha Liu[1,2]*

[1]Department of Pulmonary Function, Children's Hospital of Chongqing Medical University, Chongqing, China, [2]National Clinical Research Center for Child Health and Disorders, Ministry of Education Key Laboratory of Child Development and Disorders, Chongqing Key Laboratory of Pediatrics, Children's Hospital of Chongqing Medical University, Chongqing, China, [3]Department of Respiratory Medicine, Children's Hospital of Chongqing Medical University, Chongqing, China

Objective: To investigate the correlation between tidal breathing pulmonary function parameters combined with mixed exhaled gas nitric oxide values and the degree of airway hyperresponsiveness (AHR) in children aged 0–3 years with suspected asthma.

Methods: In this retrospective study, we collected baseline clinical data, tidal breathing pulmonary function parameters (measured before methacholine inhalation), fractional exhaled nitric oxide (FeNO) levels, and methacholine challenge test (MCT) results from 818 pediatric asthma patients treated at the Children's Hospital of Chongqing Medical University between January 2021 and June 2023. Baseline data, tidal respiratory pulmonary function parameters, and FeNO values were used to analyze their correlation with AHR. Ordinal multiclass logistic regression analysis was used to identify factors influencing AHR. The receiver operating characteristic (ROC) curve was performed to evaluate the efficacy of predicting AHR using tidal breathing pulmonary function parameters and FeNO values.

Results: Intergroup comparisons showed significant differences in age, weight, height, FeNO, TPTEF/TE, RR, TI/TE, TEF50/TIF50, and PTFE/TEF25 ($P < 0.05$). Further ordinal multiclass logistic regression analysis revealed that increases in FeNO, RR, and PTEF/TEF25 were significantly positively correlated with AHR severity ($P < 0.001$), while age was significantly negatively correlated ($P < 0.001$). FeNO showed reasonable accuracy in predicting AHR at methacholine concentrations of 8 mg/ml (AUC=0.774) and a cut-off value of 14 ppb (sensitivity 88.5%, specificity 63.8%). The combined parameters (FeNO, RR, PTFE/TEF25, and age) showed high accuracy in predicting AHR at methacholine concentrations of 0.5 mg/ml (AUC=0.847).

Conclusions: Our study revealed that Current airway inflammation and airway obstruction predicted AHR at this point.FeNO, RR, PTEF/TEF25, and age were effective predictive parameters for the degree of AHR in children aged 0–3 years with suspected asthma; FeNO >14 ppb served as an independent factor suggesting AHR in children at methacholine concentrations of 8 mg/ml, and the combined parameters showed better predictive efficacy.

KEYWORDS

infants, pulmonary function, airway inflammation, FeNO, airway hyperresponsiveness (AHR)

1 Introduction

Airway hyperresponsiveness (AHR) is a pathological state of excessive airway responsiveness to stimuli associated with chronic airway inflammation and is one of the main features of asthma (1). The methacholine challenge test (MCT) is often used to quantify the degree of AHR and evaluate the patient's condition and treatment effect. Studies have shown that the level of airway reactivity in early infancy (1 month old) is strongly correlated with asthma in childhood (6 years old) (2, 3), and increased airway reactivity in childhood is an independent risk factor for asthma in adulthood (4, 5). Therefore, early identification of airway hyperresponsiveness in infancy is crucial to detect potential asthma risks in childhood, facilitate early intervention, and reduce the probability of developing asthma in adults. However, the clinical application of MCT is limited because of its complex operation, time-consuming nature, and high risk of inducing bronchospasm. Thus, it is urgent to find the predictive parameters of AHR.

Exhaled nitric oxide (fractional exhaled nitric oxide, FeNO) is commonly used in the clinic to evaluate airway inflammation in patients with asthma and the compliance and practicality of inhaled corticosteroid (ICS) treatment (5), thereby guiding the diagnosis and treatment of asthma. Additionally, tidal pulmonary function tests are significant in the severity assessment and prognosis of respiratory diseases in infants and young children. Previous studies have shown that tidal pulmonary function parameters in newborns can predict the severity of AHR and ICS usage at the age of ten (6). Scarce research has been conducted on the prediction of airway reactivity in infants and young children and is mostly limited to the prediction of positive or negative airway reactivity. Our study aimed to analyze the correlation between tidal pulmonary function parameters combined with FeNO and the degree of AHR to predict the degree of AHR in children aged 0–3 years with asthma and provide a reference for the choice of treatment methods and prognosis assessment.

2 Patients and methods

2.1 Patients

A retrospective analysis was performed on 818 children diagnosed with asthma from January 2021 to June 2023 in the Asthma Clinic of Children's Hospital of Chongqing Medical University. All included patients underwent the FeNO test, tidal breath pulmonary function test, and MCT on the day of consultation. The inclusion criteria were as follows: (1) Aged 0–3 years old, (2) Meeting the Global Initiative for Asthma (2023 edition) diagnostic criteria for asthma in children aged 5 and below to ensure that the children are in clinical remission; Specifically, symptoms of upper respiratory tract infection lasting more than 10 days, more than 3 exacerbations per year, having received ≥2 months of low-dose ICS treatment and showing clinical improvement. (3) Children were follow-up visits and had been diagnosed with asthma by a clinical doctor at least 2 times. (4) Excluding children with chest deformities, lower respiratory tract infection in the previous 4 weeks, or upper respiratory tract infection in the previous 1 week. This study was approved by the Research Ethics Committee of the Children's Hospital of Chongqing Medical University (Table 1).

