edra

ISSN 3035-2134 | March 2025 Vol. 3, No. 1

Pediatric Respiratory Journal

Official Journal of the Italian Pediatric Respiratory Society

Official Journal of the Italian Pediatric Respiratory Society (Società Italiana per le Malattie Respiratorie Infantili – SIMRI)

via G. Quagliariello 27, 80131
Naples, Italy
Ph. 081 19578490
Fax 081 19578071
segreteria@simri.it
www.simri.it

Pediatric Respiratory Journal registered at Tribunale di Milano n. 4227, April 4, 2025

EDRA S.p.A.
via G. Spadolini, 7
20141 Milan, Italy
Ph. 0039 (0)2-88184.1
Fax 0039 (0)2-88184.301
www.edraspa.it

Vol. 3(1), 1-9, 2025 PEDIATR RESPIR J

RESEARCH ARTICLE

Assessing the efficacy of L-arginine and vitamin C supplementation in pediatric Long-COVID: a Randomized Controlled Trial

Emanuele Antonio **Liotta** [1, ¤], Giuseppe Fabio **Parisi** [1, ¤, *], Maria **Papale** [1], Giulia **Pecora** [1], Sara **Manti** [2, †], Salvatore **Leonardi** [1, †]

*** Correspondence to:**

gf.parisi@policlinico.unict.it. ORCID: https://orcid.org/0000-0003-4291-0195

Doi

10.56164/PediatrRespirJ.2024.63

[1] Department of Clinical and Experimental Medicine, Pediatric Respiratory Unit, University of Catania, San Marco Hospital, Catania, Italy

[2] Department of Human Pathology of Adult and Childhood Gaetano Barresi, Pediatric Unit, University of Messina, Messina, Italy

[¤] Should be considered joint first Author

[†] Should be considered joint senior Author

ABSTRACT

Following acute SARS-CoV2 infection, both in pediatric and adult populations, lingering or new symptoms may arise, leading to a condition known as Long-COVID. Understanding the pathophysiological basis of this condition has enabled the identification of supportive therapeutic strategies. Endothelial damage and oxidative stress seem to play a dominant role in the pathogenesis of Long-COVID. These patients experience a relative deficiency of L-arginine, and supplementation of L-arginine may improve endothelial function by promoting the activity of nitric oxide synthase (NOS). Additionally, vitamin C supplementation could reduce oxidative stress caused by the pro-inflammatory state typical of this condition. In this randomized and controlled trial, we assess the therapeutic effects of the combination of L-arginine and vitamin C in a pediatric population diagnosed with Long-COVID. The primary outcome was to evaluate the improvement in quality of life and changes in scores on the Borg scale, chosen as an indirect indicator of respiratory function. Thirty-six patients were recruited and divided into 3 treatment arms. Results showed improvement in the Borg scale at 30 ($p < 0.01$) and in quality of life ($p < 0.001$) among treated *vs*. untreated patients. Therefore, this supplementation may be considered in the treatment of Long-COVID in the pediatric age group.

HIGHLIGHTS BOX

What is already known about this topic? The use of L-arginine in Long-COVID in adulthood is supported by various scientific evidence, there are few references regarding its use and effectiveness in pediatric age. **What does this article add to our knowledge?** Arginine supplementation in the pediatric age improves the symptoms of Long- COVID, raising the quality of life of children. The use could be extended to post-viral syndrome potentially caused by other viruses. **How does this study impact current management guidelines?** There are no guidelines on the treatment of Long-COVID in the pediatric age. However, the use of L-arginine and vitamin C is an effective and safe strategy in its management, in particular improving asthenia and fatigue, allowing the recovery of psychophysical and relational performance.

KEY WORDS

Long-COVID; L-arginine; children; therapy; vitamin C.

INTRODUCTION

The coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is characterized by an acute infection and a set of symptoms and comorbidities that can remain and persist after the end of the acute phase identifying a condition called Long-COVID. Different organizations have provided similar but different diagnostic parameters to diagnose Long-COVID. The World Health Organization (WHO) suggested a clinical definition for Long-COVID-19, indicating it typically manifests around three months after the onset of COVID-19. The symptoms should endure for at least two months and cannot be attributed to an alternative diagnosis (1).

The prevalence of Long-COVID in the pediatric age, based on the data available in the literature, is variable and depends on a series of risk factors (third childhood and transition age, female sex, history of allergic diseases, other chronic underlying disease, patients with severe symptoms in the acute phase of COVID-19) (2). The most reported symptom is nasal congestion (17%), followed by headache (15%), fatigue (13%), loss of appetite (10%), insomnia (9%), cough (8%), abdominal pain (6%), confusion and lack of concentration (5.2%), skin rashes (4.9%). Fatigue, insomnia, lack of concentration and headaches are the symptoms that have the greatest impact on the daily lives of enrolled patients. Headaches and insomnia lasted longer: the first up to 4-6 months after the infection, the second up to a year later. Approximately one in ten children has multisystem involvement, presenting two or more symptoms simultaneously (3).

The mechanisms through which Long-COVID is established are not yet clear; however, various pathophysiological mechanisms involving the virus have been hypothesized (4). First, direct tissue damage caused by the virus in acute infection could contribute to developing long-term complications. The cellular entry gate for the virus angiotensin-converting enzyme 2 (ACE2) is distributed in many tissues of our body (epithelial cells, nasal goblet cells, gastrointestinal epithelial cells, pancreatic β cells, and renal podocytes), suggesting the involvement of different tissues and organs in the acute phase and the possible outcomes of the infection. In addition to the long-term consequences of cellular damage created by primary infection, there are other mechanisms (5):

- endothelial damage: endothelial cells (ECs) express ACE2, SARS-CoV-2 can directly infect and reproduce within ECs (6-8). Moreover, the simultaneous release of inflammatory cytokines like IL-6, IL-1, and TNF can activate ECs, exacerbating the situation. The activated complement system can also cause harm to ECs (9-11);
- dysregulation of the immune system due to the finding of autoreactive T cells in patients infected with COVID-19, like what is found in subjects with autoimmune diseases;
- PCR-positive persistent low-level detection of SARS-CoV-2 infection in patient with symptomatic Long-COVID (12).

Several nutraceuticals have been used to improve the clinical status of Long-COVID patients (13). Among these, L-arginine has proven to be particularly effective. This molecule plays a key role in the regulation of respiratory functions, the immune system and endothelial function. Reduced levels of L-arginine and the resulting dysfunction of nitric oxide synthase (NOS) play a direct role in various respiratory diseases, including asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, bronchopulmonary dysplasia, and pulmonary hypertension (14).

Arginine's impact extends to aspects of cellular defense function, likely through the mediation of nitric oxyde (NO) formation by cNOS (15). These beneficial effects of arginine on cellular defense function have prompted its incorporation into contemporary immune-enhancing formulas aimed at lowering infectious morbidity and mortality among critically ill and immunocompromised patients.

L-arginine can be a substrate of the NOS, producing NO with a beneficial effect on the vascular endothelium or be metabolized by the arginase enzyme into ornithine, a step associated with endothelial and immune dysfunction.

During acute SARS-CoV2 infection, the balance between the activity of these two enzymes is altered and the enzymatic activity of arginase increases, leading to a reduction in the levels of available plasma L-arginine, a lower activity of the nitric oxide synthase enzyme and lower NO production (**Figure 1**) (16). In fact, reduced levels of plasma L-arginine and increased arginase activity were found in adult patients with Long-COVID and pediatric

patients with Multisystem Inflammatory Syndrome (MIS-C) compared to healthy controls (17). Alterations of this metabolic pathway are associated with increased dysregulation of the immune system, endothelial dysfunction, inflammation, thrombosis (17).

Unlike most animals, humans cannot produce vitamin C internally, so it is necessary to obtain it through diet. The recommended daily intake of vitamin C is typically 75 mg for women and 90 mg for men. Ascorbic acid serves as a cofactor for various enzymes (such as dopamine B-monooxygenase, prolyl 4-hydroxylase, and lysyl hydroxylase) and plays a fundamental role in protecting cellular components from damage caused by free radicals produced during metabolism (18).

Similarly, recent clinical trials have highlighted the beneficial effect of vitamin C in improving oxidative imbalance and vascular remodeling resulting from endothelial dysfunction and the reduction of capillary permeability, a concept that plays an important role in infectious diseases, including COVID-19 (19). Therefore, administering vitamin C could be effective in speeding up recovery following the acute phase of the infection (20).

A study conducted by Tosato M and colleagues on 46 adults (median age 51, 65% women) found that after 28 days of receiving L-arginine plus vitamin C, supplementation improved walking performance, muscle strength, and reduced fatigue in adults with Long COVID (21).

Another interesting finding from a study on an adult population comes from a survey conducted by Izzo *et al.* with 1,390 participants. It revealed that patients in the L-Arginine + vitamin C treatment group had significantly lower scores for effort perception ($p < 0.0001$) compared to those who received the multivitamin combination (22). Based on these observations, a possible strategy for improving the symptoms of Long-COVID, even in pediatric age, is the oral supplementation of L-arginine combined with vitamin C.

The objective of this study was to evaluate whether combined therapy with L-Arginine and liposomal vitamin C (Bioarginina C, Farmaceutici Damor S.p.A., Italy) in pediatric patients with previous SARS-CoV2 infection and symptoms attributable to Long-COVID can determine a gain in terms of remission of symptoms and improved quality of life.

MATERIALS AND METHODS

Within our specialized pediatric pulmonology unit, we have established a clinic dedicated to children with a previous SARS-CoV-2 infection. We enrolled all patients who, during the outpatient evaluation (from March to October 2022), were diagnosed with Long-Covid and met the inclusion criteria (listed below). After obtaining approval from the ethics committee and obtaining informed consent, the study involved the enrollment of patients who met the following inclusion criteria: age

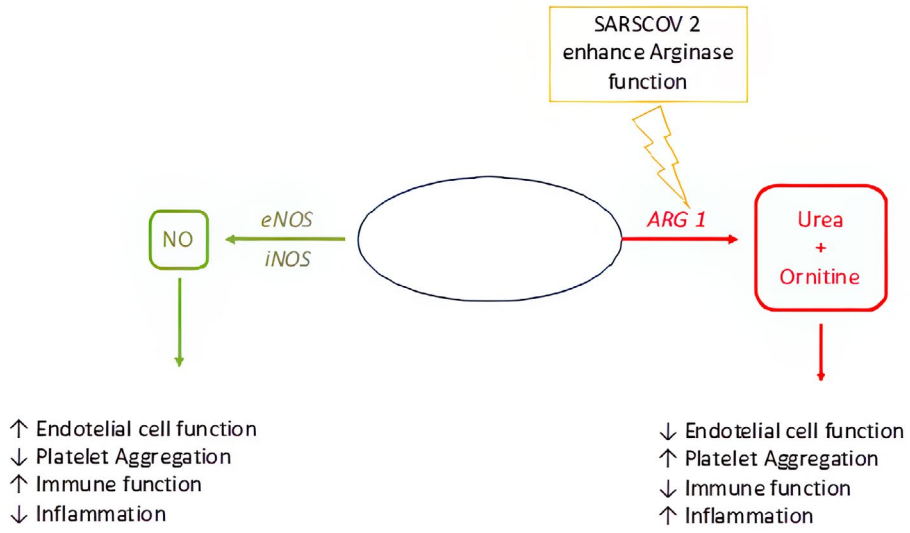

***Figure 1**. Metabolic pathways of L-arginine.*

between 5 and 14 years; previous SARS-CoV2 infection ascertained by nasopharyngeal swab; negative to SARS-CoV2 on nasopharyngeal swab for at least 3 weeks (however, for all patients, enrollment occurred at least 60 days after the primary infection); presence of symptoms, attributable to Long-COVID absent in the period preceding the SARS-CoV2 infection (asthenia, reduced exercise tolerance, respiratory symptoms, abdominal pain, headache).

The study includes 3 assessments, respectively at enrollment in the study (T0), after 30 days (T30) and after 60 days (T60). The patients with the previously described criteria and symptoms attributable to Long-COVID were enrolled, randomized into three study arms:

1) treatment with Bioarginine C, 2 vials per day for 30 days, starting from T0;
2) treatment with Bioarginine C, 2 vials per day for 30 days starting from T30;
3) control group untreated and observed for 60 days.

At T0 a clinical check-up of the child was carried out with the:

– administration of the Long-COVID follow-up questionnaire (in which for each symptom it is necessary to express a degree of severity ranging from 0, absence of the symptom, to 3, maximum degree of severity of the symptom);
– Borg cr10 scale score assigned by the caregiver after asking, including during telephone interviews, the same questions that explored the child's effort in performing daily activities compared to the period before the onset of symptoms, such as 'climbing a flight of stairs', 'playing with peers', and 'walking with the family';
– COVID-related psychological questionnaires on quality of life for specific age of the patient filled in by the doctor following instructions from the parent (Kid- & Kiddo-KINDL) (23, 24). The KINDL is a versatile tool designed to assess Health-Related Quality of Life in children and adolescents aged 3 years and above. Comprising 24 items, it is a concise, methodologically appropriate, and psychometrically reliable measure. This instrument has been translated into multiple languages and extensively utilized in both German and international studies. Norm values are established using representative data from the German National Health Interview and Examination Survey for Children and Adolescents (KiGGS).

None of the enrolled patients took any other medications during the observation period. Some received bronchodilator therapy during the acute infection.

These questionnaires were repeated, via telephone, by the same interviewer, 30 and 60 days after enrollment in the study.

Based on the data obtained from the analysis of the results of completing the questionnaires and from the assignment of a score on the Borg scale; we carried out a statistical analysis within each study arm at the different observation times (T0, T30 and T60) and between the three different study arms at the same observation time.

RESULTS

The study enrolled 36 children, with 12 patients for each of the three arms. The majority of the 36 patients

Table 1. *Enrolled patient features.*

	L-arginine + vitamin C	No treatment
No.	24	12
Median age	9.69	7.8
Female sex	18	6
Hospitalization for COVID-19	1	0

Table 2. *Sum of the severity scores obtained for each symptom within the different groups.*

	T0: GROUP 1	GROUP 2	GROUP 3
Hospitalization	1	0	0
Asthenia	10	12	6
Dyspnea	6	7	4
Chest tightness	4	9	4
Dizziness	3	3	3
Gastrointestinal symptom	6	2	4
Headache	8	8	6
Anosmia	0	0	0
Concentration difficulties	4	9	1
Sleep disorder	4	4	1
Cough	2	2	4
Rhinitis	4	0	4

Table 3. *Borg scale and quality of life (QoL) results in the 3 arms at different time points.*

		Improving of Borg scale	Quality of life questionnaires (kid- & kiddo-KINDL)
First study arms	After receiving 30 days of treatment (T0 *vs*. T30)	p < 0.030	
Second study arms	After receiving 30 days of treatment (T0 *vs*. T60)	p < 0.001	p < 0.001
Third study arms (no treatment)		Not significative	Not significative

involved in the study were female (M:F 1:2) and for 32 out of 36 cases inclusion in the study occurred more than 90 days after the primary SARS-CoV2 infection. Only one patient was hospitalized during the primary infection, the remainder developed a primary infection that passed paucisymptomatically. However, there were no substantial differences in terms of sex, age and hospitalization between patients treated with L-arginine+vitamin C compared to those not treated (**Table 1**).

In **Table 2**, we show, for each study group at T0 (enrollment), the sum of the severity of the different symptoms presented. From this perspective, the three samples exhibit a largely homogeneous distribution of the explored disorders.

In the internal analysis of the first study arm (**Table 3**), we found statistically significant differences regarding the improvement in the score attributed to the Borg scale between T0 and T30 (p < 0.030) and between T0 and T60 (p < 0.02). The internal analysis of the second arm of the study showed a statistically significant difference in the comparison between T0 and T60 regarding respectively the improvement of the scores assigned to the Borg scale (p < 0.001) and those attributed to the quality-of-life questionnaires (p < 0.001). No statistical significance was found regarding the internal analysis of the third study arm, *i.e.* those patients who did not receive any treatment and were observed for 60 days.

Regarding the comparison between different study arms at T30 (**Table 4**), a statistically significant difference emerged regarding the improvement in the Borg scale score between the first and second study arms (p < 0.004) and between the first and third study arms (p < 0.001); furthermore, at T30 there was a statistically significant difference in the improvement in quality of life between the first and second study arms (p < 0.001) and between the first and third study arms (p < 0.001). In the comparison between different study arms at T60 (**Table 4**), a statistically significant difference was highlighted regarding the improvement of the Borg scale score between the second and third study arms (p < 0.01) and between the first and third study arms (p < 0.01). Similarly, at T60, there was a statistically significant difference in the improvement in quality of life between arm 2 and arm 3 (p < 0.001) and between arm 1 and arm 3 (p < 0.001).

No adverse effects, even minimal ones, were reported in any patient treated.

DISCUSSION

The data extrapolated from the comparison between different study groups at the same timepoints show that an index susceptible to a rapid improvement (already after 30 days) following treatment with L-Arginine and liposomal vitamin C is the score on the Borg scale and

Table 4. *Borg scale and QoL results in the 3 arms at different time points.*

		Improving of Borg scale	Quality of life questionnaires (kid- & kiddo-KINDL)
T30	Study arms 1 *vs*. Study arms 2	p < 0.004	p < 0.001
	Study arms 1 *vs*. Study arms 3	p < 0.001	p < 0.001
T60	Study arms 2 *vs*. Study arms 3	p < 0.01	p < 0.001
	Study arms 1 *vs*. Study arms 3	p < 0.01	p < 0.001

that this improvement does not occur spontaneously in patients without being treated. While the scores obtained on the quality-of-life questionnaires show an observable and consistent improvement when compared between the different arms at T60. Data regarding the internal analysis of Groups 1 and 2 both show a statistically significant difference in the improvement of the Borg scale score between T0 and T60. Both groups received the combination treatment of L-arginine and vitamin C for 30 days. However, statistically significant improvements in Borg scale scores were not observed in the internal analysis of Group 3, the control group that did not receive any treatment. This comparison underscores that the improvement in treated patients was not solely due to potential spontaneous recovery within the sixty days, eliminating additional confounding factors given the homogeneity of the characteristics of the study groups.

This study confirms in the pediatric age the data already present in the literature for adults on the therapeutic effects of the association of L-arginine with vitamin C in patients with Long-COVID. Starting from the assumption of considering the dysfunction of endothelial cells (25, 26), as the main mechanism in the pathophysiology of Long-COVID, the result of damage to the vascular microcirculation established during the primary infection (given the abundant expression of ACE 2 receptors on the endothelial cell) which it is maintained once the primary infection phase has passed and can persist over time as highlighted by a clinical study in which the endothelial damage persisted even for a period longer than six months from the primary infection in the follow-up of patients affected by COVID19 compared to healthy controls (27). Furthermore, other studies have evaluated how the clinical manifestations related to Long-COVID, mainly non-respiratory ones, are more attributable, from a pathophysiological point of view to persistent endothelial dysfunction (28).

As reported above, L-arginine has pleiotropic effects, regulating the functions of the microcirculation through the production of NO via NO-synthase, allowing vessel dilation, reducing shear stress and acting as a regulator of the proliferation of immune system cells. In particular, increased levels of myeloid-derived immunosuppressive cells stimulated by the activity of argininase, the enzyme that opposes NO-synthase by metabolizing L-arginine into ornithine and urea, have been found in COVID19 patients with severe clinical manifestations (29, 30). Furthermore, an inverse correlation has been demonstrated between L-arginine levels and the level of platelet activation (31), responsible for hypercoagulability and contributing to the development of thromboembolic complications.

Oxidative stress is among the main vectors of inflammation (32), so the administration of vitamin C certainly proves useful in patients with Long-COVID in whom there is an underlying pro-inflammatory state, acting mainly on symptoms such as asthenia and fatigue (32, 34).

Our results were based not only on the mere attribution of a score to the severity of the symptoms reported, but also on a global and serial evaluation of the child through the COVID-related questionnaires on quality of life for specific age of the patient (Kid- & Kiddo-KINDL) which allowed us to investigate the relational sphere of the young patient, evaluating the relationship with the parent, the interaction with peers, the variation in school performance, essential elements in the developmental age. We observed a statistically significant improvement in the global score obtained at the evaluations performed at the end of the therapy with L-arginine + vitamin C compared to the changes in scores obtained in the evaluation of untreated controls.

Specifically, at T60, in comparison between groups that received the treatment *versus* Group 3, a statistically significant difference was found regarding the overall quality of life score, which in our case represented a secondary but very interesting outcome. Certainly, the improvement of symptoms in treated patients is intertwined and affects the quality of life of the young patient, improving the relationship sphere and school performance.

Our study has some limitations: the analysis was conducted on a limited sample of children with a diagnosis of Long-Covid, the randomization did not include double blinding, and parameters from respiratory function tests were not included in the follow-up assessments.

Regarding adverse reactions related to the administration of the L-arginine + vitamin C combination, none were observed, nor reported by parents, in our 24 treated patients, confirming the already existing safety data of the dietary supplement.

What occurs in the cellular microenvironment during Long-COVID (expression of pro-inflammatory cytokines, endothelial dysfunction) has many similarities with the

mechanisms underlying post-viral fatigue or Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) (35), conditions that can develop in children and adults following acute infection by other viruses involved in pandemics in the recent past or endemically present (36).

The insights gained from the study on the combined treatment of L-arginine and vitamin C in pediatric Long-COVID patients prompt intriguing speculations about the potential broader applicability of this treatment regimen across various viral infections. Drawing parallels between Long-COVID and other post-viral syndromes highlights the underlying commonalities in immune dysregulation, endothelial dysfunction, and inflammation that could be targeted by this therapeutic approach (37).

By considering the pleiotropic effects of L-arginine, particularly its role in regulating microcirculation, supporting immune system functions, and protecting endothelial health, it becomes apparent that these mechanisms could have implications beyond the context of Long-COVID. In viral infections where endothelial dysfunction and inflammatory responses are prominent features, the ability of L-arginine to modulate these pathways presents a promising avenue for intervention (38). Furthermore, the antioxidative properties of vitamin C hold potential in mitigating oxidative stress-induced tissue damage and inflammation commonly observed in various viral illnesses. By reducing the burden of oxidative stress, vitamin C may contribute to a more favorable recovery trajectory and potentially lessen the severity of symptoms associated with viral infections (39).

Moreover, the emphasis on improvements in quality-of-life measures observed in the study underscores the holistic benefits that this treatment approach could offer to individuals battling other viral infections. Enhanced recovery improved social interactions, and better functional outcomes could be transformative in not just symptom management but also in restoring overall well-being in patients affected by diverse viral illnesses.

In light of these considerations, the combined treatment of L-arginine and vitamin C emerges as a multifaceted therapeutic strategy with the potential to address common pathophysiological mechanisms in viral infections beyond Long-COVID. Further exploration through clinical trials and research endeavors is warranted to elucidate the full scope of effectiveness and applicability of this treatment regimen in diverse viral contexts.

CONCLUSIONS

Long-COVID represents a possible outcome of the primary SARS-CoV2 infection, which manifests itself with multisystem symptoms and can compromise the young patient's quality of life. A combination of L-arginine and vitamin C is recommended to manage these symptoms effectively and safely. This combination has been found to be particularly useful in improving asthenia and fatigue, leading to better psychophysical and relational performance. Furthermore, treatment with L-arginine may be effective in reducing symptoms following infections caused by viral pathogens other than SARS-CoV-2, which are similar in terms of clinical manifestations, pathogenesis, and long-term outcomes.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests

The Authors have declared no conflict of interests.

Financial support

There was no institutional or private funding for this article.

Authors' contributions

Conceptualization: GFP, MP, SL; methodology: MP, SM; validation: SM, GP; formal analysis: SM; investigation: EL, MP; resources: GFP; data curation: EL, SM; writing-original draft preparation: EL; writing-review and editing: GFP; visualization: SM, GP; supervision: SM, SL. All Authors have read and agreed to the published version of the manuscript.

Ethical approval

Human studies and subjects

The Ethics Committee (Comitato Etico Catania 1) approved the study by deliberation 596/2021.

Data sharing and data accessibility

The Authors confirm that the data supporting the findings of this study are available within the article.

Publication ethics

Plagiarism

Authors declare no potentially overlapping publications with the content of this manuscript and all original studies are cited as appropriate.

Data falsification and fabrication

All the data corresponds to the real.