2.2 Methods

Before all tests, children were induced into a stable sedated sleep state through oral administration of 10% chloral hydrate at a dosage of 30–50 mg/kg.

2.2.1 FeNO measurement

FeNO was measured by offline tidal-breath NO measurement using a Sunvou nitric oxide analyzer (CA2123). Here are the operation steps: The appropriate tidal mask was selected and connected to the analyzer's special sampling bag through the tidal offline sampler (to filter NO in the air). The mask was tightly fastened to the subject's nose and mouth to prevent air leakage. After collecting more than 5 tidal breaths until the sampling bag was half full, the sampling bag was connected to the instrument for offline FeNO measurement, and the unit of the test result was ppb (parts per billion). FeNO was used by a technician specialized in pulmonary function tests before MCT, and the measurement precautions were strictly following the children's guidelines (5).

2.2.2 Tidal breathing pulmonary function test

The tests were performed by a technician specialized in pulmonary function tests using a pulmonary function instrument (Jaeger MasterScreen Paed.CareFusion, San Diego, CA). The testing method strictly followed the quality control

TABLE 1 Demographic and functional parameter statistics and difference analysis of AHR degree.

Characteristic	Total $n = 818$	MCH (0.5 mg/ml) $n = 34$	MCH (2 mg/ml) $n = 252$	MCH (8 mg/ml) $n = 394$	MCH (16 mg/ml) $n = 107$	Non-responsive $n = 31$	*P*
Gender (Male)	505 (61.7)	22 (64.7)	153 (60.7)	243 (61.7)	67 (62.2)	20 (64.5)	0.984
Age (Month)	34.0 (26.0, 40.0)	23.5 (17.5, 34.0)	33.0 (24.0, 39.0)	35.0 (28.0, 40.0)	36.0 (29.0, 40.0)	39.0 (33.0, 41.0)	<0.001
Weight (kg)	14.0 (12.5, 15.0)	12.0 (10.5, 14.0)	14.0 (12.5, 15.0)	14.0 (12.5, 15.0)	14.0 (12.5, 15.5)	14.0 (13.0, 15.0)	0.001
Height (cm)	96.0 (90.0, 100.0)	87.5 (83.3, 97.0)	95.5 (89.0, 100.0)	96.0 (91.0, 100.0)	96.0 (92.0, 100.0)	99.0 (94.0, 102.0)	<0.001
FeNO (ppb)	27.0 (17.0, 39.0)	45.0 (26.5, 62.5)	32.0 (19.0, 43.0)	28.0 (20.0, 37.0)	11.0 (7.0, 20.0)	12.0 (5.0, 34.0)	<0.001
TPTEF/TE (%)	23.2 (19.5, 28.2)	23.3 (18.8, 27.1)	22.3 (19.3, 26.8)	23.0 (19.2, 28.4)	25.3 (20.5, 30.5)	24.1 (21.9, 30.9)	0.008
VPTEF/VE (%)	26.4 (23.9, 30.7)	25.6 (23.6, 29.7)	26.1 (23.8, 29.5)	26.4 (23.8, 30.8)	28.3 (24.3, 32.1)	28.1 (25.6, 33.9)	0.210
RR (pbm)	26.1 ± 4.3	28.9 ± 5.5	27.0 ± 4.1	25.4 ± 3.9	25.3 ± 4.9	25.5 ± 4.0	<0.001
VT/kg	8.9 (7.8, 9.8)	9.1 (7.4, 9.6)	8.7 (7.7, 9.8)	8.9 (7.8, 9.8)	8.9 (8.0, 9.9)	9.0 (7.4, 9.6)	0.544
tI/tE (%)	63.0 (56.0, 70.0)	62.5 (56.5, 71.0)	62.5 (55.0, 68.0)	63.0 (55.8, 69.0)	64.0 (58.0, 73.0)	68.2 (55.0, 77.0)	0.048
TEF50/TIF50 (%)	71.8 (63.3, 80.5)	68.4 (63.4, 78.8)	70.9 (62.1, 78.9)	72.5 (63.8, 80.7)	72.3 (62.8, 83.2)	74.0 (64.6, 90.4)	0.284
PTEF/TEF25 (%)	154.8 ± 25.3	162.6 ± 23.5	159.7 ± 25.2	153.4 ± 24.5	149.1 ± 26.1	144.4 ± 27.3	<0.001

Data are presented as Number (%), Median (P25, P75), or (x ± s).Comparison of Baseline Demographic Characteristics and Pre-Challenge Parameters Across AHR Severity Groups.
MCH(C%), methacholine concentration at the endpoint of methacholine challenge test; Non-responsive, methacholine challenge test methacholine concentration 16 mg/ml without an endpoint; FeNO, fractional exhaled nitric oxide; TPTEF/TE, time to reach peak tidal expiratory flow over expiratory time; VPTEF/VE, volume to reach peak tidal expiratory flow over expiratory volume; RR, respiratory rate; VT/kg, tidal volume per kilogram of body weight; TI/TE, the ratio of inspiratory time to expiratory time; TEF50/TIF50, tidal expiratory flow at 50% of expiratory volume divided by tidal inspiratory flow at 50% of inspiratory volume; PEF/TEF25, the ratio of peak expiratory flow rate to instantaneous expiratory flow rate with 25% tidal volume remaining.

requirements of the Children's Tidal Breathing Pulmonary Function Guidelines (7).

2.2.3 Methacholine challenge test

Before the test, the children's nasal secretions were cleared, and the transcutaneous arterial oxygen saturation (SPO_2) of the children was monitored at the same time. Airway responsiveness can only be measured if at least one of the two parameters TPTEF/TE and VPTEF/VE exceeds 23% and SPO2 ≥ 95%.

Testing steps: Different concentrations of methacholine (MCH, Sigma, USA) solution were inhaled from low to high through the high-frequency atomizer by participants, with concentrations of 0.5, 2, 8, and 16 mg/ml in turn. Each atomization time was 1 min, and the inhalation time between different concentration intervals was 2 min. The tidal pulmonary function test was repeated 30–60 s after each aerosol inhalation of methacholine solution until the MCT was positive or the MCH concentration was the highest, and then the challenge process was terminated. Immediately after the stimulation process is terminated, a bronchodilator (1.25 mg terbutaline sulfate, 0.125 mg ipratropium bromide) combined with 4 ml of atomized inhalation solution is administered. SPO_2, heart rate, and breathing were closely observed during the whole test. After 4–6 min, pulmonary function was retested, and the child's SPO_2, heart rate, and pulmonary signs were evaluated until the parameters returned to the basic level.

Result determination: MCT-positive results were judged based on comprehensive indicators: ① Obvious expiratory wheezing appeared on lung auscultation; ② Respiratory frequency (RR) value increased by ≥50%; ③ SPO_2 value decreased by ≥5. During the test, if any two of ①, ②, and ③ occur at the same time, it is considered an excitation endpoint. Degree classification: In the event of an excitation endpoint result, the degree was determined based on the concentration of inhaled MCH: 0.5, 2, 8, and 16 mg/ml, and no methacholine challenge test (MCT) endpoint at 16 mg/ml.

2.3 Statistical analysis

Data entry was performed using EXCEL, double entry, and check. Data analysis was performed using SPSS 27.0. Categorical variables were expressed as the number of cases (%), continuous variables conformed to a normal distribution and variance chi-square and were expressed as the mean ± standard deviation, and comparisons between multiple groups were performed using analysis of variance. Nonnormal continuous variables were expressed as M (P25, P75), and the Kruskal-Wallis H test was used for comparisons between multiple groups. Correlations between parameters were analyzed using Spearman correlation analysis. Factors with statistically significant significance in the one-way analysis of factors were included in the multifactorial regression analysis as the independent variables, and factors with a strong correlation with age were excluded (height, body weight). The four concentrations (0.5 mg/ml, 2 mg/ml, 8 mg/ml, 16 mg/ml) at which the MCT showed provocation endpoints were assigned the values of 1, 2, 3, and 4, respectively, to represent different grades of airway responsiveness, and the ordered multiclassified logistic regression model was constructed, analyzing the independent influences on the degree of AHR. ROC curves were used to evaluate the efficacy of FeNO vs. the Combined parameter prediction at different degrees of AHR. Differences were considered statistically significant at $P < 0.05$.

3 Result

3.1 Characteristics of participants

A total of 7,957 children who completed tidal breathing pulmonary function test and MCT in the pulmonary function

room of the Children's Hospital of Chongqing Medical University from January 2021 to June 2023 were selected, including 998 children who also completed FeNO on the same day. A total of 180 children whose diagnosis was unclear or who were not treated with ICS in outpatient medical records were excluded. A total of 818 children (aged 0–3 years, 505 males and 313 females) with asthma were included. Corresponding data of age, height, weight, and testing parameters were obtained. All participants were divided into 5 groups according to the severity of airway responsiveness determined by MCT. Comparisons of baseline characteristics and pre-challenge measurements (FeNO, tidal breathing parameters) among these groups are shown in Table 1. Statistically significant differences were observed in age, weight, height, FeNO, TPTEF/TE, RR, TI/TE, and PTFE/TEF25 ($P < 0.05$) (Table 1).

3.2 Correlation between age and parameters

There was a strong correlation between weight ($r = 0.658$, $P < 0.001$), height ($r = 0.803$, $P < 0.001$) and age. RR ($r = -0.206$, $P < 0.001$), TI/TE ($r = 0.173$, $P < 0.001$), TEF50/TIF50 ($r = 0.232$, $P < 0.001$), and AHR ($r = 0.184$, $P < 0.001$) were significantly related to age. There was no correlation between FeNO, TPTEF/TE, VPTEF/VT, VT/kg, PTEF/TEF25, and age ($P > 0.05$).