REFERENCES

1. Soriano JB, Murthy S, Marshall JC, Relan P, Diaz JV. A clinical case definition of post-COVID-19 condition by a Delphi consensus. Lancet Infectious Diseases. 2022;22(4):e102-7. doi: 10.1016/j.jpedsurg.2007.08.043.
2. Esposito S, Principi N, Azzari C, Cardinale F, Di Mauro G, Galli L, et al. Italian intersociety consensus on management of long covid in children. Ital J Pediatr. 2022;48(1):42. doi: 10.1186/s13052-022-01233-6.
3. Osmanov IM, Spiridonova E, Bobkova P, Gamirova A, Shikhaleva A, Andreeva M, et al. Risk factors for post-COVID-19 condition in previously hospitalised children using the ISARIC Global follow-up protocol: a prospective cohort study. Eur Respir J. 2022;59(2):2101341. doi: 10.1183/13993003.01341-2021.
4. Desai AD, Lavelle M, Boursiquot BC, Wan EY. Long-term complications of COVID-19. American Journal of Physiology-Cell Physiology. 2022;322(1):C1-11. doi: 10.1152/ajpcell.00375.2021.
5. Nalbandian A, Sehgal K, Gupta A, Madhavan MV, McGroder C, Stevens JS, et al. Post-acute COVID-19 syndrome. Nat Med. 2021;27(4):601-15. doi: 10.1038/s41591-021-01283-z.
6. Ackermann M, Verleden SE, Kuehnel M, Haverich A, Welte T, Laenger F, et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. N Engl J Med. 2020;383(2):120-8. doi: 10.1056/NEJMoa2015432.
7. Liu F, Han K, Blair R, Kenst K, Qin Z, Upcin B, et al. SARS-CoV-2 Infects Endothelial Cells In Vivo and In Vitro. Front Cell Infect Microbiol. 2021;11:701278. doi: 10.3389/fcimb.2021.701278.
8. Lei Y, Zhang J, Schiavon CR, He M, Chen L, Shen H, et al. SARS-CoV-2 Spike Protein Impairs Endothelial Function via Downregulation of ACE 2. Circ Res. 2021;128(9):1323-6. doi: 10.1161/CIRCRESAHA.121.318902.
9. Noris M, Benigni A, Remuzzi G. The case of complement activation in COVID-19 multiorgan impact. Kidney International. 2020;98(2):314-22. doi: 10.1016/j.kint.2020.05.013.
10. Flaumenhaft R, Enjyoji K, Schmaier AA. Vasculopathy in COVID-19. Blood. 2022;140(3):222-35. doi: 10.1182/blood.2021012250.
11. Wu X, Xiang M, Jing H, Wang C, Novakovic VA, Shi J. Damage to endothelial barriers and its contribution to long COVID. Angiogenesis. 2024;27(1):5-22. doi: 10.1007/s10456-023-09878-5.
12. Ngai JC, Ko FW, Ng SS, To K, Tong M, Hui DS. The long□term impact of severe acute respiratory syndrome on pulmonary function, exercise capacity and health status. Respirology. 2010;15(3):543-50. doi: 10.1111/j.1440-1843.2010.01720.x.
13. Tosato M, Ciciarello F, Zazzara MB, Pais C, Savera G, Picca A, et al. Nutraceuticals and Dietary Supplements for Older Adults with Long COVID-19. Clinics in Geriatric Medicine. 2022;38(3):565-91. doi: 10.1016/j.cger.2022.04.004.
14. Scott JA, Maarsingh H, Holguin F, Grasemann H. Arginine Therapy for Lung Diseases. Front Pharmacol. 2021;12:627503. doi: 10.3389/fphar.2021.627503.
15. Barbul A. Arginine and immune function. Nutrition. 1990;6(1):53-8; discussion 59-62. PMID: 2135757.
16. Adebayo A, Varzideh F, Wilson S, Gambardella J, Eacobacci M, Jankauskas SS, et al. l-Arginine and COVID-19: An Update. Nutrients. 2021;13(11):3951. doi: 10.3390/nu13113951.
17. Rees CA, Rostad CA, Mantus G, Anderson EJ, Chahroudi A, Jaggi P, et al. Altered amino acid profile in patients with SARS-CoV-2 infection. Proc Natl Acad Sci USA. 2021;118(25):e2101708118. doi: 10.1073/pnas.2101708118.
18. Kaźmierczak-Barańska J, Boguszewska K, Adamus-Grabicka A, Karwowski BT. Two Faces of Vitamin C-Antioxidative and Pro-Oxidative Agent. Nutrients. 2020;12(5):1501. doi: 10.3390/nu12051501.
19. Morelli MB, Gambardella J, Castellanos V, Trimarco V, Santulli G. Vitamin C and Cardiovascular Disease: An Update. Antioxidants. 2020;9(12):1227. doi: 10.3390/antiox9121227.
20. Soto ME, Guarner-Lans V, Soria-Castro E, Manzano Pech L, Pérez-Torres I. Is Antioxidant Therapy a Useful Complementary Measure for Covid-19 Treatment? An Algorithm for Its Application. Medicina. 2020;56(8):386. doi: 10.3390/medicina56080386.
21. Tosato M, Calvani R, Picca A, Ciciarello F, Galluzzo V, Coelho-Júnior HJ, et al; Gemelli against COVID-19 Post-Acute Care Team. Effects of l-Arginine Plus Vitamin C Supplementation on Physical Performance, Endothelial Function, and Persistent Fatigue in Adults with Long COVID: A Single-Blind Randomized Controlled Trial. Nutrients. 2022;14(23):4984. doi: 10.3390/nu14234984.
22. Izzo R, Trimarco V, Mone P, Aloè T, Capra Marzani M, Diana A, et al. Combining L-Arginine with vitamin C improves long-COVID symptoms: The LINCOLN Survey. Pharmacol Res. 2022;183:106360. doi: 10.1016/j.phrs.2022.106360.
23. Ravens-Sieberer U, Bullinger M. Assessing health-related quality of life in chronically ill children with the German KINDL: first psychometric and content analytical results. Qual Life Res. 1998;7(5):399-407. doi: 10.1023/a:1008853819715.
24. Bullinger M, Brütt AL, Erhart M, Ravens-Sieberer U; BELLA Study Group. Psychometric properties of the KINDL-R questionnaire: results of the BELLA study. Eur Child Adolesc Psychiatry. 2008;17(S1):125-32. doi: 10.1007/s00787-008-1014-z.
25. Zha D, Fu M, Qian Y. Vascular Endothelial Glycocalyx Damage and Potential Targeted Therapy in COVID-19. Cells. 2022;11(12):1972. doi: 10.3390/cells11121972.
26. Gambardella J, Kansakar U, Sardu C, Messina V, Jankauskas SS, Marfella R, et al. Exosomal miR-145 and miR-885 Regulate Thrombosis in COVID-19. J Pharmacol Exp Ther. 2023;384(1):109-15. doi: 10.1124/jpet.122.001209.

27. Oikonomou E, Souvaliotis N, Lampsas S, Siasos G, Poulakou G, Theofilis P, et al. Endothelial dysfunction in acute and long standing COVID−19: A prospective cohort study. Vascular Pharmacology. 2022;144:106975. doi: 10.1016/j.vph.2022.106975.
28. Charfeddine S, Ibn Hadj Amor H, Jdidi J, Torjmen S, Kraiem S, Hammami R, et al. Long COVID 19 Syndrome: Is It Related to Microcirculation and Endothelial Dysfunction? Insights From TUN-EndCOV Study. Front Cardiovasc Med. 2021;8:745758. doi: 10.3389/fcvm.2021.745758.
29. Reizine F, Lesouhaitier M, Gregoire M, Pinceaux K, Gacouin A, Maamar A, et al. SARS-CoV-2-Induced ARDS Associates with MDSC Expansion, Lymphocyte Dysfunction, and Arginine Shortage. J Clin Immunol. 2021;41(3):515-25. doi: 10.1007/s10875-020-00920-5.
30. Dean MJ, Ochoa JB, Sanchez-Pino M, Zabaleta J, Garai J, Del Valle L, et al. Transcriptome and Functions of Granulocytic Myeloid-Derived Suppressor Cells Determine their Association with Disease Severity of COVID-19. Preprint. medRxiv. 2021;2021.03.26.21254441. doi: 10.1101/2021.03.26.21254441.
31. Sacchi A, Grassi G, Notari S, Gili S, Bordoni V, Tartaglia E, et al. Expansion of Myeloid Derived Suppressor Cells Contributes to Platelet Activation by L-Arginine Deprivation during SARS-CoV-2 Infection. Cells. 2021;10(8):2111. doi: 10.3390/cells10082111.
32. Crook H, Raza S, Nowell J, Young M, Edison P. Long covid - mechanisms, risk factors, and management. BMJ. 2021;n1648. doi: 10.1136/bmj.n1648.
33. Suh SY, Bae WK, Ahn HY, Choi SE, Jung GC, Yeom CH. Intravenous Vitamin C administration reduces fatigue in office workers: a double-blind randomized controlled trial. Nutr J. 2012;11(1):7. doi: 10.1186/1475-2891-11-7.
34. Stephenson CM, Levin RD, Spector T, Lis CG. Phase I clinical trial to evaluate the safety, tolerability, and pharmacokinetics of high-dose intravenous ascorbic acid in patients with advanced cancer. Cancer Chemother Pharmacol. 2013;72(1):139-46. doi: 10.1007/s00280-013-2179-9.
35. Wirth KJ, Scheibenbogen C, Paul F. An attempt to explain the neurological symptoms of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. J Transl Med. 2021;19(1):471. doi: 10.1186/s12967-021-03143-3.
36. Islam MF, Cotler J, Jason LA. Post-viral fatigue and COVID-19: lessons from past epidemics. Fatigue: Biomedicine, Health & Behavior. 2020;8(2):61-9. doi:10.1080/21641846.2020.1778227.
37. Presti S, Manti S, Gambilonghi F, Parisi GF, Papale M, Leonardi S. Comparative Analysis of Pediatric Hospitalizations during Two Consecutive Influenza and Respiratory Virus Seasons Post-Pandemic. Viruses. 2023;15(9):1825. doi:10.3390/v15091825.
38. Parisi GF, Carota G, Castruccio Castracani C, Spampinato M, Manti S, Papale M, et al. Nutraceuticals in the Prevention of Viral Infections, including COVID-19, among the Pediatric Population: A Review of the Literature. Int J Mol Sci. 2021;22(5):2465. doi:10.3390/ijms22052465.
39. Matera L, Manti S, Petrarca L, Pierangeli A, Conti MG, Mancino E, et al. An overview on viral interference during SARS-CoV-2 pandemic. Front Pediatr. 2023;11:1308105. doi:10.3389/fped.2023.1308105.

RESEARCH ARTICLE

Assessing asthma-specific breath markers in preschool children using remote breath collection

Rémy **Bourgeois** [1, ¤], Katharina **Rohrbach** [1, ¤], Yvette **Baumann** [1], Nathan **Perkins** [2], Elias **Seidl** [1], Srdjan **Micic** [1, ¤, *], Alexander **Moeller** [1, ¤, *]

*** Correspondence to:**

alexander.moeller@kispi.uzh.ch; ORCID: https://orcid.org/0000-0001-7284-4251

micic.math@gmail.com; ORCID: https://orcid.org/0000-0002-6887-2417

ABSTRACT

Asthma is the most common chronic respiratory disease in children. However, distinguishing asthma from other respiratory complaints in preschool age remains a diagnostic challenge. Secondary electrospray ionization high resolution mass spectrometry (SESI-HRMS) together with a remote breath sample collecting method is a promising tool to overcome this diagnostic problem. This study investigated whether previously identified asthma-specific volatile organic compounds (VOCs) can be reidentified in preschool children by SESI-HRMS using an offline breath collection device.

A total of 30 patients presenting with chronic respiratory symptoms (CRS) and referred for the investigation of preschool asthma as well as 32 healthy controls between 3 and 6 years of age were recruited from the outpatient clinic at the University Children's Hospital in Zurich, Switzerland. Participants were instructed to exhale into an offline breath collection device entailing a Nalophan bag. Breath samples were transferred to SESI-HRMS in a heated box at body temperature. Samples were pumped into SESI-HRMS using a pressurized box. Detection of previously identified asthma-specific markers (mass-to-charge ratio (m/z) tolerance of 0.002 Da) and statistical analysis were performed.

Of 375 previously identified, asthma-specific m/z features, 125 were detected again in preschool children with the remote breath collection method. 16 detected m/z features showed statistically significant differences between patients with CRS and healthy controls (Wilcoxon rank-sum test, adjusted p-value <0.05). Eight of those 16 significant m/z features had been identified chemically as representatives of monosaccharide and fatty acid metabolism, lysine degradation and aldehydes in a previous study. Predictive performance of confirmed markers for distinguishing patients with CRS from healthy patients, using random forest algorithm in a repeated cross-validation, resulted in an AUC of 0.77 (95% CI: 0.60-0.93).

This is the first study to successfully reidentify asthma-specific VOCs in preschool children by SESI-HRMS using a breath collection device that entails Nalophan. Despite loss of m/z features, significant asthma-specific VOCs were identified, emphasizing the potential of this remote breath collection method for diagnosing asthma in preschool children.

Doi

10.56164/PediatrRespirJ.2025.71

[1] Department of Respiratory Medicine, University Children's Hospital Zurich, Zurich, Switzerland and Zurich Exhalomics

[2] Department of Clinical Chemistry and Biochemistry, University Children's Hospital Zurich, Zurich, Switzerland

[¤] These Authors have equally contributed to this work and RB and KR should be considered joint first; SM and AM should be considered joint last Authors.

This work has been presented as a poster at the European Respiratory Society Congress September 2024.

KEY WORDS

Preschool; asthma; breath analysis.

HIGHLIGHTS BOX

What is already known about this topic? In the past few decades, different breath markers for asthma in the form of VOCs have been identified but results could not be replicated in preschool children. **What does this article add to our knowledge?** This is the first study to successfully reidentify asthma-specific breath markers in preschool children by SESI-HRMS using a remote breath collection device. Our findings emphasize the potential for targeted breath analysis in preschool children. **How does this study impact current management guidelines?** Current asthma diagnosis guidelines request objective tests, but these are challenging in preschool children. This study lays another foundation to develop an easy to perform and reliable breath test for asthma diagnosis in younger children.

INTRODUCTION

Asthma is the most common chronic respiratory disease in children, affecting over 5 million children in Europe (1). The diagnosis remains challenging and there is no single diagnostic gold standard to diagnose asthma in children (2-4). In children aged 5-16 years, the diagnosis is usually based on a diagnostic algorithm that entails different tests, *i.e.* spirometry, bronchodilator responsiveness (BDR) testing and the measurement of exhaled nitric oxide (FeNO) (3). In preschool children, the proper diagnosis of asthma is generally not possible, since cognitively demanding breathing maneuvers necessary for spirometry and BDR testing are usually too complex to perform in this age group (5, 6). Therefore, children with a high suspicion of asthma, that are not able to perform pulmonary function testing according to current standards are usually referred as children with CRS (3). There is a need for alternative methods that are non-invasive, easy to apply and do not require complex cooperation to diagnose asthma in preschool children. A growing number of studies were published suggesting that breath analysis may offer such a possibility (2, 7).

Several different breath collecting methods for remote analysis have been developed in the last two decades (2). Some studies successfully detected asthma-specific breath markers in children using Tedlar sampling bags together with different exhalation maneuvers and analysis techniques (2). More recently, Decrue *et al*. successfully deployed a remote collection method using Nalophan bags and SESI-HRMS for breath analysis in infants (8). Nalophan proved itself as a reliable component of breath collecting devices in previous studies (9, 10), even if the breath sample was stored at room temperature over a longer period of time prior to measurement (9). Therefore, Nalophan is a reliable part of breath collecting devices for remote analysis.

In the past few decades, different breath markers for asthma in the form of volatile organic compounds (VOCs) have been identified (2, 11, 12). Typically, VOCs associated with oxidative stress, *e.g.* aldehydes, polysaturated fatty acids, ethane and pentane were identified in breath samples of asthma patients due to the inflammatory nature of the disease (13-22). Our research group more specifically demonstrated upregulated metabolisms of lysine, tyrosine, fatty acids, 2-oxocarboxylic acid and monosaccharides as well as downregulated metabolisms of arginine, proline, linoleic acid, palmitoylethanolamide (PEA) and aldehydes in school-aged children with allergic asthma (7). Thus, the above-mentioned VOCs are promising targets of asthma-related research using SESI-HRMS.

The aim of this study was to investigate whether 1) a remote breath collecting method entailing Nalophan is feasible to be used in SESI-HRMS analytics and 2) previously identified asthma-specific VOCs can be reidentified in preschool children with CRS distinguishing them from healthy controls (7).

METHODS

Study population

The EXPEDIA study (EXhalomics in PEDIatric Asthma) is a cross-sectional observational study that aims to assess the possibility of using exhaled volatile organic

compounds to diagnose asthma in children using online mass spectrometry.

For this study, 30 patients referred for the investigation of preschool asthma and presenting with CRS, *i.e.* wheezing and/or dry cough, as well as 32 healthy controls (HC) between 3 and 6 years of age were recruited from the outpatient clinic at the University Children's Hospital in Zurich, Switzerland. Children who had acute respiratory infections within the last 6 weeks, with other chronic respiratory or cardiac diseases or any condition restricting the proper execution of the breath sampling were excluded from the study.

Breath collection and transportation

Remote breath collection was conducted using a custom-made offline breath collecting device (**Figure 1**) consisting of a 0.7-liter Nalophan bag (Kalle GmbH, Wiesbaden, Germany) attached to a sealable valve piece (Swagelok Company, Ohio, OH, USA) with zip ties, a custom-made connecting tube and a mouthpiece (QuinTron Instrument Company Inc., Milwaukee, WI, USA). Children were instructed to take deep inspirations and exhale directly into the Nalophan bag through the mouthpiece until the bag was fully inflated. Whole breath samples were collected without filtering or fractioning the exhalation. A VOC filter was not used, as it was deemed impractical for preschool children (23). Participants were not strictly restricted from medication usage or eating, and physical activity was naturally limited by the hospital setting of the doctor's visit. The filled bags were sealed and transferred to SESI-HRMS in a heated box at body temperature (37 °C) within 15 minutes (**Figure 2**) to prevent condensation of the sample (24, 25).

Breath analysis

Breath analysis was conducted using a SESI source (SuperSESI, FIT FossilionTech, Madrid, Spain) combined with a high-resolution time-of-flight mass spectrometer (TripleTOF 5600+, AB Sciex, Concord, ON, Canada). Instrumental settings were previously published by our group (7, 26). In short, the sampling line (SL) temperature of the ion source was set at 130 °C, with temperatures of the ionization chambers 1 and 2 (IC1 and IC2) both at 100 °C. Ion spray voltage floating (ISVF) was set at +4500 V in positive mode and -4500 V in negative mode, respectively. For electrospray generation, Sharp Singularity™ nanoESI emitters (FIT FossilionTech, Madrid, Spain) with 20 µm inner diameter were used. The electrospray solution was 0.1% formic acid in water. During each measurement, the contents of the Nalophan bags were continuously infused into the SESI using a custom-made pressurized box (**Figure 2**) at a flow rate of 0.3 l/min for a standardized duration of 30 seconds. Data acquisition was performed in full scan mode in both positive and negative ionization mode. The mass range scanned was from 50 to 500 Da, with a scan time of 0.5 seconds per spectrum.

Data preprocessing

The recorded raw mass spectra were aligned using PeakView 2.2 (AB Sciex, Concord, ON, Canada) and converted into the mzXML file format using MSConvert (ProteoWizard v3.0.2) (27). The processing of the converted mass spectral data was performed in R (version

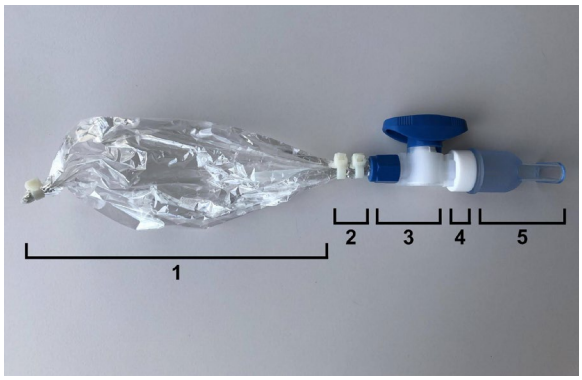

Figure 1. *Offline breath collecting device. The device consists of a Nalophan bag (1) attached to a sealable valve piece (3) with zip ties (2), a connecting tube (4) and a mouthpiece (5). The collected breath air is trapped inside the bag after closing the valve piece.*

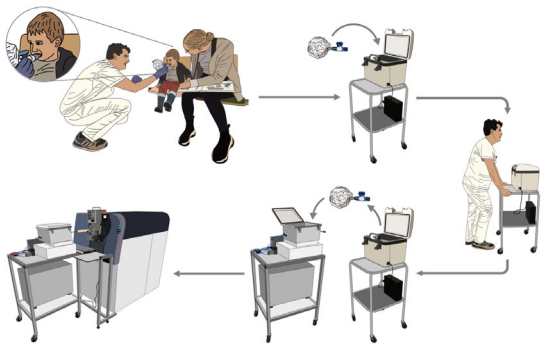

Figure 2. *Remote breath collection, transportation and measurement. Breath collection was conducted remotely using a breath collecting device (**Figure 1**). The contents were then transferred to SESI-HRMS within 15 minutes in a heated box at body temperature. Each sample was pumped into SESI using a pressurized box.*

4.2.1, R Foundation for Statistical Computing, Vienna, Austria) following the methodology outlined in detail elsewhere (7, 26). To summarize, the mass spectra was fitted onto a linearly spaced mass-to-charge ratio (m/z) axis (step size: 0.0005, 900'000 data points, 50-500 m/z range) through piecewise cubic Hermite interpolation. For each measurement, the interpolated mass spectra associated with the infusion maneuvers into the instrument were averaged. Peak picking was then performed on the averaged mass spectra using a height filter of 10 counts per second, followed by centroiding via trapezoidal integration to determine the signal intensities of m/z features. Subsequently, for further analysis, the m/z feature intensities were normalized to the total ion current (TIC), log2-transformed and organized into a data matrix representing breath profiles.

Statistical analysis

Markers previously linked to allergic asthma from our study (7) were used to filter the features within the remotely collected breath profiles of preschool children. An m/z tolerance of 0.002 Da was set for accurate mass matching. The Wilcoxon rank-sum test was used to assess the differential abundance of these features between the CRS and control groups (28). To account for multiple hypothesis testing, p-values were corrected using Storey's procedure (29). A significance level of adjusted p-value <0.05 was applied. Furthermore, to assess the predictive ability of the detected features in classifying the preschool children as healthy or with CRS, random forest algorithm was trained and tested in a 5 times repeated 10-fold cross-validation (30). To avoid training the classifiers on all detected signals, feature selection was performed in each cross-validation iteration using Meinshausen and Bühlmann stability selection as previously applied in our SESI-HRMS study (31, 32). In short, within each cross-validation loop, stability selection was performed using random 80% training data subsamples, Wilcoxon rank-sum p-values for feature ranking, and a threshold of the 20th percentile (0.2-quantile) of p-values across 100 repetitions. To retain the most important features, only those features with p-values below the 0.2-quantile threshold in at least 90 out of 100 repetitions were selected. More detailed information on statistical analysis is provided in the supplementary material.

Ethics

This study was approved by the Ethics Committee of the Canton of Zurich (KEK-ZH-Nr. 2018-00441) and written informed parental consent and oral assent of the participants were obtained prior to study participation.

RESULTS

Remotely collected exhaled breath samples of 30 children with CRS and 32 healthy controls were included in this study. The two groups had comparable age and sex distributions. Demographic and clinical characteristics of the two study cohorts are shown in **Table 1**.

In our previous exploratory SESI-HRMS study, 375 m/z features were found to be potentially associated with allergic asthma in school-aged children (7). From those features, 125 were detected again (<0.002 Da) in remotely collected breath samples of preschool children (**Table S1**). Among the detected features, 16 showed statistically significant differences between the control and CRS group (Wilcoxon rank-sum test, adjusted p-value <0.05, **Figure 3**), replicating the significance observed in the previous study between healthy and asthmatic children. Additionally, eight of the 16 significant features were chemically identified in our earlier work (7). These identified compounds belong to various metabolic pathways and chemical families, including monosaccharides and metabolites (glucuronate, glucarate), aldehydes (4-hydroxy-2-octenal, 4-hydroxy-2-hexenal), fatty acid metabolism (6-hydroxyhexanoic acid), and lysine degradation (glutarate). A complete list of all 16 significant features is provided in **Table 2**. Boxplots for the eight chemically identified markers are presented in **Figure 4** for further visualization.