3.3 Correlation between pulmonary function parameters and AHR

Using assignment 4 (AHR at methacholine concentration at 16 mg/ml) as the reference category, ordered multicategorical logistic regression analysis was used (parallel validation significance level of 0.498, $P > 0.05$), and the model fit was $P < 0.001$. The results showed that an increase in FeNO, RR, and PTEF/TEF25 was significantly and positively correlated with the severity of AHR ($P < 0.001$)). An increase in age was negatively correlated with the severity of AHR ($P < 0.001$) (Table 2).

3.4 Efficiency value analysis of predicting AHR

The ROC curve was used to evaluate the effectiveness of the independent parameter FeNO and the combined parameters (FeNO, RR, PTEF/TEF25, and age) in predicting different degrees of AHR (Table 3). The results showed that the accuracy of FeNO in predicting AHR at methacholine concentrations of 8 mg/ml was acceptable (AUC = 0.774), with a predictive threshold of 14 ppb. The accuracy of the combined parameters in predicting different degrees of AHR was acceptable and excellent in predicting AHR at methacholine concentrations of 0.5 mg/ml (AUC = 0.847) (Figure 1).

TABLE 2 Multiple ordinal logistic analysis of AHR.

Characteristic	B	*SE*	Wald x^2	*P*	OR	OR 95%CI
FeNO (ppb)	−0.04	0.004	99.519	<0.001	0.962	0.954~0.969
Age (month)	0.038	0.008	21.745	<0.001	1.039	1.022~1.055
RR (pbm)	−0.093	0.016	32.071	<0.001	0.911	0.882~0.941
TPTEF/TE (%)	0.007	0.012	0.33	0.566	1.007	0.984~1.030
PTEF/TEF25 (%)	−0.013	0.004	11.684	0.001	0.987	0.979~0.994

4 Discussion

Previous studies on the correlation between FeNO and age have mainly focused on children over 4 years old, and whether FeNO is strongly correlated with age is controversial (8, 9). Our results showed no significant correlation between FeNO and age in children under 4 years of age ($P > 0.05$). We found that AHR decreased with increasing age in infants and young children, which is consistent with previous studies (10).

Tidal breathing pulmonary function is an efficient and simple method for assessing airway obstruction in infants (11). TPTEF/TE and VPTEF/VE, which both have a high correlation, are crucial parameters indicating airway obstruction, with their decreasing values proportionally related to the severity of airway obstruction (7). However, TPTEF/TE is superior to VPTEF/VE in reflecting airway obstruction (12, 13). Our study found significant differences in TPTEF/TE with varying degrees of AHR ($P < 0.05$), whereas VPTEF/VE showed no significant variation across multiple groups, which confirmed the previous view. Geir Håland (6) demonstrated that reduced TPTEF/TE in the neonatal period is a significant predictor of severe AHR and the use of ICS at age 10. However, our study did not find a predictive value of TPTEF/TE for current AHR, suggesting that early airway conditions may be related to childhood AHR but not to current AHR. Morris (14) confirmed that the attenuation of flow over time during tidal breathing reflects respiratory system resistance. PTEF/TEF25 can quantify the rapid decline in flow during airway obstruction, and increased airway obstruction causes a decrease in TEF25 values and may lead to a shift from tidal to forced breathing, resulting in an increase in PTEF values. The inverse changes in these two parameters make the alterations in PTEF/TEF25 more pronounced, rendering it a sensitive indicator of increasing airway obstruction (15). Previous studies have also verified a high correlation between airway obstruction and AHR (16, 17), recommending FeNO combined with forced pulmonary function parameters of airways substituting for MCT in assessing AHR in asthma patients (18). Based on these known findings, our study showed significant differences in TPTEF/TE and PTEF/TEF25 across various groups, with a high correlation of PTEF/TEF25 with AHR. This suggested that airway obstruction may be the main cause of AHR in infants during asthma. Therefore, for this population, airway obstruction is a key factor of AHR, with its degree closely related to the severity of AHR. Our study highlighted the diagnostic and evaluative value of PTEF/TEF25 in infants and toddlers with asthma. Additionally, respiratory rate (RR) is a critical indicator for assessing children's physiological status and

TABLE 3 Efficacy of feNO and combined parameters in predicting the corresponding AHR at different MCH concentrations.

Characteristic	MCH (C%)	AUC	95% CI of AUC	Sensitivity %	Specificity %	*P*
FeNO	0.5	0.693	0.661–0.725	52.94	84.69	<0.001
	2	0.620	0.586–0.653	40.21	80.64	<0.001
	8	0.774	0.774–0.802	88.53	63.77	<0.001
	16	0.669	0.635–0.701	94.28	45.16	0.001
Combined parameters	0.5	0.847	0.820–0.871	97.06	56.51	<0.001
	2	0.721	0.689–0.751	57.34	78.20	<0.001
	8	0.784	0.754–0.812	85.00	64.49	<0.001
	16	0.716	0.684–0.747	64.04	74.19	<0.001

Combined parameters, FeNO, RR, PTEF/TEF25, and age.