The assessment of the confirmed markers in classifying samples as HC or CRS, using random forest algorithm in a 5-fold repeated 10-fold cross-validation, resulted in an area under the ROC curve (AUC) of 0.77 (95% CI: 0.60-0.93) (**Figure 5A**). The average classification accuracy reached 72.6% (CI: 59.2%-85%) with sensitivity of 72.9% (CI: 51.7%-94.6%) and specificity of 72.3% (CI: 52.7%-90.9%). On average, 14 ± 1.1 markers were selected in each cross-validation training data set. Markers and their selection frequencies used for classifier training are shown in **Figure 5B** and **Table S1**.

Table 1. *Demographic and clinical characteristics of the participants.*

Parameter	CRS group (n = 30)	HC group (n = 32)	Significance
Age [y]	5.5 ± 1.1	5 ± 1.2	n.s.
Female [n]	10 (33.3%)	11 (34.4%)	n.s.
Height [cm]	111.7 ± 7	109.2 ± 9.1	n.s.
Height z-score	-0.22 ± 0.98	-0.02 ± 1.08	n.s.
Weight [kg]	19.8 ± 4.1	18.8 ± 3.3	n.s.
Weight z-score	-0.02 ± 1.33	0.11 ± 0.95	n.s.
BMI [kg/m²]	15.7 ± 2.23	15.7 ± 1.4	n.s.
BMI z-score	0.09 ± 1.45	0.17 ± 1.03	n.s.
Diagnosis			
Allergic asthma [n]	12 (30%)	-	-
Suspected allergic asthma [n]	5 (16.7%)	-	-
Non-allergic asthma [n]	3 (10%)	-	-
Suspected non-allergic asthma [n]	3 (10%)	-	-
Recurrent obstructive episodes [n]	7 (23.3%)	-	-
Allergy [n]	20 (66.7%)	4 (12.5%)	***
Food allergy [n]	6 (20%)	4 (12.5%)	n.s.
Allergy to aeroallergens [n]	18 (60%)	2 (6.3%)	***
Hay fever [n]	11 (36.7%)	2 (6.3%)	**
GINA management Step			
Step 1 [n]	17 (56.7%)	-	-
Step 2 [n]	5 (16.7%)	-	-
Step 3 [n]	5 (16.7%)	-	-
No treatment [n]	3 (10%)		
Inhaled corticosteroid (ICS) therapy [n]	11 (36.7%)	-	-
Bronchodilator use			
Salbutamol (100 µg/dose, prn) [n]	25 (83.3%)	-	-

*Continuous variables are presented as mean ± standard deviation (SD) and categorical variables as counts (%). The group differences were tested with a two-sample t-test for continuous variables and a Fisher's exact test for the categorical variables. Significance levels: n.s.: >0.05, *: <0.05, **: <0.01, ***: <0.001.*

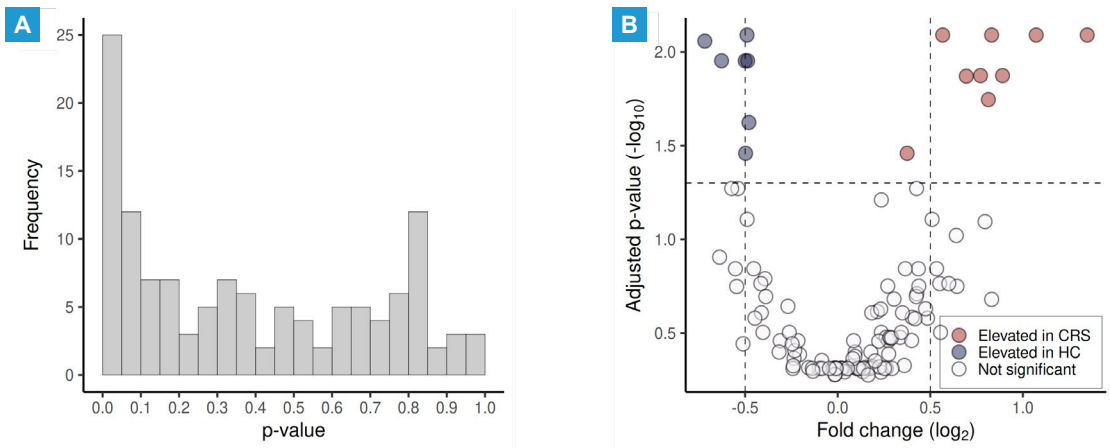

Figure 3. *Statistical analysis of detected metabolites between CRS and HC groups.* ***(A)*** *Distribution of p-values according to the Wilcoxon rank-sum test of all 125 detected m/z features;* ***(B)*** *Volcano plot for all detected m/z features. X-axis: log2 fold change (CRS vs. HC). Y-axis: -log10 of adjusted p-value. Markers are colored according to significance and fold change: red (elevated in CRS, adj. p-value <0.05), blue (elevated in HC, adj. p-value <0.05), and white (not significant).*

Table 2. *Significant asthma-associated markers from our previous study (7) detected in preschool children via remote breath collection.*

m/z	Charge	Elevated in	log2FC	p-value	Adj. p-value	Compound (mol. formula)	Metabolic pathway/chemical family
193.0355	neg	CRS	0.83	0.0003	0.0081	Glucuronate C6H10O7	Monosaccharides and metabolites
194.0305	neg	CRS	0.57	0.0006	0.0081	-	
209.0305	neg	CRS	1.07	0.0006	0.0081	Glucarate C6H10O8	Monosaccharides and metabolites
227.041	neg	CRS	1.35	0.0002	0.0081	-	
83.0335	pos	HC	-0.49	0.0005	0.0081	-	
136.021	pos	HC	-0.72	0.0008	0.0088	Benzothiazole C7H5NS	Heterocyclic compounds
115.075	pos	HC	-0.50	0.0015	0.0111	4-Hydroxy-2-hexenal C6H10O2	Aldehydes
135.101	pos	HC	-0.63	0.0013	0.0111	-	
143.106	pos	HC	-0.49	0.0014	0.0111	4-Hydroxy-2-octenal C8H14O2	Aldehydes
196.0465	neg	CRS	0.77	0.0022	0.0134	-	
226.033	neg	CRS	0.89	0.0022	0.0134	-	
167.068	pos	CRS	0.69	0.0024	0.0135	3-Ethoxybenzoate C9H10O3	Aromatic compound
183.051	neg	CRS	0.81	0.0035	0.0179	-	
151.096	pos	HC	-0.48	0.0050	0.0238	6-Hydroxyhexanoic acid C6H12O3	Fatty acid metabolism
131.035	neg	CRS	0.37	0.0083	0.0347	Glutarate (pentanedioic acid) C5H8O4	Lysine degradation
191.164	pos	HC	-0.50	0.0079	0.0347	-	

Ordered by significance in the current study (adj. p-value). m/z: mass-to-charge ratio, charge: ionization mode, log2FC: log2-fold-change, Adj. p-value: adjusted p-value (28), Compound and metabolic pathway/chemical family: putatively identified compound in our previous work (7) with the associated metabolic pathway or chemical family. Dash "-" was used in case of unknown compounds.

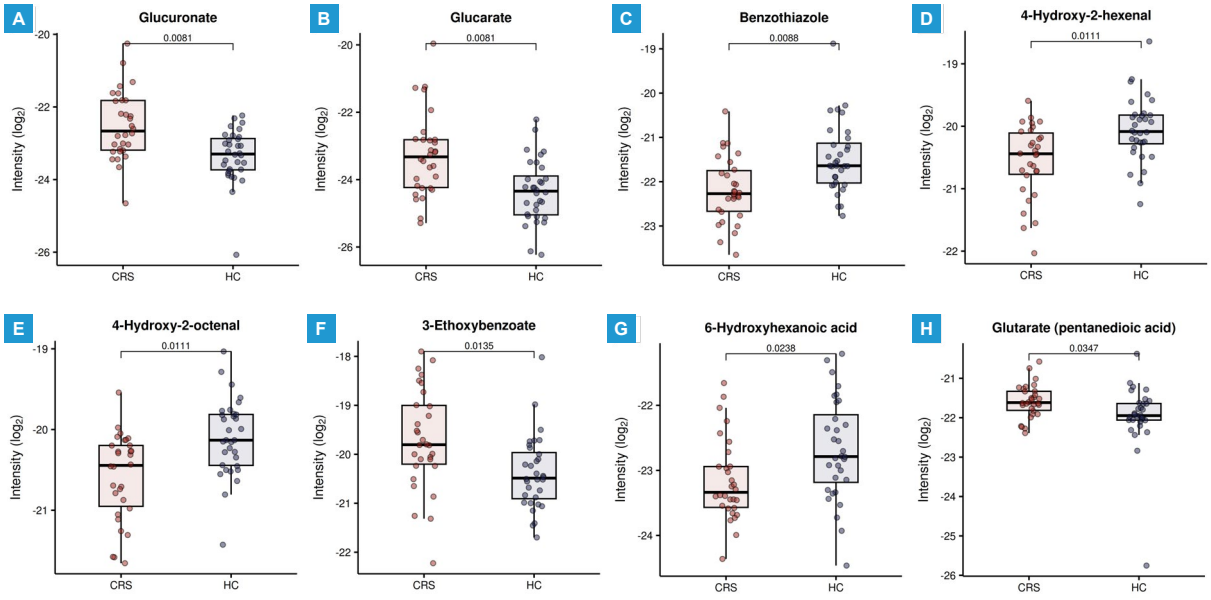

Figure 4. *Boxplots of breath molecules with significant differences between HC preschool children and those with CRS. These molecules correspond to the previously chemically identified compounds in our study using SESI-HRMS, which were associated with allergic asthma in school-aged children (7).*

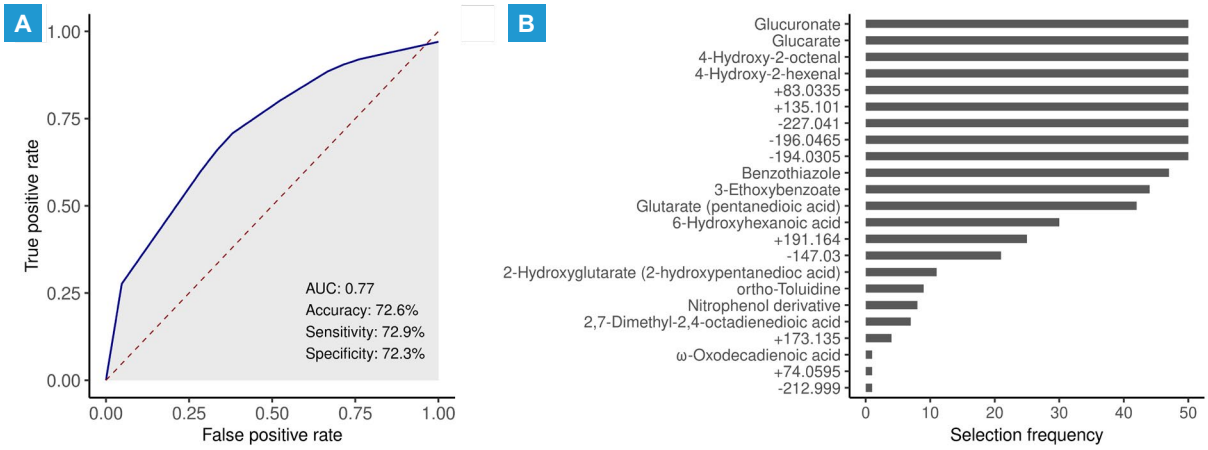

Figure 5. *Classification performance.* ***(A).*** *Average receiver operating characteristic (ROC) curve for classifying CRS and HC control samples, averaged over all cross-validation iterations (33).The diagonal red dashed line represents the line of no discrimination, the blue line represents the average ROC curve, and the gray shaded area indicates the AUC;* ***(B)*** *selection frequencies of the markers used for classification in each cross-validation iteration. The markers are ordered from top to bottom by their selection frequency. Selection frequency represents how often a marker was selected during cross-validation. Compound names were used, when possible, while unidentified markers were represented by their m/z values with the corresponding ionization mode (+/-).*

DISCUSSION

This study validates that an offline breath collecting device entailing Nalophan can be used in SESI-HRMS analytics to detect VOCs in preschool children. Furthermore, for the first time, we were able to reidentify asthma-specific VOCs in preschool children presenting with CRS, distinguishing them from healthy controls.

In a previous online breath analysis study, our research group identified 375 m/z features potentially associated with asthma in school-aged children (7). In the present study, we detected 125 of those m/z features again in a cohort of preschool children using our remote breath collection method. Of these, 16 m/z features showed statistically significant differences between children referred for the investigation of preschool asthma, presenting with wheezing and/or dry cough (CRS) and healthy control children, and 8 m/z features had previously been identified chemically (7).

Among the 8 significant, chemically reidentified markers were glucuronate and glucarate, which are both part of monosaccharide metabolism. It has been previously described that due to inflammation, airway hyperresponsiveness, hypoxia and increased breathing effort there is an increased energy demand in asthma patients leading to an altered energy metabolism (34).Depicting this alteration, an affected carbohydrate metabolism has been demonstrated both in the lungs of murine models and sera of human asthma patients (35, 36). Therefore, altered levels of glucuronate and glucarate in our preschool cohort correspond to previous findings.

Two other significant, chemically reidentified markers were 4-hydroxy-2-octenal and 4-hydroxy-2-hexenal, which both belong to the chemical group of aldehydes. Aldehydes were described as a product of lipid peroxidation in environments of oxidative stress (37), similarly observed in asthma patients. Remarkably, demonstrating the same trend as the asthma group in our study with school-aged children (7), the CRS group of the preschool cohort showed decreased instead of increased intensities for both 4-hydroxy-2-octenal and 4-hydroxy-2-hexenal. These contradictory results fall in line with the varying results of previous studies that observed both increased and decreased or even unchanged levels of aldehydes in patients with chronic respiratory symptoms compared to healthy controls (38-44).

With 6-hydroxyhexanoic acid, a representative of fatty acid metabolism was significantly different between children with CRS and healthy controls. Similar to aldehydes, the presence of fatty acid metabolites is an expression of oxidative stress (19), as observed in patients with chronic respiratory inflammation. A previous study analyzed breath profiles in patients with COPD exacerbations that showed decreased levels of fatty acids, indicating an upregulation of ω-oxidation-pathways (45). Similarly in our study population, a decreased signal intensity of

6-hydroxyhexanoic acid in CRS patients compared to healthy controls was observed, potentially explained by oxidative stress present in CRS patients.

Another significant, chemically reidentified marker was glutarate which is part of lysine degradation. Although the role of lysine degradation in the pathophysiology of asthma is not fully understood, it has been hypothesized that lysine degradation and thus accumulation of glutarate reflect the generation of energy due to increased breathing efforts in asthma patients (35). Supporting this hypothesis, elevated levels of glutarate in the sera, urine and breath of children were associated with pediatric asthma in previous studies (35, 46, 47). Therefore, elevated levels of glutarate in CRS patients of our study population correspond to preliminary findings.

Finally, two other significant, chemically reidentified m/z features were 3-Ethoxybenzoate and Benzothiazole. We interpreted these features as environmental contaminants since these substances do not occur naturally in any human metabolic pathway (48, 49). The reason for the statistically significant intensity difference for these substances between the CRS children and healthy controls is unclear. A potential reason for this difference may be differences in metabolization and elimination of environmental substances between children suffering from CRS and health controls due to an altered metabolic state underlying the chronic respiratory disease, analogous to the principles mentioned above. This hypothesis, however, should be subject of further research.

Among the remaining 109 non-significant features ($p > 0.05$), the majority were linked to endogenous pathways, such as fatty acid metabolism, lysine degradation, and aldehyde formation (**Table S2**). However, we also found three exogenous markers within this set: ortho-Toluidine (m/z 108.08), 4-Methyl-2-nitrophenol (m/z 152.0335), and a Nitrophenol derivative (m/z 166.0475). While these features did not show statistically significant differences between groups, their redetection aligns with previous observations, reflecting the complexity of breath profiles and potential environmental influences (7).

Assessment of classification performance based on machine learning resulted in an average AUC of 0.77 and an average classification accuracy of approximately 70%. Among the fourteen markers selected at least once during cross-validation, some chemically identified markers were consistently or very frequently selected (**Figure 5B**, **Table S1**). These included the endogenous compounds associated with energy metabolism (glucuronate and glucarate), aldehydes (4-hydroxy-2-hexenal and 4-hydroxy-2-octenal), and lysine degradation (glutarate), highlighting their potential contribution in classifying patients with wheezing and dry cough (CRS). However, the results indicate moderate discriminative power of the identified breath markers for differentiating between healthy controls and individuals with CRS. It is important to note that the machine learning pipeline employed in this study relied on internal validation through repeated cross-validation. External validation with an independent dataset is needed to confirm the model's performance in future studies.

Of 375 asthma-specific m/z features found in school-aged children (7), 250 were not detected again in our preschool cohort. In contrast to the previous study, that included allergic asthmatic school-aged children (7), here we present data from preschool children referred for the investigation of preschool asthma, presenting with chronic or recurrent respiratory symptoms, *i.e.* wheezing and/or dry cough, which is, a mixed population of children with confirmed and non-confirmed allergic or non-allergic asthma or recurrent obstructive bronchitis. These population differences may have contributed to differences in detected breath markers. Another potential reason may be overall metabolic changes over the course of childhood (50), leading to partially different breath patterns between preschool and school-aged children. To investigate these changes of breath patterns in children, longitudinal studies are needed.

Additional limitations to the comparability between the preschool and school-aged group were differences in quality and quantity of the exhalations. Samples from school-aged children were exhaled directly into SESI-HRMS minimizing the potential loss or alteration of volatile compounds between exhalation and detection (7). On the other hand, samples of the younger preschool children were collected using our breath collecting device and were analyzed with minimal delay. Although exhalation into our device was easier to understand and perform by preschool children compared to online breath analysis, the collection of a sample of comparable size and effort turned out to be difficult in some cases. Furthermore, the study aimed to reflect realistic condi-

tions by not strictly controlling potential confounders such as medication use, food intake or physical activity. This approach may introduce variability in VOC profiles. However, imposing such restrictions would have been impractical and ethically challenging, potentially limiting participation and compromising data collection. Further loss of asthma-specific markers may have occurred in the bag system itself. Although Nalophan showed less adsorption of VOCs compared to other materials in a previous study (10), it is likely that the intensity of a substantial number of markers was reduced due to their adhesion properties, as observed in other work (8, 9, 24). In the specific case of aldehydes, it has been shown that the functional group has an influence on the adhesion properties of the aldehyde to Nalophan (8). Furthermore, storage time between breath collection and measurement potentially facilitates oxidation processes of VOCs lowering their signal intensities (51). Finally, chemical modification of VOCs may also have occurred due to temperature changes, *e.g.* during transfer from the patient to the heated box, and humidity changes as a result of diffusion of water through Nalophan, as these processes were described in previous studies (9, 52, 53).

Despite the above-mentioned limiting factors, we were still able to detect 125 asthma-specific m/z features again in preschool children using our breath collecting device in a realistic clinical scenario. Our findings emphasize the potential of remote breath collection methods together with SESI-HRMS for targeted breath analysis in preschool children.

CONCLUSIONS

This is the first study to successfully reidentify asthma-specific breath markers in preschool children by SESI-HRMS using a breath collection device that entails Nalophan. This study lays another foundation to develop an easy to perform and reliable offline breath test in order to lower the age for diagnosing asthma in children.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests

The Authors have declared no conflict of interests.

Financial support

The Evi Diethelm-Winteler Foundation and the Dr. Geber-ten Bosch Foundation

Author contributions

Study design and concept: JM, AM. Data acquisition: RB, KR, YB. Data processing and analysis: SM, NP. Data evaluation and interpretation: AM, SM, RB, NP. Drafting of the manuscript: RB, SM, AM, ES. Funding acquisition: AM. Review and editing of the manuscript: all Authors.

Ethical approval

Human studies and subjects

The study followed the ethical standards established in the Declaration of Helsinki. It was approved by the Ethics Committee of the Canton of Zurich (KEK-ZH-Nr. 2018-00441) and written informed parental consent and oral assent of the participants were obtained prior to study participation.

Data sharing and data accessibility

Data are available upon motivated request to the Corresponding Author.

Publication ethics

Plagiarism

Authors declare no potentially overlapping publications with the content of this manuscript and all original studies are cited as appropriate.

Data falsification and fabrication

All the data corresponds to the real.

REFERENCES

1. van den Akker-van Marle ME, Bruil J, Detmar SB. Evaluation of cost of disease: assessing the burden to society of asthma in children in the European Union. Allergy. 2005;60(2):140-9. doi: 10.1111/j.1398-9995.2005.00692.x.
2. Neerincx AH, Vijverberg SJH, Bos LDJ, Brinkman P, van der Schee MP, de Vries R, et al. Breathomics from exhaled volatile organic compounds in pediatric asthma. Pediatr Pulmonol. 2017;52(12):1616-27. doi: 10.1002/ppul.23785.
3. Gaillard EA, Kuehni CE, Turner S, Goutaki M, Holden KA, de Jong CCM, et al. European Respiratory Society clinical practice guidelines for the diagnosis of asthma in children aged 5-16 years. Eur Respir J. 2021;58(5):2004173. doi: 10.1183/13993003.04173-2020.
4. Brand PLP, Caudri D, Eber E, Gaillard EA, Garcia-Marcos L, Hedlin G, et al. Classification and pharmacological treatment of preschool wheezing: changes since 2008. Eur Respir J. 2014;43(4):1172-7. doi: 10.1183/09031936.00199913.

5. Crenesse D, Berlioz M, Bourrier T, Albertini M. Spirometry in children aged 3 to 5 years: reliability of forced expiratory maneuvers. Pediatr Pulmonol. 2001;32(1):56-61. doi: 10.1002/ppul.1089.
6. Kanengiser S, Dozor AJ. Forced expiratory maneuvers in children aged 3 to 5 years. Pediatr Pulmonol. 1994;18(3):144-9. doi: 10.1002/ppul.1950180305.
7. Weber R, Streckenbach B, Welti L, Inci D, Kohler M, Perkins N, et al. Online breath analysis with SESI/HRMS for metabolic signatures in children with allergic asthma. Front Mol Biosci. 2023;10:1154536. doi: 10.3389/fmolb.2023.1154536.
8. Decrue F, Singh KD, Gisler A, Awchi M, Zeng J, Usemann J, et al. Combination of Exhaled Breath Analysis with Parallel Lung Function and FeNO Measurements in Infants. Anal Chem. 2021;93(47):15579-83. doi: 10.1021/acs.analchem.1c02036.
9. Sola-Martínez RA, Zeng J, Awchi M, Gisler A, Arnold K, Singh KD, et al. Preservation of exhaled breath samples for analysis by off-line SESI-HRMS: proof-of-concept study. J Breath Res. 2023;18(1). doi: 10.1088/1752-7163/ad10e1.
10. Liu C, Zeng J, Sinues P, Fang M, Zhou Z, Li X. Quantification of volatile organic compounds by secondary electrospray ionization-high resolution mass spectrometry. Anal Chim Acta. 2021;1180:338876. doi: 10.1016/j.aca.2021.338876.
11. Azim A, Barber C, Dennison P, Riley J, Howarth P. Exhaled volatile organic compounds in adult asthma: a systematic review. Eur Respir J. 2019;54(3). doi: 10.1183/13993003.00056-2019.m.
12. Sola-Martínez RA, Lozano-Terol G, Gallego-Jara J, Morales E, Cantero-Cano E, Sanchez-Solis M, et al. Exhaled volatilome analysis as a useful tool to discriminate asthma with other coexisting atopic diseases in women of childbearing age. Sci Rep. 2021;11(1):13823. doi: 10.1038/s41598-021-92933-2.
13. Rahman I. The role of oxidative stress in the pathogenesis of COPD: implications for therapy. Treat Respir Med. 2005;4(3):175-200. doi: 10.2165/00151829-200504030-00003.
14. Alary J, Guéraud F, Cravedi JP. Fate of 4-hydroxynonenal in vivo: disposition and metabolic pathways. Mol Aspects Med. 2003;24(4-5):177-87. doi: 10.1016/s0098-2997(03)00012-8.
15. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med. 1991;11(1):81-128. doi: 10.1016/0891-5849(91)90192-6.
16. Flor AC, Kron SJ. Lipid-derived reactive aldehydes link oxidative stress to cell senescence. Cell Death Dis. 2016;7(9):e2366. doi: 10.1038/cddis.2016.275.
17. Rahman I, Biswas SK. Non-invasive biomarkers of oxidative stress: reproducibility and methodological issues. Redox Rep. 2004;9(3):125-43. doi: 10.1179/135100004225005219.
18. Schneider C, Porter NA, Brash AR. Routes to 4-hydroxynonenal: fundamental issues in the mechanisms of lipid peroxidation. J Biol Chem. 2008;283(23):15539-43. doi: 10.1074/jbc.R800001200.
19. Dallinga JW, Robroeks CMHHT, van Berkel JJBN, Moonen EJC, Godschalk RWL, Jöbsis Q, et al. Volatile organic compounds in exhaled breath as a diagnostic tool for asthma in children. Clin Exp Allergy. 2010;40(1):68-76. doi: 10.1111/j.1365-2222.2009.03343.x.
20. Olopade CO, Zakkar M, Swedler WI, Rubinstein I. Exhaled pentane levels in acute asthma. Chest. 1997;111(4):862-5 doi: 10.1378/chest.111.4.862.
21. Paredi P, Kharitonov SA, Leak D, Shah PL, Cramer D, Hodson ME, et al. Exhaled Ethane Is Elevated in Cystic Fibrosis and Correlates with Carbon Monoxide Levels and Airway Obstruction. Am J Respir Crit Care Med. 2000;161(4):1247-51. doi: 10.1164/ajrccm.161.4.9906122.
22. Paredi P, Kharitonov SA, Barnes PJ. Analysis of expired air for oxidation products. Am J Respir Crit Care Med. 2002;166(12 Pt 2):S31-7. doi: 10.1164/rccm.2206012.
23. Weber R, Kaeslin J, Moeller S, Perkins N, Micic S, Moeller A. Effects of a Volatile Organic Compound Filter on Breath Profiles Measured by Secondary Electrospray High-Resolution Mass Spectrometry. Molecules. 2022;28(1):45. doi: doi: 10.3390/molecules28010045.
24. Gilchrist FJ, Razavi C, Webb AK, Jones AM, Spaněl P, Smith D, et al. An investigation of suitable bag materials for the collection and storage of breath samples containing hydrogen cyanide. J Breath Res. 2012;6(3):036004. doi: 10.1088/1752-7155/6/3/036004.
25. Mochalski P, King J, Unterkofler K, Amann A. Stability of selected volatile breath constituents in Tedlar, Kynar and Flexfilm sampling bags. Analyst. 2013;138(5):1405-18. doi: 10.1039/c2an36193k.
26. Kaeslin J, Micic S, Weber R, Müller S, Perkins N, Berger C, et al. Differentiation of Cystic Fibrosis-Related Pathogens by Volatile Organic Compound Analysis with Secondary Electrospray Ionization Mass Spectrometry. Metabolites. 2021;11(11). doi: 10.3390/metabo11110773
27. Kessner D, Chambers M, Burke R, Agus D, Mallick P. ProteoWizard: open-source software for rapid proteomics tools development. Bioinformatics. 2008;24(21):2534-6. doi: 10.1093/bioinformatics/btn323.
28. Mann HB, Whitney DR. On a Test of Whether one of Two Random Variables is Stochastically Larger than the Other. Ann Math Stat. 1947;18(1):50-60. doi: 10.1214/aoms/1177730491.
29. Storey JD. A direct approach to false discovery rates. J R Stat Soc Series B Stat Methodol. 2002;64(3):479-98. doi: 10.1111/1467-9868.00346.
30. Breiman L. Random Forests. Mach Learn. 2001;45(1):5-32. doi: 10.1023/A:1010933404324.
31. Weber R, Haas N, Baghdasaryan A, Bruderer T, Inci D, Micic S, et al. Volatile organic compound breath signatures of children with cystic fibrosis by real-time

SESI-HRMS. ERJ Open Res [Internet]. 2020;6(1). doi: 10.1183/23120541.00171-2019.

32. Meinshausen N, Bühlmann P. Stability selection. J R Stat Soc Series B Stat Methodol. 2010;72(4):417-73. doi: 10.1111/j.1467-9868.2010.00740.x.
33. Fawcett T. An introduction to ROC analysis. Pattern Recognit Lett. 2006;27(8):861-74.
34. Papamichael MM, Katsardis C, Sarandi E, Georgaki S, Frima ES, Varvarigou A, et al. Application of Metabolomics in Pediatric Asthma: Prediction, Diagnosis and Personalized Treatment. Metabolites. 2021;11(4). doi: 10.3390/metabo11040251.
35. Chang C, Guo ZG, He B, Yao WZ. Metabolic alterations in the sera of Chinese patients with mild persistent asthma: a GC-MS-based metabolomics analysis. Acta Pharmacol Sin. 2015;36(11):1356-66. doi: 10.1038/aps.2015.102.
36. Ho WE, Xu YJ, Xu F, Cheng C, Peh HY, Tannenbaum SR, et al. Metabolomics reveals altered metabolic pathways in experimental asthma. Am J Respir Cell Mol Biol. 2013;48(2):204-11. doi: 10.1165/rcmb.2012-0246OC.
37. Jesenak M, Zelieskova M, Babusikova E. Oxidative Stress and Bronchial Asthma in Children-Causes or Consequences? Front Pediatr. 2017;5:162. doi: 10.3389/fped.2017.00162.
38. Ibrahim B, Basanta M, Cadden P, Singh D, Douce D, Woodcock A, et al. Non-invasive phenotyping using exhaled volatile organic compounds in asthma. Thorax. 2011;66(9):804-9. doi: 10.1136/thx.2010.156695.
39. Sagdic A, Sener O, Bulucu F, Karadurmus N, Özel HE, Yamanel L, et al. Oxidative stress status and plasma trace elements in patients with asthma or allergic rhinitis. Allergol Immunopathol. 2011;39(4):200-5. doi: 10.1016/j.aller.2010.07.006.
40. Caldeira M, Perestrelo R, Barros AS, Bilelo MJ, Morête A, Câmara JS, et al. Allergic asthma exhaled breath metabolome: a challenge for comprehensive two-dimensional gas chromatography. J Chromatogr A. 2012;1254:87-97. doi: 10.1016/j.chroma.2012.07.023.
41. Riscassi S, Corradi M, Andreoli R, Maccari C, Mercolini F, Pescollderungg L, et al. Nitric oxide products and aldehydes in exhaled breath condensate in children with asthma. Clin Exp Allergy. 2022;52(4):561-4. doi: 10.1111/cea.14066.
42. Gahleitner F, Guallar-Hoyas C, Beardsmore CS, Pandya HC, Thomas CP. Metabolomics pilot study to identify volatile organic compound markers of childhood asthma in exhaled breath. Bioanalysis. 2013;5(18):2239-47. doi: 10.4155/bio.13.184.
43. van de Kant KDG, van Berkel JJBN, Jöbsis Q, Lima Passos V, Klaassen EMM, van der Sande L, et al. Exhaled breath profiling in diagnosing wheezy preschool children. Eur Respir J. 2013;41(1):183-8. doi: 10.1183/09031936.00122411.
44. Smolinska A, Klaassen EMM, Dallinga JW, van de Kant KDG, Jobsis Q, Moonen EJC, et al. Profiling of volatile organic compounds in exhaled breath as a strategy to find early predictive signatures of asthma in children. PLoS One. 2014;9(4):e95668. doi: 10.1371/journal.pone.0095668.
45. Gaugg MT, Nussbaumer-Ochsner Y, Bregy L, Engler A, Stebler N, Gaisl T, et al. Real-Time Breath Analysis Reveals Specific Metabolic Signatures of COPD Exacerbations. Chest. 2019;156(2):269-76. doi: 10.1016/j.chest.2018.12.023.
46. Saude EJ, Skappak CD, Regush S, Cook K, Ben-Zvi A, Becker A, et al. Metabolomic profiling of asthma: diagnostic utility of urine nuclear magnetic resonance spectroscopy. J Allergy Clin Immunol. 2011;127(3):757-64.e1–6. doi: 10.1016/j.jaci.2010.12.1077.
47. Carraro S, Bozzetto S, Giordano G, El Mazloum D, Stocchero M, Pirillo P, et al. Wheezing preschool children with early-onset asthma reveal a specific metabolomic profile. Pediatr Allergy Immunol. 2018;29(4):375-82. doi: 10.1111/pai.12879.
48. PubChem Compound Summary for CID 6951193, 3-Ethoxybenzoate. National Center for Biotechnology Information. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/3-Ethoxybenzoate. Accessed: May 26, 2024.
49. PubChem Compound Summary for CID 7222, Benzothiazole [Internet]. National Center for Biotechnology Information. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Benzothiazole. Accessed: May 26, 2024.
50. Cominetti O, Hosking J, Jeffery A, Pinkney J, Martin FP. Contributions of Fat and Carbohydrate Metabolism to Glucose Homeostasis in Childhood Change With Age and Puberty: A 12-Years Cohort Study (EARLYBIRD 77). Frontiers in Nutrition. 2020;7. Available from: https://www.frontiersin.org/articles/10.3389/fnut.2020.00139. Accessed: Jan 31, 2025.
51. Harshman SW, Mani N, Geier BA, Kwak J, Shepard P, Fan M, et al. Storage stability of exhaled breath on Tenax TA. J Breath Res. 2016;10(4):046008. doi: 10.1088/1752-7155/10/4/046008.
52. Ghimenti S, Lomonaco T, Bellagambi FG, Tabucchi S, Onor M, Trivella MG, et al. Comparison of sampling bags for the analysis of volatile organic compounds in breath. J Breath Res. 2015;9(4):047110. doi: 10.1088/1752-7155/9/4/047110.
53. Beauchamp J, Herbig J, Gutmann R, Hansel A. On the use of Tedlar® bags for breath-gas sampling and analysis. J Breath Res. 2008;2(4):046001. doi: 10.1088/1752-7155/2/4/046001.

REVIEW

Global impact of nirsevimab on respiratory syncytial virus: Valle d'Aosta and beyond

Laura **Apprato** [1, *], Alessandra **Consolati** [1], Maria Paola **Farinelli** [1], Paolo **Serravalle** [2], Christine **Rollandin** [3, 4], Salvatore **Bongiorno** [1]

*** Correspondence to:**

lapprato@ausl.vda.it.

Doi

10.56164/PediatrRespirJ.2024.57

[1] Prevention Department, Hygiene and Public Health Aosta, Azienda USL Valle d'Aosta, Italy

[2] Maternal and Infantile Department, Complex Facility Pediatrics and Neonatology, Azienda USL Valle d'Aosta, Italy

[3] Graduate School of Health Statistics and Biometrics, University of Turin, Turin, Italy

[4] Departmental Simple Structure Preventive Medicine and Risk Management, Strategic Direction, Azienda USL Valle d'Aosta, Italy

ABSTRACT

Respiratory syncytial virus (RSV) is a leading cause of bronchiolitis and pneumonia in infants, with significant morbidity and mortality worldwide. Despite advancements in medical care, RSV continues to impose a substantial economic and emotional burden on families. Current preventive measures, such as the monoclonal antibody palivizumab, are limited to high-risk populations due to cost and administration frequency. Recently, a new prophylactic agent, nirsevimab, has become available for protection of all infants and children during their first RSV season. Nirsevimab is a humanized monoclonal antibody that targets the fusion (F) protein of RSV in its prefusion conformation, a critical structure for viral infectivity. Nirsevimab has shown an extended half-life and broad neutralizing activity against various RSV strains, including those resistant to other antibodies.

This review focuses on implementation of universal nirsevimab prophylaxis, including the pivotal Italian experience in the Valle d'Aosta region, during the 2023-2024 epidemic season. Data from the region indicates a significant reduction in RSV hospitalizations among infants who received nirsevimab, with no hospitalizations reported in this group compared to 9.7% hospitalization rate in the non-prophylaxis group. The side effects were mild and short-lived. The findings suggest that universal nirsevimab prophylaxis is an effective and safe strategy for reducing the burden of RSV in infants, aligning with successful programs in other countries, such as USA, Spain and France. Further research is needed to explore its long-term impact and cost-effectiveness. By addressing these questions, nirsevimab can play a crucial role in improving infant health outcomes and reducing the burden of RSV.

IMPACT STATEMENT: The review highlights the significant reduction in RSV hospitalizations among infants following the implementation of universal prophylaxis with nirsevimab, demonstrating its effectiveness and safety. This strategy has the potential to substantially alleviate the global burden of RSV, improving infant health outcomes.

KEY WORDS

RSV; nirsevimab; bronchiolitis; monoclonal antibody; universal immunization strategy.

INTRODUCTION

Respiratory syncytial virus (RSV) is the leading cause of bronchiolitis and pneumonia in infants worldwide, with infection rates peaking in the winter months. Beyond acute illness, RSV can lead to long-term respiratory problems like recur-

rent wheezing and asthma. There is a critical need for effective preventive strategies to reduce the impact of RSV on infant health. Despite advancements in medical care, RSV-related morbidity and mortality remain high, causing substantial economic and emotional strain on families (1).

GLOBAL IMPACT OF RSV

Globally, RSV causes about 33 million cases of lower respiratory tract infections requiring medical attention, 3.6 million hospitalizations and more than 100,000 deaths in children aged 0-5 years each year (**Figure 1**). In fact, RSV infection is the leading cause of hospitalization for respiratory infection among children under 1 year of age and is the second leading cause of child mortality after malaria (2).

Considering all infants and children in their first epidemic season, more than 20% require outpatient medical care (3, 4), and 4% are hospitalized (including nearly 1/5 in intensive care) (5).

If we consider the Italian cohort of nearly 400.000 new births (http://dati.istat.it/), >80.000 children will require outpatient medical care, 24.000 access emergency rooms, 16.000 will be hospitalized and 3.200 admitted to intensive care (6).

RSV not only causes an acute respiratory illness that requires medical attention, such as bronchiolitis, but also increases the risk of needing care in the medium and long term: in fact, 30-40% of children who do RSV bronchiolitis in the first year of life develop wheezing and/or asthma in early childhood (7, 8).

Most of the children hospitalized for RSV lower respiratory tract infection, such as bronchiolitis, were born at term and born healthy, for whom, therefore, there was still no possibility of prevention. In fact, in Italy 87% of RSV hospitalizations occur in children born healthy and/or at term (3), while 97% of children with bronchiolitis seen by the Family Pediatrician are healthy children and 92% are children born at term. The serious clinical burden of respiratory infections, such as bronchiolitis, is not only at the hospital level, but is also at ambulatory level (9).

Therefore, the main risk factors for developing a respiratory RSV infection requiring outpatient medical care and/or hospitalization are seasonality and age, two factors that affect all children (1).

Currently, the main preventive measure against RSV is the monoclonal antibody palivizumab. However, its use is limited to specific high-risk populations, such as preterm infants, those with significant heart disease, or infants with compromised immune systems (10). Palivizumab's high cost and need for frequent administration further restrict its widespread use. As a result, there is a pressing need for alternative prevention strategies that are both effective and accessible.

NIRSEVIMAB: A PROMISING PREVENTIVE STRATEGY

One promising development is nirsevimab, a humanized monoclonal antibody targeting the RSV fusion (F) protein in its pre-fusion state, crucial for the virus's infectivity. Nirsevimab has shown an extended half-life and

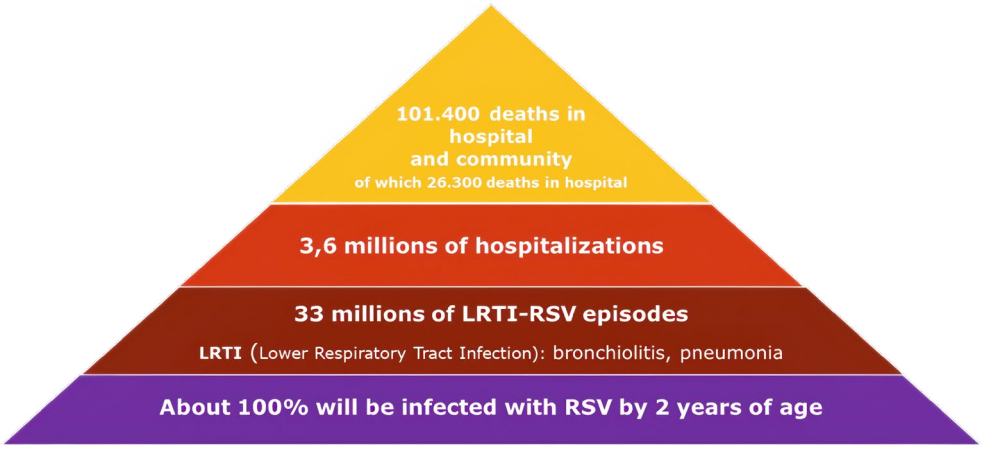

***Figure 1**. Annual worldwide RSV burden among children 0-5 years of age.*

broad neutralizing activity against various RSV strains, including those resistant to other antibodies (11). Clinical trials have demonstrated its efficacy in reducing medically attended RSV lower respiratory tract infections (LRTIs) and hospitalizations, with a favorable safety profile (11-15). Following its approval by the European Medical Agency (EMA) (16), several countries have implemented the immunization campaign with nirsevimab: USA, Spain, France, Luxembourg, Chile and Australia, aiming to reduce the burden of RSV among infants (17-22).

This is a paradigm shift in prevention because it is the first time that a monoclonal antibody has been used as a preventive tool, like a vaccine, for instance, and not as a therapy (23).

Implementation of nirsevimab in Valle d'Aosta

Many other countries, including Italy are poised to start the immunization campaign in the 2024/2025 season (24-28). In Italy, there is only one region that has started the immunization campaign as early as the 2023-2024 season: Valle d'Aosta (29).

In Valle d'Aosta, RSV infections have surged in recent epidemic seasons, with hospitalization rates exceeding the national average. To address this, Valle d'Aosta pioneered universal nirsevimab prophylaxis for newborns during the 2023-2024 epidemic season. Data and results of the immunization campaign were published in the journal Vaccines and presented at the ESPID 2024 (The 42nd Annual Meeting European Society for Pediatric Infectious Diseases) congress (29, 30).

In this article we report results from our prospective observational cohort study, comparing the incidence of hospitalization for RSV bronchiolitis or pneumonia in infants who received nirsevimab prophylaxis against those who did not. The study also monitors the safety of nirsevimab by tracking short-term adverse effects.

Infants born between 1 May 2023 and 31 March 2024 were included, excluding those with existing risk factors already receiving palivizumab because, in consideration of the late availability of nirsevimab (from 20 December), we decided to continue the immunization with palivizumab. Extensive training sessions for healthcare workers were conducted to ensure program adherence. Nirsevimab was imported from France, and parents were informed and summoned through postal services. Prophylaxis was administered at the Aosta hospital for the *in-season* babies (born from 12/19/2023 to 03/31/2024) and at the Hygiene and Public Health Services (SISP) for the 'catch-up' babies (born from 05/01/2023 to 12/18/2023) and monitored through follow-up telephone interviews and local health unit data. Out of 615 eligible neonates, 71.5% adhered to nirsevimab prophylaxis.

Hospitalizations for RSV bronchiolitis significantly decreased in the prophylaxis group compared to previous seasons. Among those who received nirsevimab, none were hospitalized for RSV bronchiolitis, while the non-prophylaxis group saw a 9.7% hospitalization rate (**Figure 2**). Side effects were generally mild and short-lived, including fever and local reactions at the injection site.

The study highlights the substantial reduction in RSV-related hospitalizations due to universal nirsevimab prophylaxis. The findings align with similar successful programs in other countries, such as USA, Spain, France and Luxembourg, demonstrating the efficacy and safety of nirsevimab. The cost-effectiveness of nirsevimab, considering reduced hospital admissions and social care costs, makes it a viable preventive strategy. The program's success in Valle d'Aosta underscores the importance of collaborative healthcare efforts and proactive policies in implementing innovative health measures.

Evidence from Spain and other countries

Valle d'Aosta data fits with other evidence that has been generated in countries that have implemented the universal immunization campaign in the 2023/2024 season (29).

In Galicia, coverage and hospitalization rates recorded throughout the immunization campaign were monitored weekly in the NIRSE-GAL study (31). It was achieved:

- 95.4% coverage in children born during the RSV season (October 2023-March 2024). who were immunized in the hospital at birth;
- 89.9% of coverage in children born out of season (born between April-September 2023) who were recalled for in-hospital immunization before the start of the RSV season (almost all of whom were already immunized in October 2023);
- 97% coverage in the highest risk infants (preterm <29 gw, CHD, CLD).

Recorded nirsevimab effectiveness in reducing RSV-related LRTI hospitalizations was 82%.

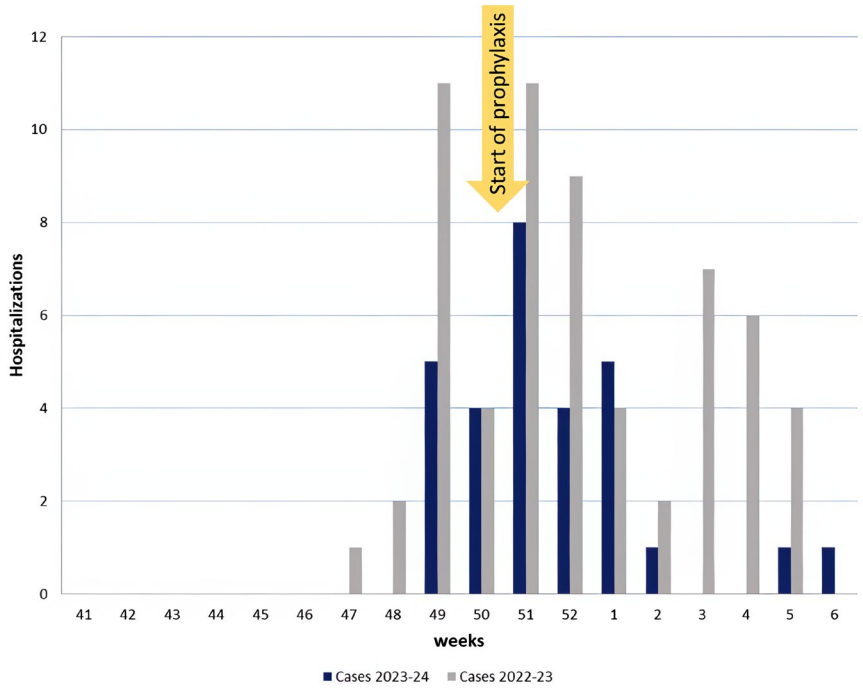

***Figure 2**. Hospitalizations for RSV bronchiolitis in Valle d'Aosta in the last two epidemic seasons.*

The effectiveness of nirsevimab in preventing hospitalizations for confirmed RSV infection and the impact of a birth immunization strategy were also evaluated in other regions of Spain.

In Navarre, of the 1177 infants analyzed in the cohort study, 1083 (92.0%) received nirsevimab, 95.6% of whom were immunized within seven days of birth.

The risk of RSV hospitalization was 8.5% (8/94) among non-immunized infants and 0.7% (8/1083) among immunized infants. The risks of ICU admission were 2.1% (2/94) and 0.3% (3/1083), respectively. The estimated effectiveness of immunoprophylaxis in preventing hospitalization for lower respiratory tract infection due to RSV was 88.7% (95% CI, 69.6-95.8). Efficacy estimates were similarly high in preventing emergency department visits (87.9%; 95% CI, 70.3-95.1) or cases requiring ICU admission (85.9%; 95% CI, 13.2-97.7) (32).

A multicenter active surveillance study was conducted in nine hospitals in three Spain regions: Valencia, Murcia, Valladolid.

A total of 166 infants admitted to one of the enrolled hospitals were included. Of the admitted infants, 95 were RSV positive, 56 of whom (59%) had been immunized.

The study used two methodological approaches: the screening method and the test-negative design. The screening method compared the proportion of infants immunized with nirsevimab among those hospitalized for RSV-positive LRTIs with the proportion immunized in the catchment area. The test-negative design compared the odds of being immunized with nirsevimab among infants hospitalized for RSV-positive versus RSV-negative LRTIs.

Both methods indicated that nirsevimab provided a high level of protection, with a reduction of at least 70%, against hospitalizations for RSV-positive LRTIs in immunized infants (33).

A study was conducted in Catalogna in which 26.525 infants were included. By the end of the study period, 23,127 infants (87.2%) had been immunized against RSV. The control group showed higher incidence rates for all outcomes, particularly for severe cases, including RSV-specific and all-cause outcomes, in both outpatient and hospital settings (34).