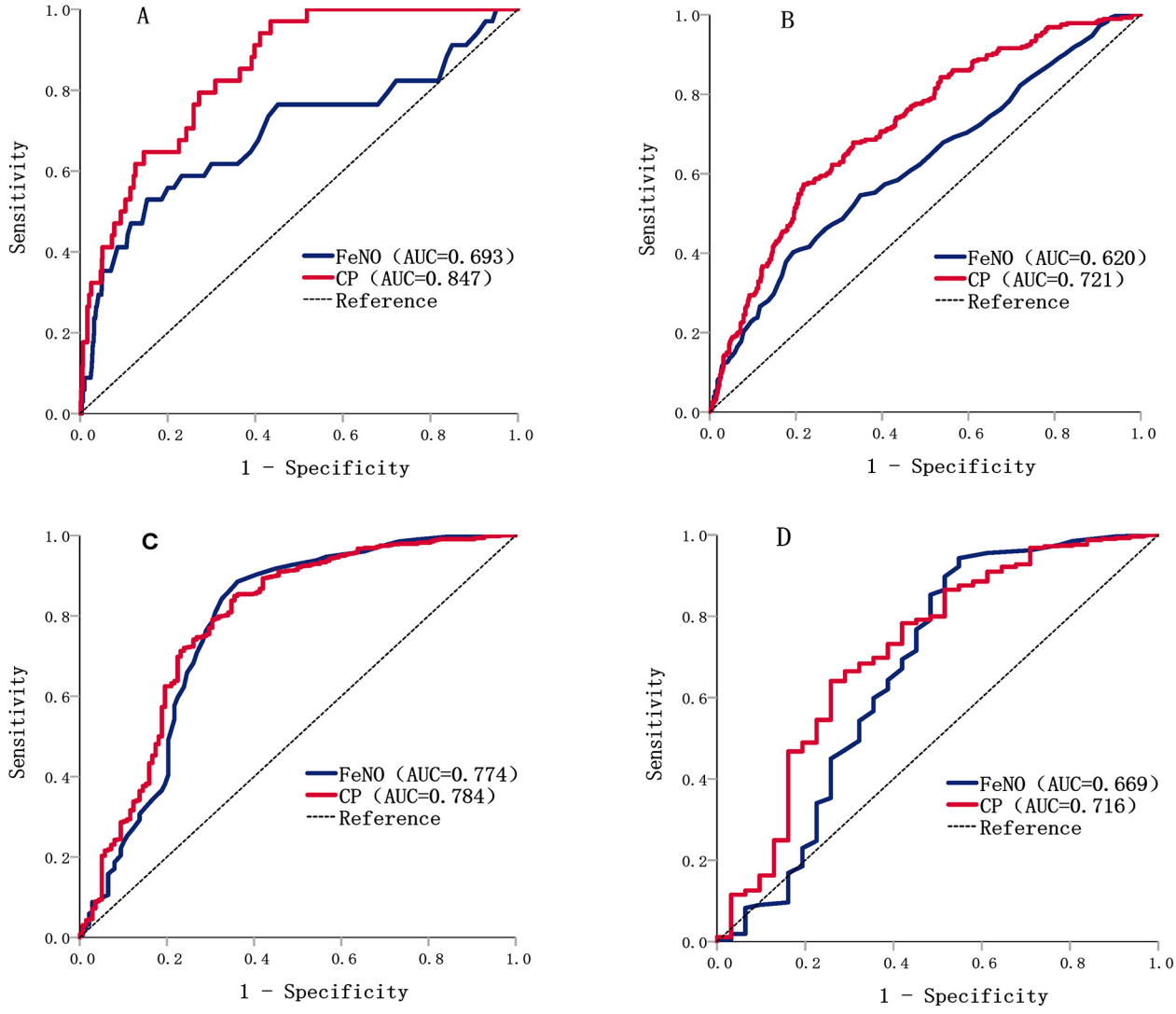


FIGURE 1
ROC curves of FeNO vs. combined parameters in predicting different degrees of AHR. **(A)** ROC curves of FeNO and combined parameters in predicting AHR at methacholine concentrations of 0.5 mg/ml; **(B)** ROC curves of FeNO and combined parameters in predicting AHR at methacholine concentrations of 2 mg/ml; **(C)** ROC curves of FeNO and combined parameters in predicting AHR at methacholine concentrations of 8 mg/ml; **(D)** ROC curves of FeNO and combined parameters in predicting AHR at methacholine concentrations of 16 mg/ml.

can be used for early warning scores of disease exacerbation (19). RR showed significant differences among children with varying degrees of airway reactivity, suggesting its potential as a predictive indicator for assessing the severity of asthma. Above all, it is recommended to pay special attention to the values of PTEF/TEF25 and RR when evaluating the effectiveness of asthma treatment in infants.