In the universal immunization campaign that began in the 2023-2024 season, nirsevimab administration demonstrated a good safety profile in line with that reported in the authorization.

In fact, of the more than 200,000 children who received nirsevimab in Spain, no new risks were identified beyond those listed in the data sheet: rash, pyrexia, and injection site reactions (19).

In the USA, the Centers for Disease Control and Prevention (CDC) estimated that in the US nirsevimab was 90% effective in preventing and reducing RSV-associated hospitalizations in all infants and children in their first season of RSV. In this multicenter analysis in which 699 infants hospitalized with ARI during their first RSV season were included, nirsevimab treatment was shown to be 90% effective against RSV-associated hospitalization. This early efficacy estimate supports existing recommendations for the prevention of severe VRS disease in infants in their first season of RSV (35).

A prospective, multicenter, case-control study was also conducted in France that analyzed the efficacy of nirsevimab against hospitalization for RSV-associated bronchiolitis in infants younger than 12 months of age in a real-world setting (36).

The study included 1035 infants, including 690 cases and 345 controls:

– cases were infants younger than 12 months of age who were hospitalized for RSV-associated bronchiolitis between October 15 and December 10, 2023;
– controls were infants with clinic visits to the same hospitals for conditions unrelated to RSV infection.

Infants at higher risk of severe RSV disease, such as: premature infants <6 months of age, infants with chronic lung disease of the premature, and congenital heart disease, were also included in the study.

A total of 60 case patients (8.7%) and 97 control patients (28.1%) were immunized with nirsevimab. Nirsevimab demonstrated an efficacy against hospitalization for RSV-associated bronchiolitis of 83.0%.

In addition, nirsevimab demonstrated efficacy of:

– 69.6% in the reduction of RSV-associated bronchiolitis that required ICU hospitalization;
– 67.2% in the reduction of RSV-associated bronchiolitis that involved ventilatory support.

Given the excellent results obtained (**Table 1**) and the good safety profile also confirmed in real-world, the International Health Authorities recommended nirsevimab in all infants and children at the first season of RSV.

Spanish Health Minister confirmed the use of nirsevimab for next season as well. In Germany, STIKO recommend the use of nirsevimab in all infants and children during their first RSV season, regardless of the presence of risk factors (24).

GLOBAL RECOMMENDATIONS AND

Table 1. *Reduction of hospitalization and coverage upon nirsevimab implementation during the 2023/204 RSV season in Spain, USA and France.*

Countries	Coverage	Reduction of hospitalization
Galicia	91%	82%
Navarre	92%	85.9%
Valencia, Murcia, Valladolid	59%	70%
Catalogna	87.2%	87.6%
USA	68%	90%
France	16%	83%

FUTURE DIRECTIONS

These studies provide compelling evidence for the universal use of nirsevimab in preventing RSV bronchiolitis in children, demonstrating its effectiveness and safety. Results from regions like Galicia are promising, and achieving similar outcomes will require substantial organizational efforts to ensure high levels of immunization adherence. This is crucial for replicating the efficacy observed in clinical trials and early implementation studies.

Ongoing surveillance will be necessary to comprehensively assess RSV circulation and detect any shifts in RSV epidemiology following widespread nirsevimab use. Additional data, including long-term effects and epidemiological trends, are essential to confirm and complement the already proven effectiveness of the product. To comprehensively evaluate nirsevimab, continuous research and surveillance in diverse settings are imperative.

Further research is needed to explore its long-term impact, cost-effectiveness, and performance in different clinical contexts. By addressing these questions, nirsevimab can play a crucial role in improving infant health outcomes and reducing the global burden of RSV-related illnesses.

COMPLIANCE WITH ETHICAL STANDARDS

Conflicts of interests

The Authors declare no conflicts of interest.

Fundings

The publication of this article was financially supported by Sanofi.

Ethical approval

Human studies and subjects

N/A.

Data sharing and data accessibility

Data are available upon motivated request to the Corresponding Author.

Publication ethics

Plagiarism

Authors declare no potentially overlapping publications with the content of this manuscript and all original studies are cited as appropriate.

Data falsification and fabrication

All the data correspond to the real.

REFERENCES

1. Azzari C, Baraldi E, Bonanni P, Bozzola E, Coscia A, Lanari M, et al. Epidemiology and prevention of respiratory syncytial virus infections in children in Italy. Ital J Pediatr. 2021;47(1):198. doi: 10.1186/s13052-021-01148-8.
2. Li Y, Wang X, Blau DM, Caballero MT, Feikin DR, Gill CJ, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in children younger than 5 years in 2019: a systematic analysis. Lancet. 2022;399(10340):2047-64. doi:10.1016/S0140-6736(22)00478-0.
3. Lively JY, Curns AT, Weinberg GA, Edwards KM, Staat MA, Prill MM, et al. Respiratory Syncytial Virus-Associated Outpatient Visits Among Children Younger Than 24 Months. J Pediatric Infect Dis Soc. 2019;8(3):284-6. doi: 10.1093/jpids/piz011.
4. Heppe Montero M, Gil-Prieto R, Walter S, Aleixandre Blanquer F, Gil De Miguel Á. Burden of severe bronchiolitis in children up to 2 years of age in Spain from 2012 to 2017. Hum Vaccin Immunother. 2022;18(1):1883379. doi: 10.1080/21645515.2021.1883379
5. Barbati F, Moriondo M, Pisano L, Calistri E, Lodi L, Ricci S, et al. Epidemiology of Respiratory Syncytial Virus-Related Hospitalization Over a 5-Year Period in Italy: Evaluation of Seasonality and Age Distribution Before Vaccine Introduction. Vaccines. 2020;8(1):15. doi: 10.3390/vaccines8010015.
6. Italian National Institute of Statistics (ISTAT). Demo − Resident population by sex, age, and marital status as of January 1. Available from: https://demo.istat.it/app/?i=FE3&l=it. Accessed: Aug 22, 2024.
7. Manti S, Staiano A, Orfeo L, Midulla F, Marseglia GL, Ghizzi C, et al. UPDATE - 2022 Italian guidelines on the management of bronchiolitis in infants. Ital J Pediatr. 2023;49(1):19. doi: 10.1186/s13052-022-01392-6.
8. Wu P, Hartert TV. Evidence for a causal relationship between respiratory syncytial virus infection and asthma. Expert Rev Anti Infect Ther. 2011;9(9):731-45. doi: 10.1586/eri.11.92.
9. Barbieri E, Cavagnis S, Scamarcia A, Cantarutti L, Bertizzolo L, Bangert M, et al. Assessing the burden of bronchiolitis and lower respiratory tract infections in children ≤24 months of age in Italy, 2012-2019. Front Pediatr. 203;11:1143735. doi: 10.3389/fped.2023.1143735.
10. European Medicines Agency. Palivizumab, Summary of Product Characteristics. Available from: https://www.ema.europa.eu/en/documents/product-information/synagis-epar-product-information_en.pdf. Accessed: Aug 3, 2024.
11. AIFA. Beyfortus. RCP. Available from: https://farmaci.agenziafarmaco.gov.it/bancadatifarmaci/farmaco?farmaco=050403. Accessed: Aug 3, 2024.
12. Griffin MP, Yuan Y, Takas T, Domachowske JB, Madhi SA, Manzoni P, et al; Nirsevimab Study Group. Single-Dose Nirsevimab for Prevention of RSV in Preterm Infants. N Engl J Med. 2020;383(5):415-25. doi: 10.1056/NEJMoa1913556. Erratum in: N Engl J Med. 2020;383(7):698. doi: 10.1056/NEJMx200019.
13. Hammitt LL, Dagan R, Yuan Y, Baca Cots M, Bosheva M, Madhi SA, et al; MELODY Study Group. Nirsevimab for Prevention of RSV in Healthy Late-Preterm and Term Infants. N Engl J Med. 2022;386(9):837-46. doi: 10.1056/NEJMoa2110275.
14. Muller WJ, Madhi SA, Seoane Nuñez B, Baca Cots M, Bosheva M, Dagan R, et al; MELODY Study Group. Nirsevimab for Prevention of RSV in Term and Late-Preterm Infants. N Engl J Med. 2023;388(16):1533-4. doi: 10.1056/NEJMc2214773.
15. Domachowske J, Madhi SA, Simões EAF, Atanasova V, Cabañas F, Furuno K, et al; MEDLEY Study Group. Safety of Nirsevimab for RSV in Infants with Heart or Lung Disease or Prematurity. N Engl J Med. 2022;386(9):892-4. doi: 10.1056/NEJMc2112186.
16. European Medicines Agency. Beyfortus. EPAR. Available from: https://www.ema.europa.eu/en/medicines/human/EPAR/beyfortus. Accessed: Jan 22, 2025.
17. Centers for Disease Control and Prevention. CDC Recommends a Powerful New Tool to Protect Infants from the Leading Cause of Hospitalization. Available from: https://www.cdc.gov/media/releases/2023/p-0803-new-tool-prevent-infant-hospitalization-.html. Accessed: June 22, 2024.
18. Position de la SP2A sur les stratégies de prévention de la bronchiolite − SP2A. Available from: https://www.sp2a.

fr/spa_actualites/position-strategies-prevention-bronchiolite/. Accessed: Jan 22, 2025.

19. Health Ministry of Spain. Recomendaciones de utilización de nirsevimab para la temporada 2024-2025 en España. Available from: https://www.sanidad.gob.es/areas/promocionPrevencion/vacunaciones/comoTrabajamos/docs/Nirsevimab.pdf. Accessed: Jan 22, 2025.
20. Ernst C, Bejko D, Gaasch L, Hannelas E, Kahn I, Pierron C, Del Lero N, et al. Impact of nirsevimab prophylaxis on paediatric respiratory syncytial virus (RSV)-related hospitalisations during the initial 2023/24 season in Luxembourg. Euro Surveill. 2024;29(4):pii=2400033. doi: 10.2807/1560-7917.ES.2024.29.4.2400033.
21. Ministerio de Salud, Gobierno de Chile. Departamento de Estadisticas e Informaciòn de Salud. Available from: https://deis.minsal.cl/. Accessed: Jan 22, 2025.
22. Western Australian children first to access protection from RSV. Western Australian Government. Available from: https://www.wa.gov.au/government/media-statements/Cook-Labor-Government/Western-Australian-children-first-to-access-protection-from-RSV-20240305#:~:text=In%20an%20Australian%20first%2C%20Western,infant%20hospitalisation%20in%20the%20country. Accessed: Jan 20, 2025.
23. American Academy of Pediatrics. About Nirsevimab. Available from: https://www.aap.org/en/patient-care/respiratory-syncytial-virus-rsv-prevention/nirsevimab-frequently-asked-questions/. Accessed: Jan 22, 2025.
24. Robert Koch Institute. Epidemiologisches Bulletin 26/2024. Available from: https://www.rki.de/DE/Content/Infekt/EpidBull/Archiv/2024/Ausgaben/26_24.pdf?__blob=publicationFile. Accessed: Jan 23, 2025.
25. Bundesministerium. Erste Lieferung der RSV-Prophylaxe. Available from: https://www.sozialministerium.at/Services/Neuigkeiten-und-Termine/RSV-Prophylaxe.html. Accessed: Jan 20, 2025.
26. Public Health Agency of Canada. An Advisory Committee Statement (ACS) National Advisory Committee on Immunization (NACI). Available from: https://www.canada.ca/content/dam/phac-aspc/documents/services/publications/vaccines-immunization/palivizumab-respiratory-syncitial-virus-infection-infants/palivizumab-resp-infection-infants-eng.pdf. Accessed: Jan 22, 2025.
27. Superior Health Council. Preventive strategies against RSV disease in children. Available from: https://www.health.belgium.be/sites/default/files/uploads/fields/fpshealth_theme_file/20231220_shc-9760_advice_rsv_children_vweb.pdf. Accessed: Jan 22, 2025.
28. Health Ministry of Italy. Misure di prevenzione e immunizzazione contro il virus respiratorio sinciziale (VRS). Available from: https://www.trovanorme.salute.gov.it/norme/renderNormsanPdf?anno=2024&codLeg=99716&parte=1%20&serie=null. Accessed: Jan 22, 2025.
29. Consolati A, Farinelli M, Serravalle P, Rollandin C, Apprato L, Esposito S, et al. Safety and Efficacy of Nirsevimab in a Universal Prevention Program of Respiratory Syncytial Virus Bronchiolitis in Newborns and Infants in the First Year of Life in the Valle d'Aosta Region, Italy, in the 2023-2024 Epidemic Season. Vaccines. 2024;12(5):549. doi: 10.3390/vaccines12050549.
30. EP675 - Coverage and effectiveness of nirsevimab in a universal prevention program against RSV: preliminary data from Valle d'Aosta Region of Italy - The 42nd Annual Meeting of the European Society for Paediatric Infectious Diseases. Available from: ctimeetingtech.com. Accessed: Jan 22, 2024.
31. Ares-Gómez S, Mallah N, Santiago-Pérez MI, Pardo-Seco J, Pérez-Martínez O, Otero-Barrós MT, et al; NIRSE-GAL study group. Effectiveness and impact of universal prophylaxis with nirsevimab in infants against hospitalisation for respiratory syncytial virus in Galicia, Spain: initial results of a population-based longitudinal study. Lancet Infect Dis. 2024;24(8):817-828. doi: 10.1016/S1473-3099(24)00215-9. Erratum in: Lancet Infect Dis. 2024;24(7):e419. doi: 10.1016/S1473-3099(24)00355-4.
32. Ezpeleta G, Navascués A, Viguria N, Herranz-Aguirre M, Juan Belloc SE, Gimeno Ballester J, et al. Effectiveness of Nirsevimab Immunoprophylaxis Administered at Birth to Prevent Infant Hospitalisation for Respiratory Syncytial Virus Infection: A Population-Based Cohort Study. Vaccines. 2024;12(4):383. doi: 10.3390/vaccines12040383.
33. López-Lacort M, Muñoz-Quiles C, Mira-Iglesias A, López-Labrador FX, Mengual-Chuliá B, Fernández-García C, et al. Early estimates of nirsevimab immunoprophylaxis effectiveness against hospital admission for respiratory syncytial virus lower respiratory tract infections in infants, Spain, October 2023 to January 2024. Euro Surveill. 2024;29(6):2400046. doi: 10.2807/1560-7917.ES.2024.29.6.2400046
34. Coma E, Martinez-Marcos M, Hermosilla E, Mendioroz J, Reñé A, Fina F, et al. Effectiveness of nirsevimab immunoprophylaxis against respiratory syncytial virus-related outcomes in hospital and primary care settings: a retrospective cohort study in infants in Catalonia. Arch Dis Child. 2024;109(9):736-41. doi: 10.1136/archdischild-2024-327153.
35. Early Estimate of Nirsevimab Effectiveness for Prevention of Respiratory Syncytial Virus–Associated Hospitalization Among Infants Entering Their First Respiratory Syncytial Virus Season — New Vaccine Surveillance Network, October 2023-February 2024. MMWR. Available from: cdc.gov. Accessed: Jan 22, 2025.
36. Paireau J, Durand C, Raimbault S, Cazaubon J, Mortamet G, Viriot D, et al. Nirsevimab Effectiveness Against Cases of Respiratory Syncytial Virus Bronchiolitis Hospitalised in Paediatric Intensive Care Units in France, September 2023-January 2024. Influenza Other Respir Viruses. 2024;18(6):e13311. doi: 10.1111/irv.13311.

PEDIATR RESPIR J Vol. 3(1), 28-35, 2025

REVIEW

Update in the diagnosis and management of preschool wheezing disorders

Sejal **Saglani** *

*** Correspondence to:**

s.saglani@imperial.ac.uk. ORCID: https://orcid.org/0000-0001-5192-6418

Doi

10.56164/PediatrRespirJ.2025.69

National Heart and Lung Institute, Centre for Pediatrics and Child Health, Imperial College London, London, United Kingdom

ABSTRACT

Recurrent episodes of wheezing in children aged under 6 years are common and preschool children account for the majority of all childhood hospitalizations for acute wheezing. This results in significant morbidity, has an impact on the child and family's quality of life and places a significant demand on healthcare resources. Moreover, frequent preschool wheeze attacks are associated with an early loss in lung function which may track to adulthood. The focus of this review is to provide a structured approach to diagnosis and management of recurrent preschool wheezing, to prevent frequent attacks and minimize disease burden.

A detailed history and examination are critical to confirm wheezing as the predominant symptom, and to ensure alternative symptoms such as stridor, or chronic wet cough which may result from alternative diagnoses have been excluded. The constellation of wheezing with breathlessness, difficulty breathing and/or cough, supports a diagnosis of recurrent preschool wheeze. However, it is important to undertake some investigations to define the type of wheezing a child has and help decide optimal management.

In contrast to school-age asthma, preschool children with recurrent wheezing may not have an allergic, eosinophilic phenotype which will respond to maintenance inhaled corticosteroids (ICS). Assessment for aeroallergen sensitization and elevated blood eosinophils (>300 cells/mcl) when the child is well and in between episodes, helps to identify children more likely to improve with daily ICS. If neither of these tests are positive, ICS may be less effective, and assessments for lower airway bacterial infection may be helpful to decide whether treatment with targeted antibiotics is beneficial. There is preliminary evidence that oral or sublingual mixed bacterial lysates may also reduce symptoms and attacks in non-sensitized children, especially those who only have symptoms precipitated by upper respiratory infections. Recurrent preschool wheeze is heterogeneous, and management to prevent attacks should be tailored for each child. We have biomarkers to identify children who are most likely to have steroid responsive wheezing. However, evidence-based biomarkers and treatments for children with non-allergic, non-eosinophilic recurrent wheezing remain a significant unmet need.

IMPACT STATEMENT: Following the recent publication of a European Respiratory Society research statement, this review provides an update and overview of the approach to diagnosing and managing recurrent preschool wheezing. The increasing importance of using biomarkers to help guide treatments and to achieve a personalized therapeutic approach, together with current gaps in knowledge are explored.

KEY WORDS

Preschool; asthma; biomarkers; wheeze; management.

INTRODUCTION

Preschool children account for most childhood hospitalizations for acute wheeze exacerbations. Although up to 50% of children may have one episode of wheezing by their 6th birthday, only approximately 30% of those will go on to have recurrent episodes of wheezing. Recurrent symptoms, frequent exacerbations and hospital admissions in preschoolers account for one-third of healthcare costs for childhood asthma. Recurrent wheeze attacks and poor symptom control, if not effectively treated, may contribute to lower lung function trajectory to adulthood (1) and increased mortality and morbidity later in life from both respiratory and cardiovascular conditions (2).

Given the significant morbidity that results from recurrent preschool wheezing, we need a step-change in our approach to diagnosis and management to prevent attacks of preschool wheeze. Current treatment strategies tend to adopt a 'one size fits all' approach, which assumes all children with preschool wheeze will respond similarly to the same treatment, which is maintenance ICS. However, we have good evidence that preschool wheeze is heterogeneous, and not all children will respond to ICS. We therefore need to alter our approach and introduce tailored therapy by identifying those children most likely to respond to ICS and considering alternative treatments for children who are unlikely to respond. This review will summarize a practical approach to the diagnosis and management of preschool wheeze. The focus is on optimal management to prevent attacks, emphasizing the importance of both accurate clinical phenotyping and the need to investigations to identify objective biomarkers of likely treatment response.

DEFINITION OF PRESCHOOL WHEEZING

It is important to have a clear definition for preschool wheezing so that an accurate diagnosis can be made. A recent European Respiratory Society (ERS) Task Force has proposed three key criteria that should be included in the definition to ensure a consistent approach: 1. age-range 0-6 years, 2. objective confirmation of wheeze, 3. recurrent episodes of wheeze (more than one episode ever) (3).

This avoids confusion with bronchiolitis (which is often the first episode in the first few months of life). If the child then develops recurrent episodes of wheezing, this would be termed recurrent preschool wheezing. This definition allows the inclusion of children who are under a year old and ensures that wheezing is the main symptom, to minimize misdiagnosis.

CLINICAL PRESENTATION OF PRESCHOOL WHEEZE AND DIFFERENTIAL DIAGNOSIS

Before progressing to any treatments, it is essential that objective confirmation of wheezing is made (3). This may mean the child needs to be seen and examined when acutely unwell, with documentation of wheezing. If the child is being seen in a clinic setting, it is helpful to ask parents to record the child's breathing when they are unwell, or to show them a video of examples of wheezing (4), or a sound clip of a child with wheezing.

Once wheezing is confirmed, the next step is to be certain that any alternative diagnosis, that may also result in wheezing with other associated signs and symptoms, has been actively considered and excluded. A very careful and thorough history and examination are essential to ensure the diagnosis is correct. Clinical features from history or examination that should raise concern and suggest an alternative diagnosis are highlighted in **Tables 1** and **2**.

If, after a thorough history and examination, it is apparent that the child is having recurrent episodes of wheezing that are not explained by an alternative diagnosis, then an approach to management can be considered.

IS IT PRESCHOOL WHEEZE OR PRESCHOOL ASTHMA?

The specific diagnostic label that is used for a child aged under 6 years who is having recurrent episodes of confirmed wheezing, as either recurrent preschool wheeze or preschool asthma does not matter. The critical point is to understand and identify any treatable traits that are associated with the child's episodes. The Lancet commission, which brought together international experts in both adult and childhood airways diseases, has recommended the term 'asthma' should only be used as a descriptive label for a collection of symptoms, with no assumptions about underlying pathophysiology (5). The symptoms include wheezing, breathlessness, difficulty in breathing and/or cough. So, any patient that is having episodes that include this constellation of symptoms

***Table 1**. Unusual findings from history and examination that should alert to an alternative diagnosis.*

History	Examination
Symptoms present from birth/unexplained neonatal respiratory distress	Nasal polyps
Excessive vomiting or posseting	Stridor
Persistent wet or productive cough	Abnormal voice/cry
Family history of unusual chest disease	Failure to thrive
Failure to respond to conventional treatment with inhaled corticosteroids (400 mcg/day budesonide or equivalent)	Finger clubbing
Persistent/unremitting symptoms at all times	Abnormal chest shape or deformity/ recession when well
Parental anxiety	
Sudden onset, having previously been completely well	

***Table 2**. Differential diagnoses for recurrent preschool wheezing episodes.*

Tracheo/bronchomalacia
Developmental anomalies (vascular ring)
Cystic fibrosis/chronic suppurative lung disease
Recurrent aspiration – Gastroesophageal reflux/dysphagia and swallowing disorders
Chronic lung disease of prematurity
Foreign body
Immunodeficiency

may be labelled as ‘preschool wheeze’ or ‘preschool asthma’. However, what matters most is to then confirm the type of asthma or wheezing the child has.

The fundamental physiological abnormality seen in asthma is the presence of airflow obstruction, which is reversible after bronchodilator. This can be objectively measured in school-age children using spirometry and is now recommended as part of the diagnostic algorithm for school-age asthma in multiple international guidelines (6). However, for preschool children, assessments of lung function are a challenge, and are mainly undertaken in research settings (7). However, challenges around testing should not prevent attempts to confirm bronchodilator reversibility. The most practical way of doing this is to clinically assess a child for wheezing and respiratory distress before and after bronchodilator when they are acutely unwell. A documented clinical response helps to confirm the child has evidence of reversible airflow obstruction.

However, the presence of bronchodilator reversibility alone is not sufficient to guide management in this age group. It helps to confirm the diagnosis of recurrent preschool wheeze and shows a child will respond to bronchodilators when they are acutely unwell but does not help to decide what type of preschool wheeze is present, and whether the child may benefit from maintenance ICS to prevent future acute episodes. The most common pathological abnormality that is seen in most school-age children with asthma is airway inflammation, which is predominantly driven by type-2 immunity and characterized by eosinophilia (8). Most of the school-age asthma is allergic and therefore associated with eosinophilia. Maintenance ICSs are effective in school-age because they dampen airway eosinophilia. However, in contrast, it is very important to remember that preschool wheeze/asthma is not always allergic or associated with type-2 immunity. Approximately 40% of all recurrent preschool wheezers have evidence of aeroallergen sensitization, and airway eosinophilia, thus making them likely to be steroid responsive. However, a significant proportion of children with preschool wheeze do not have any evidence of allergic sensitization and may not have type-2 driven disease.

PRESCHOOL WHEEZE IS HETEROGENEOUS

Before making decisions about appropriate maintenance therapy to prevent attacks for children with preschool wheeze, it is important to use information from history, examination and to undertake additional investigations to define the type of preschool wheeze a child has.

It has been proposed, from expert consensus, that a child's symptom pattern may help to determine response to ICS (9). It is suggested that children who only wheeze during acute episodes precipitated by upper respiratory infections, may be less likely to respond to maintenance ICS, while those who wheeze during and between episodes will respond to ICS better. However, this includes an assumption that the phenotypes determined by symptom pattern alone will remain stable, which we know is not the case (10). It also includes an assumption that phenotype determined by symptom pattern relates to airway inflammation and pathology, which is also not true (11). This can be illustrated by the Case descriptions summarized in **Box 1** and **2** below.

As can be seen, both patients have been prescribed maintenance ICS to try to prevent recurrent episodes of wheeze, and despite this, both continue to have severe episodes requiring hospitalizations and intravenous therapy. So how can we decide which child is on the correct treatment? There are several differences between the cases that can be highlighted from history. The child in Case 1 only ever has episodes with an upper respiratory infection, she is completely well in between. She also does not have any personal history of atopy or allergic disease, but she does have a family history of atopy and asthma. In contrast, Case 2 has symptoms that are triggered by exercise and exposure to cats, in addition to when she has an upper respiratory infection, she also has eczema and food allergies. If we now define the type of preschool wheeze for each case from history, we can say:

– Case 1: non-atopic with recurrent infection (viral) induced wheeze.
– Case 2: atopic with recurrent (persistent) preschool wheeze with food allergies and eczema.

A good history has helped to define the type of wheezing the two patients have and suggests Case 1 may not have type-2, eosinophilic airways disease and Case 2 is more likely to have allergic, type-2, eosinophilic airways disease. But Case 1 does have a family history of atopy and asthma, and they both continue to have severe episodes, so how can we be more certain about the best approach to management?

Box 1. Case 1.

- 2-year-old Caucasian girl
- Recurrent episodes of breathlessness/wheezing
- Always at the onset of a cold, well in between
- 10 acute admissions since 14 months old
- Worst episode needed Optiflow and iv MgSO4
- No eczema/no food allergies
- Father: asthma, eczema, hay fever, peanut allergy
- Prescribed Beclometasone 100 mcg 2 puffs bd and prn salbutamol

Box 2. Case 2.

- 3-year-old Afro-Caribbean girl
- Recurrent wheeze episodes since first year of life triggered by viral infections
- 4 acute attacks in the last 6 months needing iv MgSO4, oxygen
- Symptoms triggered by exercise and exposure to cats
- Food allergies: egg, fish, cashews, sesame
- Eczema
- Prescribed Seretide 125/25 mcg 1 puff bd and prn salbutamol

OBJECTIVE TESTS TO HELP DEFINE THE TYPE OF PRESCHOOL WHEEZE

Although it is very likely that Case 2 above has allergic, eosinophilic preschool wheeze. It is becoming apparent that investigations are helpful, in addition to a good history, to identify children who are differential responders to maintenance ICS.

One of the first studies to demonstrate the utility of objective tests in preschool children to identify differential responders to daily ICS was the Individualized Therapy for Asthma in Toddlers, 'INFANT' study, which was a multicenter, randomized, double-blind, clinical trial in children aged 12 to 59 months with asthma, who needed daily controller therapy (12). Children had 3 crossover periods with daily ICS, daily leukotriene receptor antagonists, and as-needed ICS. The primary outcome was

differential response to medication based on a composite measure of asthma control. The findings showed that 25% of preschool wheezers showed no response to daily ICS, and the others showed a spectrum of response (12). Good response to daily ICS was demonstrated in children with either aeroallergen sensitization or blood eosinophils ≥300 cells/μL. The best response was seen in those who were positive for both biomarkers. This showed objective phenotyping with aeroallergen sensitization and blood eosinophil counts is useful for guiding treatment selection and identifies children with frequent exacerbations for whom treatment with a daily ICS is beneficial.

In the Cases above, these tests would be helpful to understand whether both patients should continue maintenance ICS.

BASICS OF ASTHMA MANAGEMENT ARE AS IMPORTANT FOR PRESCHOOL AS SCHOOL-AGE CHILDREN

The interesting point about the cases is that the child with the allergic, eosinophilic phenotype (Case 2) continued to have severe exacerbations despite being prescribed high-dose maintenance treatment. When this occurs, it is essential to remember that ensuring the basics of management have been optimized for children with preschool wheeze/asthma just as we do for school-age children with difficult-to-treat asthma (13). If the phenotype is confirmed as being steroid responsive, then the next step should not be automatic therapy escalation, but should include assessment of inhaler technique, parent/caregiver education to ensure they understand the prescription and why the ICS administration is important and obtaining evidence of adherence to the ICS. Simply relying on parental reports about adherence is not sufficient, as up to 50% of wheezers, even those with severe disease, have been shown to have sub-optimal adherence when assessed using electronic monitors (14). In Case 2, when the basics were checked, it became apparent that the child was not receiving the ICS regularly, and this was the main explanation for the repeated and severe presentations.

Other factors that are critical for all preschool children with recurrent wheeze that will contribute to exacerbations and poor symptom control, include avoidance of exposure to cigarette smoke and vaping (15). In addition, for those who have allergic sensitization, avoidance of the allergens to which they are sensitized is as important in this age group as in school-age children. A latent class analysis of 5 clinical trials that investigated efficacy of ICS in preschool wheeze showed although each trial had shown benefit from ICS compared to placebo in all children as a group, in research setting of high adherences, daily ICS did not affect exacerbations rates compared to placebo in children with minimal sensitization and those with tobacco smoke exposure (16).

MANAGEMENT OF PRESCHOOL WHEEZE IN NON-ALLERGIC CHILDREN WITHOUT PERIPHERAL EOSINOPHILIA

Although we know how to identify children who are most likely to respond to daily ICS treatment, the biggest remaining challenge is how we should manage preschool children with recurrent wheezing who do not have allergic sensitization or evidence of elevated blood eosinophils. It is becoming increasingly apparent that for some children with recurrent wheezing, lower airway infection may be the main cause of their symptoms. Unbiased analysis of lower airway inflammation in children aged 1-16 years with severe wheezing and asthma has shown two distinct lower airway inflammation clusters in children under 5 years. A cluster with predominant allergic sensitization which was steroid responsive and a second cluster that was predominantly neutrophilic and steroid refractory (17). In a further study which assessed lower airway inflammation and infection in recurrent severe wheeze, four clusters were identified, an atopic cluster with associated blood eosinophilia, a non-atopic cluster with low infection rate and high use of ICS, a non-atopic cluster with high rates of both bacterial and viral infection with an associated lower airway neutrophilia and a non-atopic cluster with low infection rate and no use of ICSs (11). This suggests a sub-group of recurrent wheezers, who are non-atopic, have lower airway bacterial infection that may respond to targeted antibiotics. A further cluster analysis of preschool wheezers who had “treatment-refractory” symptoms, not responsive to ICS, has shown 4 clusters: airway malacia, gastroesophageal reflux, lower airway rhinovirus predominant, and type-2high inflammation (18).

Evidence for both viral and bacterial infection and neutrophils playing a role in recurrent wheezing is becom-

ing increasingly apparent. Very consistently, three bacteria are most commonly cultured from the lower airways of preschool wheezers. These are *Hemophilus influenzae*, *Streptococcus pneumoniae* and *Moraxella catarrhalis* (11,19). Importantly, the bacteria are identified when children are clinically stable and well, suggesting the picture of persistent bacterial bronchitis which may precipitate acute attacks upon acute exposure to viruses or allergens. An observational study of severe, recurrent wheeze has shown prolonged treatment with targeted antibiotics for between 2-16 weeks in preschool children who had lower airway bacterial infection resulted in fewer episodes of dyspnea and fewer hospitalizations in the subsequent year (19). Therefore, in children without allergic sensitization, or blood eosinophilia, bacterial infection may be an important factor that contributes to recurrent wheeze, however, clinical trials of efficacy are currently lacking.

Another approach that has shown promise, specifically for preschool children with infection induced wheezing and no allergic sensitization, is the use of mixed bacterial lysates. These are orally or sublingually administered lysates of mixed respiratory pathogenic bacteria which have, to date, been used in many parts of Europe and Southeast Asia to prevent recurrent respiratory tract infections (20). Although we are unaware of the precise mechanism of action of the bacterial lysate compounds, it has been proposed from animal studies that there may be two mechanisms of action. The first is a skewing of early-life immune responses away from predominantly type-2 responses, towards stronger type1 responses, needed to fight infections. The second is the concept of trained immunity (21, 22). Numerous meta-analyses have been undertaken to understand their efficacy for the prevention of both respiratory tract infections and wheeze episodes in young children, however, the data remain conflicting, and currently no strong recommendations can be made from the existing literature, because of the high heterogeneity randomized trials and systematic reviews (23). A recently randomized, placebo-controlled trial including 120 children aged less than 3 years, with at least 3 wheezing episodes in the previous year, in which children with aeroallergen sensitization were excluded, assessed the efficacy of sublingual mixed bacterial lysates given for 6 months on the primary outcome of wheeze exacerbations at 1 year (24). There were significantly fewer exacerbations, fewer symptoms and better medication scores for 1 year in the children who received the mixed bacterial lysates. This provides very encouraging evidence that an intervention, given for non-allergic preschool wheezing has shown benefit not just for the duration of the treatment, but a sustained benefit up to 6 months after the treatment was stopped. This is even more encouraging than the data we have for ICS, in terms of sustained efficacy of an intervention after treatment has stopped, and potential for preventing progression of disease. Although 6 months after the intervention is a relatively short period, especially because of the potential influence of a seasonal effect in these children, where the Spring and Summer months are often a period of 'natural resolution' this does show encouraging results.

There are several clinical trials that are currently ongoing to confirm these preliminary findings of efficacy of bacterial lysates for non-allergic preschool wheeze. The Oral Bacterial Extract for the prevention of Wheezing Lower Respiratory Tract Illnesses (ORBEX) trial is currently ongoing. This is a randomized trial investigating the impact of an oral mixed bacterial lysate compound, Bronchovaxom®, on the prevention of wheezing lower respiratory illnesses. Children aged 5-17 months, with at least 1 parent with asthma, or the child with eczema, were randomized to receive Bronchovaxom® or placebo for 2 years, and the primary outcome to be assessed is wheezing 3 years later. Robust clinical evidence is needed before recommendations can be made for either the use of bacterial lysates or antibiotics to prevent attacks of preschool wheeze in non-allergic children who do not have blood eosinophilia and are unlikely to respond to ICS.

SUMMARY

Recurrent wheezing episodes in preschool children are among the most common reasons for unscheduled healthcare attendance and hospitalizations globally. Moreover, longitudinal studies show children with frequent and severe attacks are at risk of developing low lung function by school-age, which tracks a low trajectory to adulthood. The need to reduce acute episodes and disease burden is an urgent priority that requires effective interventions. After confirming recurrent wheeze objectively, and excluding alternative diagnoses, it is important to look for objective markers of response to ICS, such

as aeroallergen sensitization and blood eosinophils. The current gap in knowledge centers is specifically for children who do not have a pathological phenotype that is steroid responsive. We have increasing evidence of potential therapies that can be used, but robust clinical trial evidence for the efficacy of antibiotics and bacterial lysates in children who do not have the steroid-responsive phenotype is needed. It is critical to ensure interventional studies are designed using precision medicine, such that treatments are targeted at the individual child's risk factors and disease pathophysiology. This means we need to consider innovative trial designs and analysis approaches that can be used to understand the efficacy of interventions in relatively small populations.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests

The Author has declared no conflict of interests.

Financial support

There was no institutional or private funding for this article.

Author contributions

SS is the only Author of the article and wrote it.

Ethical approval

Human studies and subjects

N/A.

Data sharing and data accessibility

The Author confirms that the data are available within the article.

Publication ethics

Plagiarism

Author declares no potentially overlapping publications with the content of this manuscript and all original studies are cited as appropriate.

Data falsification and fabrication

All the data corresponds to the real.

REFERENCES

1. Berry CE, Billheimer D, Jenkins IC, Lu ZJ, Stern DA, Gerald LB, et al. A Distinct Low Lung Function Trajectory from Childhood to the Fourth Decade of Life. Am J Respir Crit Care Med. 2016;194(5):607-12. doi: 10.1164/rccm.201604-0753OC.
2. Agustí A, Noell G, Brugada J, Faner R. Lung function in early adulthood and health in later life: a transgenerational cohort analysis. Lancet Respir Med. 2017;5(12):935-45. doi: 10.1016/S2213-2600(17)30434-4.
3. Makrinioti H, Fainardi V, Bonnelykke K, Custovic A, Cicutto L, Coleman C, et al. European Respiratory Society statement on preschool wheezing disorders: updated definitions, knowledge gaps and proposed future research directions. Eur Respir J. 2024;64(3):2400624. doi: 10.1183/13993003.00624-2024.
4. Saglani S, McKenzie SA, Bush A, Payne DNR. A video questionnaire identifies upper airway abnormalities in preschool children with reported wheeze. Arch Dis Child. 2005;90(9):961-4. doi: 10.1136/adc.2004.071134.
5. Pavord ID, Beasley R, Agusti A, Anderson GP, Bel E, Brusselle G, et al. After asthma: redefining airways diseases. Lancet. 2018;391(10118):350-400. doi: 10.1016/S0140-6736(17)30879-6.
6. Roberts G, Valovirta E, Halken S, Eng PA, Mäkelä MJ, Lødrup Carlsen KC, et al. Diagnosing new-onset asthma in a paediatric clinical trial setting in school-age children. Front Allergy. 2024;5:1418922. doi: 10.3389/falgy.2024.1418922.
7. Wong MD, Condon K, Robinson PD, Suresh S, Zahir SF, Sly PD, et al. Assessment of bronchodilator response in preschoolers: A systematic review. Pediatr Pulmonol. 2024. doi: 10.1002/ppul.27112.
8. Papadopoulos NG, Bacharier LB, Jackson DJ, Deschildre A, Phipatanakul W, Szefler SJ, et al. Type 2 Inflammation and Asthma in Children: A Narrative Review. J Allergy Clin Immunol Pract. 2024;12(9):2310-24. doi: 10.1016/j.jaip.2024.06.010.
9. Brand PLP, Baraldi E, Bisgaard H, Boner AL, Castro-Rodriguez JA, Custovic A, et al. Definition, assessment and treatment of wheezing disorders in preschool children: an evidence-based approach. Eur Respir J. 2008;32(4):1096-110. doi: 10.1183/09031936.00002108.
10. van Wonderen KE, Geskus RB, van Aalderen WMC, Mohrs J, Bindels PJE, van der Mark LB, et al. Stability and predictiveness of multiple trigger and episodic viral wheeze in preschoolers. Clin Exp Allergy. 2016;46(6):837-47. doi: 10.1111/cea.12660.
11. Robinson PFM, Fontanella S, Ananth S, Martin Alonso A, Cook J, Kaya-de Vries D, et al. Recurrent Severe Preschool Wheeze: From Prespecified Diagnostic Labels to Underlying Endotypes. Am J Respir Crit Care Med. 2021;204(5):523-35. doi: 10.1164/rccm.202009-3696OC.
12. Fitzpatrick AM, Jackson DJ, Mauger DT, Boehmer SJ, Phipatanakul W, Sheehan WJ, et al. Individualized therapy for persistent asthma in young children. J Allergy Clin Immunol. 2016;138(6):1608-1618.e12. doi: 10.1016/j.jaci.2016.09.028.
13. Scotney E, Burchett S, Goddard T, Saglani S. Pediatric problematic severe asthma: Recent advances in man-

agement. Pediatr Allergy Immunol. 2021;32(7):1405-15. doi: 10.1111/pai.13543.

14. Bingham Y, Sanghani N, Cook J, Hall P, Jamalzadeh A, Moore-Crouch R, et al. Electronic adherence monitoring identifies severe preschool wheezers who are steroid responsive. Pediatr Pulmonol. 2020;55(9):2254-60. doi: 10.1002/ppul.24943.
15. O'Dowd A. Vaping is causing rise in asthma and wheezing episodes in children, MPs hear. BMJ. 2023;381:1503. doi: 10.1136/bmj.p1503.
16. Fitzpatrick AM, Bacharier LB, Guilbert TW, Jackson DJ, Szefler SJ, Beigelman A, et al. Phenotypes of Recurrent Wheezing in Preschool Children: Identification by Latent Class Analysis and Utility in Prediction of Future Exacerbation. J Allergy Clin Immunol Pract. 2019;7(3):915-924.e7. doi: 10.1016/j.jaip.2018.09.016.
17. Guiddir T, Saint-Pierre P, Purenne-Denis E, Lambert N, Laoudi Y, Couderc R, et al. Neutrophilic Steroid-Refractory Recurrent Wheeze and Eosinophilic Steroid-Refractory Asthma in Children. J Allergy Clin Immunol Pract. 2017;5(5):1351-1361.e2. doi: 10.1016/j.jaip.2017.02.003.
18. Teague WG, Lawrence MG, Williams S, Garrod AS, Froh D, Early SV, et al. Novel Treatment-Refractory Preschool Wheeze Phenotypes Identified by Cluster Analysis of Lung Lavage Constituents. J Allergy Clin Immunol Pract. 2021;9(7):2792-801.e4. doi: 10.1016/j.jaip.2021.03.059.
19. Schwerk N, Brinkmann F, Soudah B, Kabesch M, Hansen G. Wheeze in preschool age is associated with pulmonary bacterial infection and resolves after antibiotic therapy. PLoS One. 2011;6(11):e27913. doi: 10.1371/journal.pone.0027913.
20. Nelson HS. The return of the mixed respiratory bacterial vaccine. Allergy Asthma Proc. 2022;43(6):501-8. doi: 10.2500/aap.2022.43.220053.
21. von Mutius E, Smits HH. Primary prevention of asthma: from risk and protective factors to targeted strategies for prevention. Lancet. 2020;396(10254):854-66. doi: 10.1016/S0140-6736(20)31861-4.
22. Michael AN, Pivniouk O, Ezeh PC, Banskar S, Hahn S, DeVries A, et al. Administration of a bacterial lysate to the airway compartment is sufficient to inhibit allergen-induced lung eosinophilia in germ-free mice. J Leukoc Biol. 2024;116(2):392-7. doi: 10.1093/jleuko/qiae047.
23. Castro-Rodriguez JA, Turi KN, Forno E. A critical analysis of the effect of OM-85 for the prevention of recurrent respiratory tract infections or wheezing/asthma from systematic reviews with meta-analysis. Pediatr Allergy Immunol. 2024;35(7):e14186. doi: 10.1111/pai.14186.
24. Nieto A, Mazón A, Nieto M, Calderón R, Calaforra S, Selva B, et al. Bacterial Mucosal Immunotherapy with MV130 Prevents Recurrent Wheezing in Children: A Randomized, Double-Blind, Placebo-controlled Clinical Trial. Am J Respir Crit Care Med. 2021;204(4):462-72. doi: 10.1164/rccm.202003-0520OC.

PEDIATR RESPIR J Vol. 3(1), 36-44, 2025

REVIEW

Effect of music therapy in preterm infants

Francesco **Barbato** [1, 2], Angelo **Barbato** [3, *]

*** Correspondence to:**

angelo.barbato@unipd.it. ORCID: https://orcid.org/0000-0002-6762-3822

Doi

10.56164/PediatrRespirJ.2024.64

[1] Bachelor's Degree of Art in Modern Music, Middlesex University London, London, United Kingdom

[2] Giovanni Ferrari School of Music Therapy, Padua, Italy

[3] Professor of Pediatrics and Pediatric Pulmonologist, Scholar of University of Padua, Padua, Italy

ABSTRACT

Music therapy has been recognized as a supportive intervention for the neurosensory and cognitive development of both full-term and preterm newborns. The objective of this article is to provide an updated overview of the use of music therapy in preterm infants and to discuss the potential neurological pathways through which this technique may affect the vital parameters of these infants.

Studies were selected based on outcomes measured with electromedical equipment. A total of 12 studies were identified: 1 cross-sectional survey, 6 randomized controlled trials (RCTs), and 5 reviews. The 5 reviews, which analyzed 232 trials, consistently reported positive effects of music therapy on the basic vital functions of infants, such as heart rate (HR), respiratory rate (RR), oxygen saturation, feeding, and length of hospital stay.

The neural and humoral interactions stimulated by music therapy in infants may explain the improvement in their basic vital functions during and after sessions, resulting from the relaxing music programmed by the music therapist.

IMPACT STATEMENT: Music therapy positively influences preterm infants' vital functions, including heart rate, respiratory rate, and oxygen saturation, by stimulating neural and humoral pathways. This article highlights its potential as an effective intervention for supporting neonatal development and reducing hospital stays.

KEY WORDS

Pre-term infant; neonatal intensive care unit; music-therapy.

INTRODUCTION

'Preterm birth' refers to infants born before 37 weeks of gestation and is categorized by gestational age into three subgroups: extremely preterm (less than 28 weeks), very preterm (28 to less than 32 weeks), and moderate to late preterm (32 to 37 weeks). In 2020, an estimated 13.4 million babies were born prematurely, representing more than 1 in 10 births globally (1). According to a recent UNICEF report, 10.6% of all births are preterm, leading to approximately 3.1 million deaths worldwide (2). Many of the survivors face lifelong challenges, including cognitive disabilities, as well as visual and hearing impairments. In developed countries, preterm births are relatively common, partly due to declining birth rates and the difficulties some couples encounter with conception. In some of these countries preterm birth rates have been reported to range from 5% to 7% of live births, but these rates are likely underestimates and appear to be increasing (3).

Premature infants, with underdeveloped vascular and neurological systems, are abruptly transitioned from the protective environment of the womb into the unprotected world of the Neonatal Intensive Care Unit (NICU). They are often unable to manage many of the stimuli essential for sustaining life. This environment exposes them to bright lights, loud sounds, physical touch, and other unfamiliar stressors (4). Each of these stimuli can be perceived as a source of stress by the immature nervous system of a premature infant, potentially leading to impaired oxygenation, altered blood flow, abnormal heart rate, and adverse behavioral responses (5). Additionally, damage to the auditory system can result from harmful stimuli in the environment, such as ototoxic medications and excessive noise (6). Cochlear hair cells can lose their sensitivity to pitch when exposed to background sound levels of 60 decibels (dB) or higher (7). To help prevent hearing damage or hearing loss in infants hospitalized in the NICU, the American Academy of Pediatrics (AAP) has recommended keeping noise levels below 45 dB. However, a quiet room typically measures around 47 dB, and the advanced technological equipment used to care for premature infants in the NICU often exceeds these guidelines. The elevated noise levels associated with routine care for preterm infants may put them at risk of auditory system damage and instability in their basic vital functions (8).

Music is a combination of sounds organized according to melody, harmony, and rhythm, which are perceived and processed by the human brain (9). Music therapy involves the use of sound and music with the aim of achieving specific therapeutic goals (*e.g.*, reducing stress) that contribute to the improvement of the patient's clinical condition (10). Music therapy has been recognized as beneficial for the neurosensory and cognitive development of both full-term and preterm newborns (11). Studies on its effects are often conducted on hospitalized children, making preterm infants particularly suitable for such analysis, as they remain monitored in Grow Care Units or in NICU for extended periods − ranging from weeks to months − until they reach the vital parameters needed to survive without medical assistance (12).

The aim of this study is to provide an updated overview of the use of music therapy in preterm infants and to explore the potential neurological pathways involved in its influence on their vital parameters.

METHODS

PubMed, Embase, and Cochrane databases were utilized to identify the most relevant studies conducted over the past 20 years (from 2004 to 2024). Additional scientific papers were included as they appeared in the selected articles. The selection of papers for analysis followed the PRISMA guidelines (13).

For the search we utilized the following keywords: 'preterm infants', 'music therapy', and 'intensive care unit', focusing on objective data related to heart rate (HR), respiratory rate (RR), and oxygen saturation, outcomes objectively measurable with electro-medical equipment. Papers addressing topics such as parent-infant bonding, effects on mothers, pain, and long-term developmental outcomes were excluded

The selected articles met the following criteria:

– published in peer-reviewed scientific medical journals in English;
– article types: randomized controlled trials, reviews, and systematic reviews.

The analysis presented in this paper is narrative, and no meta-analysis was conducted.

RESULTS

A total of 42 papers initially met the selection criteria. An additional 2 papers were included from citations found within the reports, bringing the total to 44. Of these, 30 reports were excluded from the selection for not meeting the inclusion criteria, or because their outcomes could not be objectively measured with electro-medical equipment. Additionally, 2 papers were deemed irrelevant by the two reviewers (FB and AB).