Tidal breathing NO measurement in infants offers the advantage of not requiring cooperation from the child and easy collection (20). However, as it involves collecting a mixed sample of oral and nasal gases, it may be affected by nasal NO (21). Previous research has indicated that the sinuses of infants are not fully developed and that tidal breathing methods are less affected by nasal NO (22), which has been confirmed by related studies (23). Online measurement of FeNO has been standardized, and offline methods have been proven applicable in infants (24). Bult (25) first functionally differentiated three subtypes of the enzyme system responsible for NO production in 1990, and activation of inducible NOS (iNOS) by airway inflammation might lead to increased NO production and thus escalating AHR (26). Relevant literature has also demonstrated that FeNO is related to AHR in asthma patients (27). Our study showed that FeNO >14 ppb could be used as an independent indicator of AHR with a sensitivity of 88.5% and a specificity of 63.8% when reacting to AHR with methacholine concentrations of 8 mg/ml. The combination of FeNO, RR, PTEF/TEF25, and age was a better predictor of AHR at methacholine concentrations of 0.5 mg/ml and 2 mg/ml than the independent parameter FeNO. It was reported that ventilation heterogeneity in asthmatic patients is determined by both inflammation and airway obstruction (28). PTEF/TEF25 could reflect the airway obstruction, which means that higher PTEF/TEF25 values indicate more severe obstruction. Meanwhile, the flow rate of FeNO depends on the airway diameter, which is positively correlated with the level of FeNO. Our results showed that the AUC of predicting AHR at methacholine concentrations of 0.5 mg/ml was 0.847. Thus, the combined parameters showed better predictive results in predicting stronger AHR, suggesting that as the severity of AHR increases, the influences on predicting the degree of AHR increase accordingly.

To clarify how this translates into clinical decision-making, we analysed dose-specific operating characteristics. At methacholine concentrations of 0.5 mg/ml the combined model is almost fail-safe for detecting strong airway hyper-responsiveness (sensitivity 97.06%), while FeNO alone is highly specific (84.69%). Thus the combined model is ideal for ruling out severe cases, with FeNO used afterwards to confirm truly positive children and avoid overtreatment.At methacholine concentrations of 16 mg/ml the pattern reverses: FeNO becomes the high-sensitivity screen (94.28%), and the combined model supplies the specificity needed (74.19%) to trim the large false-positive pool before starting inhaled corticosteroids or referral.This two-step approach pairs each model's strength, so clinicians minimise both missed diagnoses and unnecessary treatment.

This study is retrospective and shows some sample variation among groups, which could lead to bias due to unbalanced samples. However, the samples used in this study are consistent with the actual situation of this population. We did not include a saline control before the provocative process in our study, as doing so would not improve test safety but could reduce the accuracy of AHR measurements (29). Additionally, the inhalation time is shortened by increasing the MCH inhalation concentration (1 min), which has been proven feasible (30). We defined the study population as "suspected asthmatic children", which was based on the GINA (2023 edition) criteria for the diagnosis of asthma at the age of 5 years and below and the diagnosis made by the clinicians. Although we used strict inclusion criteria, it is important to acknowledge that the diagnosis of asthma in children aged 0–3 years is controversial in the medical community, which constitutes a limitation of this study. Our study subjects were infants diagnosed with asthma and treated with ICS for more than 2 months, which is of great significance for monitoring and managing. Although there is no direct medical basis to support the hierarchical information that defines the four concentrations of 0.5/2/8/16 mg/ml as the degree of airway hyperresponsiveness, we have made this assumption based on a general understanding of airway responsiveness and the physiological effects that may result from changes in concentration. We acknowledge the limitations of this approach and view it as a preliminary exploration. Future studies should further validate the reasonableness of this hierarchy.

In conclusion, airway inflammation and airway obstruction were strongly associated with the degree of AHR and were responsible for the development of AHR in infants with suspected asthma during the remission period. Meanwhile, the effect of age on AHR needs to be calibrated in interpreting MCT results in this age range. FeNO, RR, PTFE/TEF25, and age as combined parameters have certain accuracy in predicting different degrees of AHR. Therefore, the noninvasive airway inflammation marker FeNO combined with baseline tidal breathing pulmonary function parameters is valuable in predicting the degree of AHR.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by Institutional Review Board of Children's Hospital of Chongqing Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants

or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

JQ: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Visualization, Writing – original draft, Writing – review & editing. FL: Data curation, Investigation, Writing – original draft. TW: Supervision, Writing – review & editing. ZF: Validation, Writing – review & editing. YL: Investigation, Writing – original draft. XW: Software, Writing – original draft. JZ: Investigation, Writing – original draft. SL: Funding acquisition, Resources, Supervision, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. A joint project of Chongqing Health Commission and Science and Technology Bureau, No. 2022MSXM043.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Pulmonary Function Group, R. B. of C. P. S. of C. M. A.; Pediatrics, E. B. of C. J. of A. C. Series guidelines for pediatric pulmonary function(part VI): bronchial provocation test. *Chin J Appl Clin Pediatr*. (2017) 32(4):263–9. doi: 10.3760/cma.j.issn.2095-428X.2017.04.007