Thus, 12 studies were selected for this review. The selection criteria are detailed in the flowchart (**Figure 1**), following PRISMA guidelines (13), and the studies are listed in **Table 1** (14-25) based on publication date. Notably, the systematic review by Costa S.V. *et al*., which included 39 studies, and the paper by Mohan A. *et al*., which analyzed results from 12 systematic reviews, 14 randomized trials, and 7 observational studies, led to the exclusion of numerous duplicates from the initial selection.

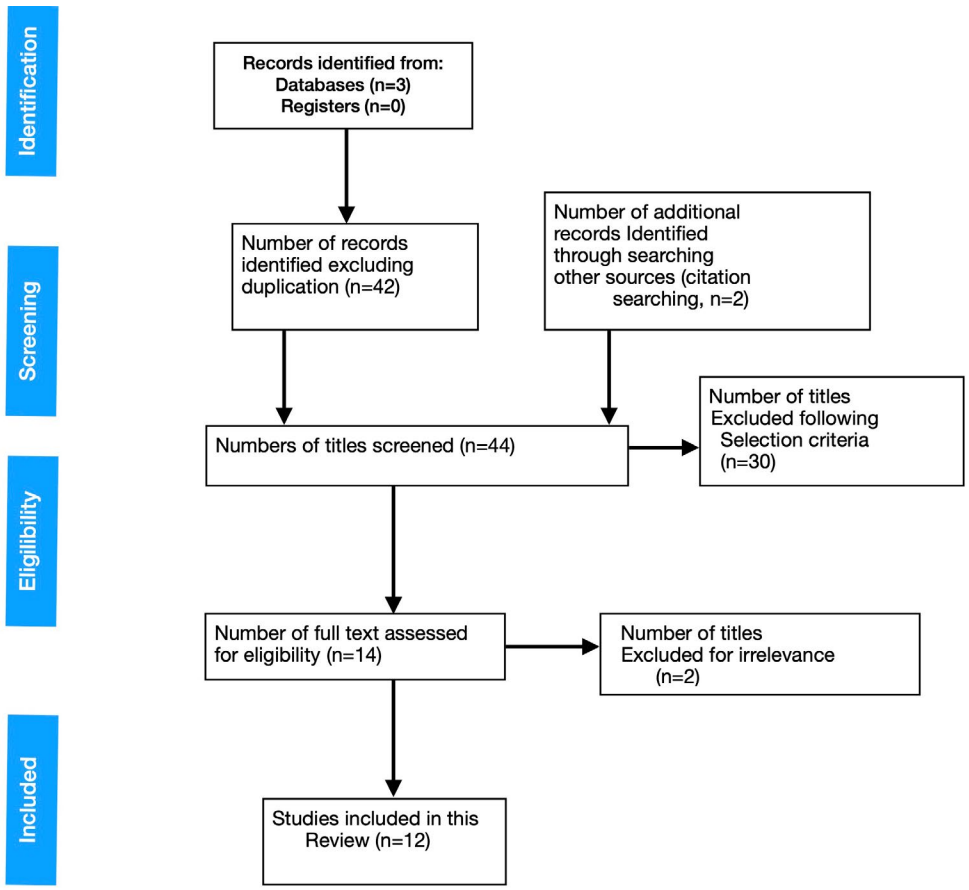

Figure 1. *PRISMA flow chart for papers' inclusion.*

The papers studied in our review included 1 cross-sectional survey, 6 randomized controlled trials (RCTs), and 5 reviews. The cross-sectional survey (1) involved a NICU staff with the administration of questionnaires evaluating the effects of music on infants and staff members. The feedback from medical and nursing staff indicated a highly positive impact of music therapy on the small patients.

The total number of preterm infants studied in the 6 randomized controlled trials (RCTs) is 268. Among these, study 5 examines cardiac function through continuous ECG to assess heart rate variability. This study suggests that music therapy, influencing subcortical and cortical brain stimulation, may have a positive effect on cardiac function in preterm infants.

Study 12 evaluates the impact of music therapy on preterm infants during their hospitalization by assessing the integrity of the brain's white matter through MRI. Results indicate a positive increase in white matter integrity in infants who received music therapy compared to controls.

Among the remaining 4 RCTs, study 2 reports no significant changes in basic physiological functions such as heart rate (HR), respiratory rate (RR), and oxygen saturation between preterm infants receiving music therapy and those in the control group. Conversely, studies 3, 4, and 7 demonstrate improvements of these parameters: study 3 and 4 show enhanced oxygen saturation in infants during the music therapy sessions, particularly when the mother is present; instead study 7 indicates a reduction in HR and RR, along with increased oxygen saturation during sleep over the course of music therapy. Study 8 assesses EEG variations during sleep in infants undergoing music therapy, revealing improvements in EEG patterns during sleep.

Table 1. *RCT, Systematic Reviews and a survey included in this study.*

N. (ref.)	First Author	Year	Type of paper	Number of participants	Type of music (dB)	Duration of music therapy (dB when reported)	Outcomes
1.-(14)	Kemper K.	2004	Cross sectional survey of NICU staff	37 physicians and 150 nurses for a total of 187 questionnaires	Recorded music (but preferred live music)	Winter 2023: Music continuously played in NICU	Staff reported in preterm infants <Stress <Crying >Sleep
2.-(15)	Alipour Z.	2013	RCT	90 premature infants: G1-30 lullabies G2-30 silence CG-30 control	Lullabies played by headphones	Each session of 20 minutes lullaby treatment (50-60 dB) per day or silence	No effects on HR, RR, oxygen saturation immediately and 10 minutes after each session
3.-(16)	Dearn T.	2014	RCT	22 preterm infants born at >28 wg and enrolled at >32 wg (+/- their mothers): 10 Study Group = SG 12 Control Group = CG	Listen to recorded lullabies and ambient sound	6 minutes of ambient sound alternating with 2x6 minutes recorded lullaby music	SG > oxygen saturation (pre-term with mother present) than CG
4.-(17)	O' Toole A.	2017	Review	N. 12 papers in the previous 5 years in pre-term infants	Different types of music, and parental involvement	Receptive MT	<HR <RR >Oxygen saturation >Feeding >Length of stay >Pain relief <Parental stress
5.-(18)	Hasegawa Y.	2020	RCT	N. 30 pre-term infants	Lullabies for a baby, delivered through a speaker in the incubator	Evaluation of HR variability before, during and after each intervention	LF and HF values decreased during the MT condition, but not LF/HF
6.-(19)	Mohan A.	2021	Systematic reviews and meta-analysis of RCTs	- 12 systematic reviews - 14 RCT - 7 observational studies	Different types of music (lullabies, recorded music, *et al*.)	Different musical approach	<HR <RR <Maternal anxiety >Feeding volume
7.-(20)	Kobus S.	2021	RCT	20 (<32 wg) Tot.: 307 MT sessions	Improvised singing / use of sansula instrument	2 individual music session per week (from 2 week of life until discharged).	<HR <RR >Oxygen saturation better during sleep
8.-(21)	Giordano V.	2021	RCT	64 (in 3 groups: G1 = live music G = 2 recorded music group G3 = control group	Live or recorded music	Amplitude-integrated EEG, 20 min after the appearance of the first quiet-sleep phase	Improvement within the first and second quiet-sleep epochs in G1 and G2
9.-(22)	Yue W.	2021	Systematic review of trials and meta-analysis	13 trials involving 1,093 participants	Music therapy with recorded music or music obtained with different techniques and/or instruments	Music sessions 1 or more times a week	<HR <RR >Oral feeding volume

→

N. (ref.)	First Author	Year	Type of paper	Number of participants	Type of music (dB)	Duration of music therapy (dB when reported)	Outcomes
10.-(23)	Costa V. S.	2022	Review	39 trials, 13 on analgesic effect and 26 evaluated the physiological and behavioral effects of music (977 participants).	Recorded music or lullaby, live music with instruments (harp) or lullaby and parents' song.	In 3 RCTs the MT session was single, in the other each MT session lasted from 10' to 60' 2-3 times a week, until discharge from the hospital (30-70 dB)	>Pain relief <HR <RR >Oxygen saturation >Weight gain and eating behavior
11.-(24)	Haslebeck F. B.	2023	Systematic review	25 trials, recruiting 1532 infants and 691 parents	Music and voice were calm, soft, in lullaby style, and the mother's voice live or recorded	Different musical approach	During MT session: - stable oxygen sat - stable RR - <HR during and post each MT session
12.-(25)	Dewan M. V.	2024	RCT	80 infants enrolled, 42 were eligible for diffusion tensor imaging analysis (MT22 = G1; ST20 = CG)	Live MT (LM)	LM was provided twice weekly from the second postnatal week onwards by a trained music therapist	>Effect of MT on white matter microstructures on cranial RMN scan, between G1 and CG

MT: Music Therapy; RCT: Randomized Controlled Trial; G 1-2: Groups of Patients; CG: Control Group; SG: Study Group; HR: Heart Rate; RR: Respiratory Rate; EEG: Electroencephalography; LF: Low Heart Frequency, HF: High Heart Frequency During Prolonged ECG.

The authors of the 5 reviews (4, 6, 9-11) listed in **Table 1**, studied 232 RCTs, all consistently reporting a positive effect of music therapy on the basic vital functions of infants. These reviews indicate improvements in HR, RR, oxygen saturation, feeding, and length of hospital stay in the preterm infants.

In the systemic review made by Mohan A. *et al*. (6), 13 RCT studies evaluating the analgesic effects of music are reported; however, these are not directly relevant to our survey.

Some reviews also document additional benefits of music therapy, including pain relief during medical procedures and reduction in parental stress. Review 11, a Cochrane review, included 25 trials recruiting 1532 infants and 691 parents. Within the trials, the music intervention varied widely in type, delivery, frequency, and duration. Music and voice were mainly characterized by calm, soft musical parameters in lullaby style, often integrating the song with mother's voice live or recorded. This review shows stable parameters in 1,532 preterm infants concerning RR and oxygen saturation, with a significant reduction in HR observed during and after each music session.

DISCUSSION

Our review demonstrates that music therapy seems to acquire the characteristics of a therapeutic practice to be used even in hospital settings for selected patients, such as preterm infants.

The studies we evaluated show increasing interest in this type of therapy, but they also demonstrate that patients are difficult to select in a homogeneous way, and numerous publications present biases that make them difficult to compare with others, involving patients with the same type of clinical condition. This makes it challenging to conduct meta-analyses in systematic literature reviews. Another aspect that significantly reduces the possibility of conducting research on large numbers of subjects is the lack of funding from pharmaceutical companies, which are not interested in this type of therapeutic procedure (26). However, with our review, we have highlighted that even though RCTs often involve small numbers of patients

and thus carry reduced scientific weight, systematic literature reviews which gather data from thousands of patients are able to demonstrate the scientific value of this therapy, which should therefore be encouraged and expanded. There are already some therapeutic protocols studied and published in literature, waiting only for adequate funding to be applied in pediatric hospital settings (27).

In the studies we analyzed, the music therapist was rarely involved in the study. The involvement of a music therapist is advisable to assess the choice of music and its method of delivery. In fact, music therapy is characterized by personally tailored music interventions initiated by a trained and qualified music therapist, which distinguishes music therapy from other music interventions, such as 'music medicine'.

Possible effect of music therapy on the nervous system of preterm infants

Neurophysiological research has also given a significant boost to music therapy by studying the neurological connections between various subcortical and cortical neuron nuclei that are stimulated through music, even in preterm infants.

Recent experimental studies have demonstrated how the auditory stimulus is transmitted from the outer hair cells and inner hair cells of the cochlea to the axons of the acoustic nerve, which convey the neural stimulus to the cochlear nuclei, both dorsal and ventral, at the level of the medulla oblongata. Other axons originating from the bulbar olivocochlear nuclei collect the auditory stimulus from the same cochlear cells. The MOC fibers (bundles of the medial olivocochlear system) carry electrical stimuli predominantly to the contralateral but also to the ipsilateral nuclei of the olivocochlear system, while the LOC fibers (bundles of the lateral olivocochlear system) carry them only to the ipsilateral nuclei of the same system, which is located below the fourth cerebral ventricle. The olivocochlear system sends and receives inputs (ascending through the brainstem, diencephalon, and brain) from neuronal axons of the lateral lemniscus nuclei and inferior colliculi, from the thalamic geniculate nuclei, from the auditory cortical center in the temporal lobe, from the prefrontal area, from the motor and sensory areas, from the visual area, and from the cerebellum. All these nerve cell nuclei present in various subcortical and cortical areas participate in the reception of the musical stimulus, as well as its tactile, sensory, visual, and motor processing (28).

At the level of the bilateral olivocortical nuclei in the brainstem, there are axons that send auditory-derived inputs to other cellular nuclei that control blood pH, form the respiratory center, and regulate the vasomotor center, which governs heart rate, vascular tone, blood pressure, and the systolic ejection force of the myocardium (28). Moreover, axons present in the inferior colliculus and thalamus release neurotransmitters that amplify or reduce axonal inputs, which also enter the bloodstream.

These complex nervous interactions, involving different areas of the central nervous system, may therefore explain the various functions that musical vibrations can have on preterm infants.

Role of neurotransmitters

One of the various pathogenetic mechanisms invoked to explain the beneficial effects of music therapy in neonates is the interaction of neurotransmitters, specifically the interaction between oxytocin and dopamine, and between oxytocin and cortisol (29). It is believed that music increases dopamine levels in the brain, which positively affects cognitive, psychological, and motor functions. Dopamine is produced by neurons in the basal nuclei that interact with neurons in the prefrontal cortex and other basal nuclei through four pathways of stimulus transmission: the 'meso-limbic pathway', the 'nigro-striatal pathway', the 'meso-cortical pathway' and the 'tubero-infundibular pathway'. The neurological stimulus induced by music particularly follows the 'meso-cortical pathway' of dopamine, which connects the 'ventral tegmental area' of the midbrain to the prefrontal cortex. This pathway is also activated in the regulation of emotions and feelings (30).

In these same areas, an interaction between cells that produce dopamine and those that produce oxytocin has been demonstrated, which together influence various social behaviors in humans, including sexual stimuli, mating behaviors, and mood swings. Some researchers define these neurological circuits as 'Circuits regulating pleasure and happiness' (31). On the other hand, music can significantly increase oxytocin levels and reduce salivary cortisol, as demonstrated by measuring salivary oxytocin concentrations in a group of subjects after lis-

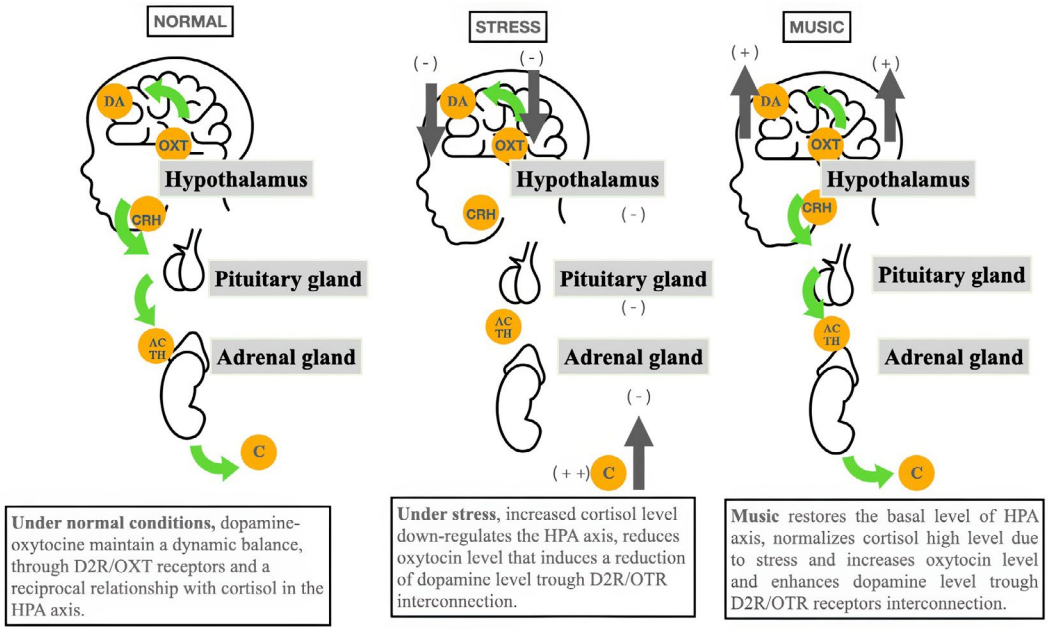

***Figure 2**. Music therapy and the hypothalamic-pituitary-adrenal (HPA) axis. Modified from: Dewan MV, et al. (25).*

DA: Dopamine; OXT: Oxytocin; HPA: Hypothalamus-Pituitary Gland-Adrenal Gland Axis; C: Cortisol; CHD: Corticotropin-Realising Hormon; ACTH: Adrenocorticotropic.

tening to slow-tempo relaxing music and fast-tempo exciting music (32). Other studies have shown a correlation between oxytocin production and a reduction in cortisol levels in subjects stimulated by music. When a subject is under stress, cortisol levels increase, which has a negative feedback mechanism on the hypothalamic-pituitary-adrenal axis, leading to a decrease in oxytocin levels. This would result in a down-regulation of dopamine through the interaction of D2R/OTR receptors (33, 34). However, musical stimulation reduces stress, normalizes cortisol levels, restores the dynamic balance of the hypothalamic-pituitary-adrenal axis, up-regulates oxytocin, and consequently increases dopamine levels through the interaction of D2R-OTR receptors (34-36) (**Figure 2**). Therefore, it is hypothesized that in children, music stimulates the nerve cells that produce these neurotransmitters, which are part of the happiness and pleasure circuit, affecting both the basal nuclei and various cortical areas, including the frontal cortical area. From here, pyramidal and extrapyramidal fibers originate, which can influence not only mood and social interactions but also the respiratory centers, heart rate, and the striated and smooth musculature of various organs and systems of the human body.

CONCLUSIONS

Neural and humoral interactions can explain the reduction in respiratory rate, heart rate, and the increase in oxygen saturation observed in preterm neonates during and after music therapy sessions, as an effect of the relaxing properties of music. In the studies we analyzed, lullaby music has so far proven to be the most commonly used. Also, this kind of music is not a drug, but it can induce the production of mediators similarly to a drug and can be used to properly maintain the vital functions in pre-term infants for its antistress effect. Recently it has been demonstrated also in subjects over 18 years of age that music therapy has an overall medium-to-large effect on stress-related outcomes (37). The numerous potential benefits and the absence of known side effects reported in newborns, suitable for this therapy and clinically stable, are a strong argument in favor of its use in NICU and Grow Care Units.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests

The Authors have declared no conflict of interests.

Financial support

There was no institutional or private funding for this article.

Authors' contributions

FB and AB wrote the manuscript.

Ethical approval

Human studies and subjects

N/A.

Data sharing and data accessibility

Data are available upon motivated request to the Corresponding Author.

Publication ethics

Plagiarism

Authors declare no potentially overlapping publications with the content of this manuscript and all original studies are cited as appropriate.

Data falsification and fabrication

All the data corresponds to the real.

REFERENCES

1. World Health Organization. Preterm birth. May 10, 2023. Available from: https://www.who.int/news-room/fact-sheets/detail/preterm-birth. Accessed: Jan 23, 2025.
2. UNICEF 2023. Levels and trends in child mortality: Report 2022 (reprinted). Available from: https://childmortality.org/wp-content/uploads/2023/01/UN-IGME-Child-Mortality-Report-2022.pdf. Accessed: Jan 23, 2025.
3. Lawn JE, Cousens SN, Darmstadt GL, Bhutta ZA, Martines J, Paul V, et al. 1 year after The Lancet Neonatal Survival Series − was the call for action heard? Lancet. 2006;367(9521):1541-7. doi: 10.1016/S0140-6736(06)68587-5.
4. Kuhn P, Zores C, Pebayle T, Hoeft A, Langlet C, Escande B, Astruc D, Dufour A. Infants born very preterm react to variations of the acoustic environment in their incubator from a minimum signal-to-noise ratio threshold of 5 to 10 dBA. Pediatr Res. 2012;71(4 Pt 1):386-92. doi: 10.1038/pr.2011.76.
5. Wachman EM, Lahav A. The effects of noise on preterm infants in the NICU. Arch Dis Child Fetal Neonatal Ed. 2011;96(4):F305-9. doi: 10.1136/adc.2009.182014.
6. Hall JW 3rd. Development of the ear and hearing. J Perinatol. 2000;20(8 Pt 2):S12-20. doi: 10.1038/sj.jp.7200439.
7. Noise: a hazard for the fetus and newborn. American Academy of Pediatrics. Committee on Environmental Health. Pediatrics. 1997;100(4):724-7.
8. Wachman EM, Lahav A. The effects of noise on preterm infants in the NICU. Arch Dis Child Fetal Neonatal Ed. 2011;96(4):F305-9. doi: 10.1136/adc.2009.182014.
9. Levitin DJ. This is your brain on music. The science of human obsession. USA: Edit. PLUME-Pinguin BOOK-USA, 2006; 13-65.
10. Stouffer JW, Shirk BJ, Polomano RC. Practice guidelines for music interventions with hospitalized pediatric patients. J Pediatr Nurs. 2007;22(6):448-56. doi: 10.1016/j.pedn.2007.04.011.
11. Parikh NA. Advanced neuroimaging and its role in predicting neurodevelopmental outcomes in very preterm infants. Semin Perinatol. 2016;40(8):530-41. doi: 10.1053/j.semperi.2016.09.005.
12. Costa VS, Bündchen DC, Sousa H, Bündchen Pires L, Felippetti FA.Clinical benefits of music-based interventions on preterm infants' health: A systematic review of randomized trials. Acta Paediatr 2022;111(3):478-89. doi: 10.1111/apa.16222.
13. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71. doi: 10.1136/bmj.n71.
14. Kemper K, Martin K, Block SM, Shoaf R, Woods C. Attitudes and expectations about music therapy for premature infants among staff in a neonatal intensive care unit. Altern Ther Health Med. 2004;10(2):50-4. PMID: 15055094.
15. Alipour Z, Eskandari N, Tehran HA, Seyed Kamal Eshagh Hossaini Hossaini SKE, Sangi S. Effects of music on physiological and behavioral responses of premature infants: a randomized controlled trial. Complement Ther Clin Pract. 2013;19(3):128-32. doi: 10.1016/j.ctcp.2013.02.007.
16. Dearn T, Shoemark H. The effect of maternal presence on premature infant response to recorded music. J Obstet Gynecol Neonatal Nurs. 2014;43(3):341-50. doi: 10.1111/1552-6909.12303.
17. O'Toole A, Francis K, Pigsley L. Does Music Positively Impact Preterm Infant Outcomes?Adv Neonatal Care. 2017;17(3):192-202. doi: 10.1097/ANC.0000000000000394.
18. Hasegawa Y, Hoshiyama M. Effect of environmental music on autonomic function in infants in intensive and growing care units. J Neonatal Perinatal Med. 2020;13(3):395-401. doi: 10.3233/NPM-180174.
19. Mohan A, Gokulakrishnan G, El-Saie A, Brickley A, Hagan J, Pammi M. Music therapy for preterm neonates in the neonatal intensive care unit: An overview of systematic reviews. Acta Paediatrica. 2021;110(12):3180-200. doi:10.1111/apa16055.
20. Kobus S, Diesel M, Dewan VM, Huening B, Date AK, Felderhoff-Mueser U, et al. Music Therapy Is Effective during Sleep in Preterm Infants. Int J Environ Res Public Health. 2021;18(16):8245. doi: 10.3390/ijerph18168245.
21. Giordano V, Goeral K, Schrage-Leitner L, Berger A, Olischar M. The Effect of Music on a EEG Cyclicity in Preterm Neonates. Children. 2021;8(3):208. doi: 10.3390/children8030208.
22. Yue W, Han X, Leo J, Zeng Z, Yang M. Effect of music therapy on preterm infants in neonatal intensive care unit: Sys-

tematic review and meta-analysis of randomized controlled trials. JAN. 2021;77(2):635-52. doi: 10.1111/jan.14630.