2. Cox DW, Mullane D, Zhang GC, Turner SW, Hayden CM, Goldblatt J, et al. Longitudinal assessment of airway responsiveness from 1 month to 18 years in the PIAF birth cohort. *Eur Respir J*. (2015) 46(6):1654–61. doi: 10.1183/13993003.00397-2015

3. Palmer LJ, Rye PJ, Gibson NA, Burton PR, Landau LI, Lesouëf PN. Airway responsiveness in early infancy predicts asthma, lung function, and respiratory symptoms by school age. *Am J Respir Crit Care Med*. (2001) 163(1):37–42. doi: 10.1164/ajrccm.163.1.2005013

4. Sears MR, Greene JM, Willan AR, Wiecek EM, Taylor DR, Flannery EM, et al. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. *N Engl J Med*. (2003) 349(15):1414–22. doi: 10.1056/NEJMoa022363

5. Pulmonary Function Group, R. B. of C. P. S. of C. M. A.; Pediatrics, E. B. of C. J. of A. C. Series guidelines for pediatric pulmonary function and non-invasive airway inflammation marker(part VII): fractional exhaled nitric oxide monitoring. *Chin J Appl Clin Pediatr*. (2017) 32(21):1622–7. doi: 10.3760/cma.j.issn.2095-428X.2017.21.006

6. Håland G, Carlsen KCL, Sandvik L, Devulapalli CS, Munthe-Kaas MC, Pettersen M, et al. Reduced lung function at birth and the risk of asthma at 10 years of age. *N Engl J Med*. (2006) 355(16):1682–9. doi: 10.1056/NEJMoa052885

7. Pulmonary Function Group, R. B. of C. P. S. of C. M. A.; Pediatrics, E. B. of C. J. of A. C. Series guidelines for pediatric pulmonary function (part IV): tidal breathing lung function. *Chin J Appl Clin Pediatr*. (2016) 31(21):1617–21. doi: 10.3760/cma.j.issn.2095-428X.2016.21.005

8. Kovesi T, Kulka R, Dales R. Exhaled nitric oxide concentration is affected by age, height, and race in healthy 9- to 12-year-old children. *Chest*. (2008) 133(1):169–75. doi: 10.1378/chest.07-1177

9. Buchvald F, Baraldi E, Carraro S, Gaston B, De Jongste J, Pijnenburg MWH, et al. Measurements of exhaled nitric oxide in healthy subjects age 4 to 17 years. *J Allergy Clin Immunol*. (2005) 115(6):1130–6. doi: 10.1016/j.jaci.2005.03.020

10. Montgomery GL, Tepper RS. Changes in airway reactivity with age in normal infants and young children. *Am Rev Respir Dis*. (1990) 142(6 Pt 1):1372–6. doi: 10.1164/ajrccm/142.6_Pt_1.1372

11. Bates JH, Schmalisch G, Filbrun D, Stocks J. Tidal breath analysis for infant pulmonary function testing. *ERS/ATS Task Force on Standards for Infant Respiratory Function Testing. European Respiratory Society/American Thoracic Society. Eur Respirator J*. (2000) 16(6):1180–92. doi: 10.1034/j.1399-3003.2000.16f26.x

12. Banovcin P, Seidenberg J, Von der Hardt H. Assessment of tidal breathing patterns for monitoring of bronchial obstruction in infants. *Pediatr Res*. (1995) 38(2):218–20. doi: 10.1203/00006450-199508000-00014

13. Lavizzari A, Zannin E, Ophorst M, Ciuffini F, Gangi S, Farolfi A, et al. Tidal breathing measurements in former preterm infants: a retrospective longitudinal study. *J Pediatr*. (2021) 230:112–118.e4. doi: 10.1016/j.jpeds.2020.11.050

14. Morris MJ, Madgwick RG, Collyer I, Denby F, Lane DJ. Analysis of expiratory tidal flow patterns as a diagnostic tool in airflow obstruction. *Eur Respir J*. (1998) 12(5):1113–7. doi: 10.1183/09031936.98.12051113

15. Totapally BR, Demerci C, Zureikat G, Nolan B. Tidal breathing flow-volume loops in bronchiolitis in infancy: the effect of albuterol [ISRCTN47364493]. *Crit Care*. (2002) 6(2):160–5. doi: 10.1186/cc1476

16. Keen C, Olin A-C, Wennergren G, Gustafsson P. Small airway function, exhaled NO and airway hyper-responsiveness in pediatric asthma. *Respir Med*. (2011) 105(10):1476–84. doi: 10.1016/j.rmed.2011.04.004

17. Yi L, Zhao Y, Guo Z, Li Q, Zhang G, Tian X, et al. The role of small airway function parameters in preschool asthmatic children. *BMC Pulm Med*. (2023) 23(1):219. doi: 10.1186/s12890-023-02515-3