23. Costa VS, Bundchen DC, Sousa H, Bundchen Pires L, Felipetti FA. Clinical benefits of music-based interventions on preterm infants' health: A systematic review of randomised trials. Acta Paediatr. 2022;111(3):478-89. doi: 10.1111/apa.16222.
24. Haslbeck FB, Mueller K, Karen T, Loewy J, Meerpohl JJ, Bassler D. Musical and vocal interventions to improve neurodevelopmental outcomes for preterm infants. Cochrane Database Syst Rev. 2023;9(9):CD013472. doi: 10.1002/14651858.CD013472.pub2.
25. Dewan MV, Jungilligens J, Kobus S, Diezel M, Dathe AK, Schweiger B, Hüning B, Felderhoff-Müser U, Bruns N. The effect of live music therapy on white matter microstructure in very preterm infants - A randomized controlled trial. Eur J Paediatr Neurol. 2024;51:132-139. doi: 10.1016/j.ejpn.2024.06.009.
26. Howick J. Exploring the Asymmetrical Relationship Between the Power of Finance Bias and Evidence. Perspect Biol Med. 2019;62(1):159-87. doi: 10.1353/pbm.2019.0009.
27. Erdei C, Schlesinger K, Pizzi MR, Inder TE. Music Therapy in the Neonatal Intensive Care Unit: A Center's Experience with Program Development, Implementation, and Preliminary Outcomes. Children. 2024;11(5):533. doi: 10.3390/children11050533.
28. Brett R Schofield B. R. Structural organization of the descending auditory pathway. Oxford Academics. 2012;43-64. doi: 10.1093/oxfordhb/9780199233281.013.0003.
29. Chen Y, Sun J, Tao J, Sun T. Treatments and regulatory mechanisms of acoustic stimuli on mood disorders and neurological diseases. Front Neurosci. 2024:17:1322486. doi: 10.3389/fnins.2023.1322486.
30. Baskerville TA, Douglas AJ. Dopamine and Oxytocin Interactions Underlying Behaviors: Potential Contributions to Behavioral Disorders. CNS Neurosci Ther. 2010;16(3):e92-e123. doi: 10.1111/j.1755-5949.2010.00154.x
31. Loonen AJM, Ivanova SA. Circuits regulating pleasure and happiness: evolution and role in mental disorders. Acta Neuropsychiatr. 2018;30(1):29-42. doi: 10.1017/neu.2017.8.
32. Ooishi Y, Mukai H, Watanabe K, Kawato S, Kashino M. Increase in salivary oxytocin and decrease in salivary cortisol after listening to relaxing slow-tempo and exiciting fast tempo music. PLoS One. 2017;12(12):e0189075. doi: 10.1371/journal.pone.0189075.
33. Alley J, Diamond LM, Lipschitz DL, Grewen K. Associations between oxytocin and cortisol reactivity and recovery in response to psychological stress and sexual arousal. Psychoneuroendocrinology. 2019;106:47-56. doi: 10.1016/j.psyneuen.2019.03.031.
34. Takayanagi Y, Onaka T. Roles of Oxytocin in Stress Responses, Allostasis and Resilience. Int J Mol Sci. 2021;23(1):150. doi: 10.3390/ijms23010150.
35. Okyay EK, Ukar T. The effect of emotional freedom technique and music applied to pregnant women who experienced prenatal loss on psychological growth, well-being, and cortisol level: A randomized controlled trial. Arch Psychiatr Nurs. 2023:45:101-12. doi: 10.1016/j.apnu.2023.04.027.
36. Yuhi T, Kyuta H, Mori HA, Murakami C, Furuhara K, Okuno M, et al. Salivary Oxytocin Concentration Changes during a Group Drumming Intervention for Maltreated School Children. Brain Sci. 2017;7(11):152. doi: 10.3390/brainsci7110152.
37. de Witte M, Pinho ADS, Stams GJ, Moonen X, Bos AER, van Hooren S. Music therapy for stress reduction: a systematic review and meta-analysis. Health Psychol Rev. 2022;16(1):134-159. doi: 10.1080/17437199.2020.1846580.

CASE REPORT

Connected to the wrong pipe: esophageal bronchi mimicking bilateral bronchial atresia

Philipp **Peters** [1], Elias **Seidl** [2,*], Jochen **Hubertus** [3], Dietrich **von Schweinitz** [4], Matthias **Griese** [1], Birgit **Kammer** [5], Karl **Reiter** [1]

*** Correspondence to:**

Elias.Seidl@ksipi.uzh.ch. ORCID: https://orcid.org/0000-0001-6610-3756

Doi

10.56164/PediatrRespirJ.2024.54

[1] Department of Pediatrics, Dr. von Hauner Children's Hospital, University Hospital, LMU Munich, Munich, Germany

[2] Department of Respiratory Medicine, University Children's Hospital Zurich, Zurich, Switzerland

[3] Department of Pediatric Surgery, Marien Hospital Witten, Ruhr-University Bochum, Witten, Germany

[4] Department of Pediatric Surgery, Dr. von Hauner Children's Hospital, University Hospital, LMU Munich, Munich, Germany

[5] Department of Radiology, University Hospital, LMU Munich, Munich, Germany

ABSTRACT

Infants frequently present with respiratory symptoms, but diagnosing the underlying pulmonary condition is sometimes challenging.

A 2-week-old neonate was referred to a postnatal history of tachypnea, poor feeding and elevated plasma inflammation markers. The patient was presented with mild episodes of coughing after feeding and diminished breath sounds of the right upper lung. A chest radiograph revealed bilateral consolidations. Whereas bronchoscopy was suggestive of bilateral bronchial atresia, computed tomography supported bronchial atresia of the right upper and middle lobe and a left-sided broncho-esophageal communication. Surprisingly, an upper gastrointestinal series revealed bilateral esophageal insertion of bronchi, and the diagnosis of a communicating bronchopulmonary foregut malformation (CBPFM) was made. Two-stage lobectomy of the affected lobes and segments was performed on days 31 and 41 after birth. Histopathological examination exhibited hamartomatous lung tissue with purulent bronchopneumonia. At a follow-up examination after 4 years, the patient was asymptomatic and thriving well with oral feeds.

CBPFM are rare malformations. This case highlights the clinical challenge of diagnosing this rare condition. There is a need to raise awareness for such uncommon conditions and improve diagnostic accuracy. For optimal management a multidisciplinary approach is essential.

IMPACT STATEMENT: This manuscript presents a complex case of a neonate referring to a postnatal history of tachypnea, poor feeding, and elevated plasma inflammatory markers. While initial diagnostics suggested bronchial atresia, further evaluation led to the diagnosis of a communicating bronchopulmonary foregut malformation. This case highlights the diagnostic challenges associated with rare neonatal conditions, emphasizing the need for increased awareness and improved diagnostic accuracy. Optimal management requires a multidisciplinary approach to ensure comprehensive care.

KEY WORDS

Esophageal bronchus; communicating bronchopulmonary foregut malformation; rare disease; neonatology.

INTRODUCTION

Neonates presenting with pulmonary symptoms within the first days of life are not infrequent (1, 2). While the clinical presentation is usually non-specific, diagnosing the underlying pulmonary condition can be challenging (3). However, delayed diagnosis

may result in considerable complications (4). Here, we describe an infant with prolonged neonatal respiratory distress, the investigations performed, how the correct diagnosis was made and the patient treated successfully. The aim of this case report is to show that the diagnosis of rare respiratory conditions is challenging, but a favorable long-term clinical outcome can be achieved when diagnosed and treated early.

CASE PRESENTATION

The male patient was born via vaginal delivery as the first child of healthy parents. During his first two weeks of life, tachypnea, fever, poor feeding and elevated plasma inflammation markers were noted (C-reactive protein and erythrocyte sedimentation rate). Also, the patient presented with mild coughing episodes after feeding and diminished breath sounds of the right upper lung. A chest radiograph was performed revealing bilateral consolidations (**Figure 1A**). Late-onset neonatal sepsis (LONS) was suspected and an empiric, intravenous antibiotic treatment started. As clinical symptoms persisted, bronchoscopy as well as high-resolution computed tomography (HR-CT) was performed during the same anesthesia. Whereas bronchoscopy was suggestive of bilateral bronchial atresia, HR-CT supported bronchial atresia of the right upper and middle lobe (**Figures 1B**, **C**) and a left-sided broncho-esophageal communication (**Figure 1D**, red arrow). Due to persisting coughing episodes after feeding, in a multidisciplinary team board meeting the need for further investigations were discussed. For the better visualization of the esophagus, it was decided to perform upper gastrointestinal series. The investigation revealed a bilateral esophageal insertion of bronchi (**Figures 1E, F**) and the diagnosis of a communicating bronchopulmonary foregut malformation (CBPFM) was made. A two-stage lobectomy of the affected lobes and segments was performed at the age of 31 and 41 days. Histopathological examination exhibited hamartomatous lung tissue with purulent bronchopneumonia. The latest follow-up chest x-ray with 10 months of age showed a good surgical outcome (**Figure 2**). At 4 years of age, the patient was asymptomatic and thriving well with oral feeds.

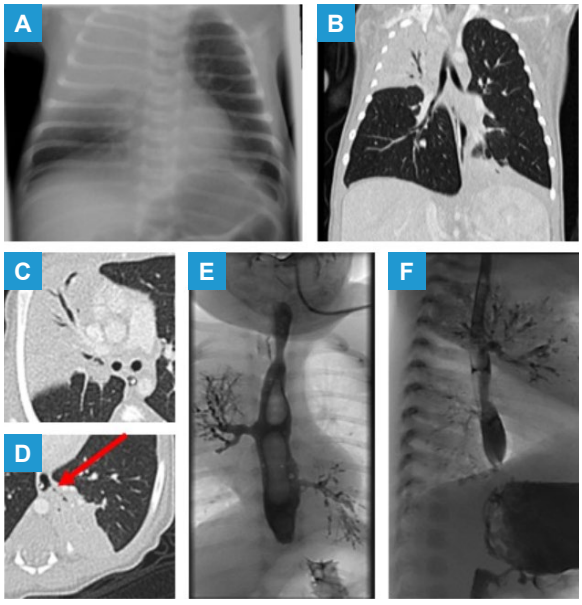

***Figure 1**. Chest X-ray (**A**) showing consolidations in the upper two thirds of the right lung and behind the heart on the left side. Computed tomography (**B** and **C**) suggests atresia of the right upper and middle lobe bronchus, probably arising from a common bud. However, on the left side, all bronchi except for the superior and the posterior-basal left lower lobe bronchi are patent. The course of the latter is suspicious of an esophageal bronchus (**D**, arrow). The upper gastrointestinal series shows esophageal insertion of a common right upper and middle lobe bronchus, as well as a common bronchus of segments VI and X on the left side (**E** and **F**).*

DISCUSSION

Here, we report a patient who presented with non-specific respiratory symptoms, tachypnea and coughing during feeding. In a multidisciplinary team meeting, different investigations were discussed. It was decided that for this patient, the advantage of a better visualization of lung tissue and esophagus outweighed the disadvan-

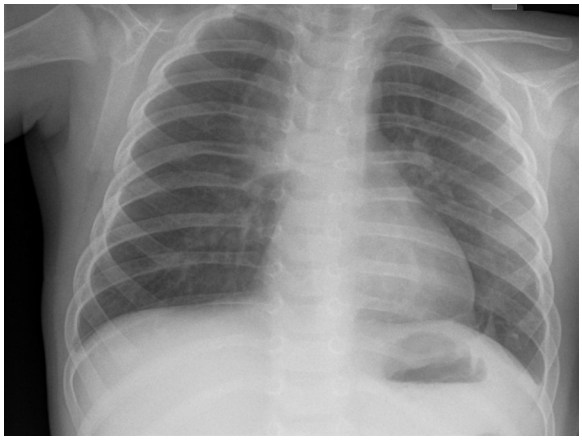

***Figure 2**. Chest X-ray at 10 months of age showing a good surgical result.*

tage of anesthesia as well as extended radiation and CBFM as underlying condition was diagnosed. Successful surgery resulted in an excellent long-term outcome. CBPFM is a rare congenital anomaly, that is defined by a patent congenital communication between the esophagus and the respiratory tract (5). The malformation is often labeled esophageal bronchus or esophageal lung (6). No data on incidence or prevalence rates has been published, yet. The embryogenesis of CBPFM is not fully understood, but a focal mesodermal defect is suspected, making CBPFM and esophageal atresia variations of the same spectrum of malformations (5, 7). CBPFM is reported to be more common in females and usually occurs unilaterally, while it is occasionally associated with other malformations, including cardiovascular anomalies, VACTERL association, skeletal malformation, anorectal malformation, or diaphragmatic hernia (6, 8). In accordance with earlier reports, the initial presentation of the patient described here was non-specific, and the diagnosis challenging. A recently published systematic review analyzed the clinical characteristics of CBPFM (5). Most children presented with respiratory symptoms after birth including respiratory distress, cough/ choking following food intake, recurrent respiratory infection, or hemoptysis. In almost two thirds of the cases, patients were diagnosed with upper gastrointestinal series. Initial misdiagnosis was common, while the reported mortality rate was high (13.1%).

In 1992, a classification system for CBPFM has been introduced: 1) combination with esophageal atresia and tracheoesophageal fistula; 2) absence of a main stem bronchus arising from the trachea as well as the total sequestered lung communicating with the lower esophagus; 3) communication of an isolated part of the lung, and 4) communication between a normal bronchial system and the esophagus (6). Of note, when CBPFM is combined with esophageal atresia, the bronchial malformation might be missed preoperatively, as no upper gastrointestinal series can be performed (16, 17).

There is the potential of compensatory alveolar growth following surgical resection of congenital thoracic malformations (CTM) performed in infancy that is believed to decrease with age (9-13). However, data on long-term pulmonary outcome following the resection of CTMs are scarce and there is an uncertainty of the most appropriate outcome measurement (14). Most used for the assessment of long-term pulmonary outcome is pulmonary function testing (PFT) (14) with the known limitations being age- and cooperation depending, reference values being based on testing results from healthy individuals that vary according to age, height, sex, and ethnicity, as well as the variability of individual measurements (15). Moreover, contradictory results of PFT results following the surgical resection of CTMs have been published (14) and there is a need for further studies investigating long-term outcome of patients with CTM after surgery.

CONCLUSIONS

CBPFM are rare malformations. This case highlights the clinical challenge of diagnosing this rare condition. It aims to raise awareness and improve diagnostic accuracy. For optimal management a multidisciplinary approach is essential.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests

The Authors have declared no conflict of interests.

Financial support

There was no institutional or private funding for this article.

Author contributions

PP, ES, and KR developed the theoretical framework and performed the data interpretation. PP, ES, JH, DvS, BK, MG and KR were primary care physicians. KR supervised the project. ES took the lead in writing the manuscript. PP, JH, DvS, BK, MG and KR contributed to the manuscript providing critical feedback.

Ethical approval

Human studies and subjects

This case report was determined not to require Ethics Committee review.

Data sharing and data accessibility

Data are available upon motivated request to the Corresponding Author.

Publication ethics

Plagiarism

Authors declare no potentially overlapping publications with the content of this manuscript and all original studies are cited as appropriate.

Data falsification and fabrication

All the data corresponds to the real.

REFERENCES

1. Kumar A, Bhatnagar V. Respiratory distress in neonates. Indian J Pediatr. 2005;72(5):425-8. doi: 10.1007/BF02731741.
2. Gallacher DJ, Hart K, Kotecha S. Common respiratory conditions of the newborn. Breathe. 2016;12(1):30-42. doi: 10.1183/20734735.000716.
3. Pramanik AK, Rangaswamy N, Gates T. Neonatal respiratory distress: a practical approach to its diagnosis and management. Pediatr Clin. 2015;62(2):453-69. doi: 10.1016/j.pcl.2014.11.008.
4. John BM, Venkateshwar V, Dagar V. Predictors of Outcome in Neonates with Respiratory Distress. J Nepal Paediatr Society. 2015;35(1):31-7. doi: 10.3126/jnps.v35i1.11868.
5. Yang G, Chen L, Xu C, Yuan M, Li Y. Congenital bronchopulmonary foregut malformation: systematic review of the literature. BMC Pediatr. 2019;19(1):305. doi: 10.1186/s12887-019-1686-1.
6. Srikanth MS, Ford EG, Stanley P, Mahour GH. Communicating bronchopulmonary foregut malformations: Classification and embryogenesis. J Pediatr Surg. 1992;27(6):732-6. doi: 10.1016/s0022-3468(05)80103-4.
7. Qi BQ, Beasley SW. Communicating bronchopulmonary foregut malformations in the adriamycin-induced rat model of oesophageal atresia. Aust NZ J Surg. 1999;69(1):56-9. doi: 10.1046/j.1440-1622.1999.01494.x.
8. Gerle RD, Jaretzki A, Ashley CA, Berne AS. Congenital Bronchopulmonary-Foregut Malformation. New Engl J Med. 1968;278(26):1413-9. doi: 10.1056/NEJM196806272782602.
9. Zeidan S, Hery G, Lacroix F, Gorincour G, Potier A, Dubus JC, et al. Intralobar sequestration associated with cystic adenomatoid malformation: diagnostic and thoracoscopic pitfalls. Surg Endosc. 2009;23(8):1750-3. doi: 10.1007/s00464-008-0183-7.
10. Joshi S, Kotecha S. Lung growth and development. Early Hum Dev. 2007;83(12):789-94. doi: 10.1016/j.earlhumdev.2007.09.007.
11. Thurlbeck WM. Postnatal human lung growth. Thorax. 1982;37(8):564-71. doi: 10.1136/thx.37.8.564.
12. Zeltner TB, Caduff JH, Gehr P, Pfenninger J, Burri PH. The postnatal development and growth of the human lung. I. Morphometry. Respir Physiol. 1987;67(3):247-67. doi: 10.1016/0034-5687(87)90057-0.
13. Nakajima C, Kijimoto C, Yokoyama Y, Miyakawa T, Tsuchiya Y, Kuroda T, et al. Longitudinal follow-up of pulmonary function after lobectomy in childhood - factors affecting lung growth. Pediatr Surg Int. 1998;13(5-6):341-5. doi: 10.1007/s003830050334.
14. Davenport M, Eber E. Long term respiratory outcomes of congenital thoracic malformations. Semin Fetal Neonatal Med. 2012;17(2):99-104. doi: 10.1016/j.siny.2012.01.011.
15. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. Interpretative strategies for lung function tests. Eur Respir J. 2005;26(5):948-68. doi: 10.1183/09031936.05.00035205.
16. He Q ming, Xiao S jie, Zhu X chun, Xiao W qiang, Wang Z, Zhong W, et al. Communicating bronchopulmonary foregut malformation type IA: radiologic anatomy and clinical dilemmas. Surg Radiol Anat. 2015;37(10):1251-6. doi: 10.1007/s00276-015-1504-x.
17. Colleran GC, Ryan CE, Lee EY, Sweeney B, Rea D, Brenner C. Computed tomography and upper gastrointestinal series findings of esophageal bronchi in infants. Pediatr Radiol. 2017;47(2):154-60. doi: 10.1007/s00247-016-3724-6.

BRIEF REPORT

Digital technologies in Pediatric Respiratory Medicine: insights from a preliminary survey by the Italian Pediatric Respiratory Society

Giulia **Roberto** [1, *], Beatrice **Andrenacci** [2], Carlo **Capristo** [3], Giuliana **Ferrante** [4, 5], Valentina Agnese **Ferraro** [6], Stefania **La Grutta** [5], Velia **Malizia** [5], Sara **Manti** [7], Matteo **Naso** [8], Raffaella **Nenna** [9], Alessandro **Onofri** [10], Alessandra **Pandolfo** [6], Giuseppe Fabio **Parisi** [11], Michele **Piazza** [4], Laura **Venditto** [4], Amelia **Licari** [12, 5] on behalf of the study group Pediatric Digital Technologies for Respiratory Care of the Italian Pediatric Respiratory Society

*** Correspondence to:**

g.roberto91@gmail.com. ORCID: https://orcid.org/0000-0002-9234-5561

ABSTRACT

In early 2024, the Italian Pediatric Respiratory Society study group 'Pediatric Digital Technologies for Respiratory Care, PeDiTCare' conducted a survey to assess the awareness and utilization of digital health technologies (DHTs) in the management of pediatric respiratory diseases. The survey was disseminated online through the Society newsletter and social media platforms, collecting responses from 46 society members from most regions of Italy over a three-week period. A preliminary descriptive analysis of the data revealed notable use of electronic health records, telemedicine, and home ventilation systems, particularly for asthma management. Key barriers to broad DHTs adoption include limited resources, high costs, and complexity of use. Respondents emphasized the need for increased financial support and training to expand DHTs integration and improve pediatric respiratory care.

IMPACT STATEMENT: This study highlights the transformative potential of digital health technologies in pediatric respiratory care, while also emphasizing the need to overcome barriers such as limited financial resources and training to fully integrate these tools and improve outcomes.

INTRODUCTION

Digital health technologies (DHTs) are transforming healthcare systems worldwide, offering new opportunities to enhance clinical care, streamline patient management, and foster collaborative research. In pediatric respiratory medicine, where early detection, consistent monitoring, and timely interventions are crucial for managing chronic conditions like asthma and respiratory failure, DHTs have the potential to revolutionize patient outcomes. Despite these advancements, the adoption and integration of digital technologies in pediatric care, particularly within respiratory medicine, remain uneven across different regions and healthcare settings. The Italian Pediatric Respiratory Society has recognized the importance of addressing these challenges and promoting the use of DHTs in pediatric respiratory care. To this end, the study group named 'Pediatric Digital Technologies for Respiratory

Doi

10.56164/PediatrRespirJ.2025.72

[1] SC Pediatric Pulmonology, Regina Margherita Pediatric Hospital, Città della Salute e della Scienza, Turin, Italy
[2] SC Pediatric, Pneumoinfectivology, IRCCS Ca' Granda, Policlinico of Milan, Milan, Italy
[3] Department of Women, Children, and General and Specialty Surgery, Luigi Vanvitelli
University of Campania, Italy
[4] Department of Surgical, Odontostomatologic and Maternal and Child Sciences, University of Verona, Verona, Italy
[5] Institute of Translational Pharmacology, National Research Council, Palermo, Italy
[6] Department of Women's and Children's Health, University of Padua, Padua, Italy
[7] UOC Pediatrics, University of Messina, Messina, Italy
[8] UOC Allergology, IRCCS G. Gaslini, Genoa, Italy
[9] Department of Maternal and Child Health and Urological Sciences, La Sapienza University of Roma, Rome, Italy
[10] UOC Pneumology and Cystic Fibrosis, Bambino Gesù Pediatric Hospital, Rome, Italy
[11] UOC Pediatric Bronchopneumology, San Marco Pediatric Hospital, University of Catania, Catania, Italy
[12] Department of Clinical-Surgical, Diagnostic and Pediatric Sciences, University of Pavia, Pavia, Italy

KEY WORDS

E-health; pediatric respiratory diseases; survey; digital health technologies.

Care, PeDiTCare' was launched in early 2024 as part of the Society broader effort to drive research and innovation in this area. PeDiTCare is a multidisciplinary initiative aimed at building a national network to advance the knowledge and application of digital healthcare for pediatric respiratory diseases. The initiative seeks to map current practices, identify barriers to adoption, and create a framework for expanding the use of DHTs in pediatric pulmonology. The primary goals of PeDiTCare group include evaluating the current landscape of digital technology use in pediatric pulmonology centers across Italy and identifying key enablers and obstacles to broader implementation. By understanding the level of awareness, application, and experience with digital tools such as telemedicine, electronic health records, and telemonitoring systems, the initiative aims to guide future efforts toward improving care quality for children with respiratory conditions. Furthermore, the project seeks to provide healthcare professionals with actionable insights on how to overcome the financial, technical, and educational challenges that currently limit the widespread adoption of DHTs in pediatric respiratory medicine.

MATERIAL AND METHODS

A survey was designed to collect data on the current levels of awareness, adoption, and practical experience with DHTs among pediatric respiratory specialists in Italy, while also identifying potential barriers and facilitators for their broad implementation in clinical settings. The survey was distributed online through the Society's newsletter and social media platforms, with responses collected over a three-week period. Following this, an initial descriptive analysis of the data was carried out to provide a preliminary understanding of the findings.

RESULTS

The survey included 46 participants, representing a significant proportion of the 53 pediatric pulmonology centers listed by the Italian Pediatric Respiratory Society. The geographical distribution covered most Italian regions, with notable participation from centers located in Piedmont, Lazio, Sicily, Lombardy and Emilia Romagna. The majority found to be women (76.1%) aged between 40 and 49 years (39.1%). Most respondents (84.8%) were pediatricians with over 10 years of experience, predominantly working in tertiary hospitals. The respiratory conditions most