18. Bao W, Zhang X, Yin J, Han L, Huang Z, Bao L, et al. Small-airway function variables in spirometry, fractional exhaled nitric oxide, and circulating eosinophils predicted airway hyperresponsiveness in patients with mild asthma. *J Asthma Allergy*. (2021) 14:415–26. doi: 10.2147/JAA.S295345

19. Akre M, Finkelstein M, Erickson M, Liu M, Vanderbilt L, Billman G. Sensitivity of the pediatric early warning score to identify patient deterioration. *Pediatrics*. (2010) 125(4):e763–769. doi: 10.1542/peds.2009-0338

20. Franklin PJ, Turner SW, Mutch RC, Stick SM. Comparison of single-breath and tidal breathing exhaled nitric oxide levels in infants. *Eur Respir J*. (2004) 23(3):369–72. doi: 10.1183/09031936.04.00084604

21. Baraldi E, de Jongste JC, European Respiratory Society/American Thoracic Society (ERS/ATS) Task Force. Measurement of exhaled nitric oxide in children, 2001. *Eur Respir J*. (2002) 20 (1):223–37. doi: 10.1183/09031936.02.00293102

22. Lundberg JO, Farkas-Szallasi T, Weitzberg E, Rinder J, Lidholm J, Anggård A, et al. High nitric oxide production in human paranasal sinuses. *Nat Med*. (1995) 1(4):370–3. doi: 10.1038/nm0495-370

23. Franklin PJ, Turner SW, Mutch RC, Stick SM. Measuring exhaled nitric oxide in infants during tidal breathing: methodological issues. *Pediatr Pulmonol*. (2004) 37(1):24–30. doi: 10.1002/ppul.10382

24. American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline

measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med*. (2005) 171(8):912–30. doi: 10.1164/rccm.200406-710ST

25. Bult H, Boeckxstaens GE, Pelckmans PA, Jordaens FH, Van Maercke YM, Herman AG. Nitric oxide as an inhibitory non-adrenergic non-cholinergic neurotransmitter. *Nature*. (1990) 345(6273):346–7. doi: 10.1038/345346a0

26. Ricciardolo FLM, Sterk PJ, Gaston B, Folkerts G. Nitric oxide in health and disease of the respiratory system. *Physiol Rev*. (2004) 84(3):731–65. doi: 10.1152/physrev.00034.2003

27. Jatakanon A, Lim S, Kharitonov SA, Chung KF, Barnes PJ. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax*. (1998) 53(2):91–5. doi: 10.1136/thx.53.2.91

28. Hardaker KM, Downie SR, Kermode JA, Farah CS, Brown NJ, Berend N, et al. Predictors of airway hyperresponsiveness differ between old and young patients with asthma. *Chest*. (2011) 139(6):1395–401. doi: 10.1378/chest.10-1839

29. Coates AL, Wanger J, Cockcroft DW, Culver BH, Bronchoprovocation Testing Task Force: Kai-Håkon Carlsen, Diamant Z, et al. ERS Technical standard on bronchial challenge testing: general considerations and performance of methacholine challenge tests. *Eur Respir J*. (2017) 49(5):1601526. doi: 10.1183/13993003.01526-2016

30. Cockcroft DW, Davis BE, Smycniuk AJ. Development of a methacholine challenge method to minimize methacholine waste. *Chest*. (2003) 124(4):1522–5. doi: 10.1378/chest.124.4.1522

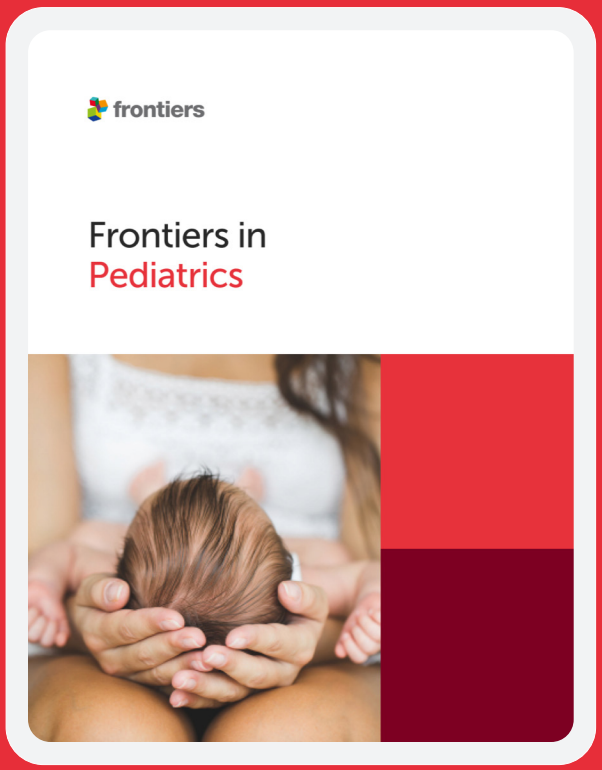
Frontiers in Pediatrics

Addresses ongoing challenges in child health and patient care

Explores research that meets ongoing challenges in pediatric patient care and child health, from neonatal screening to adolescent development.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact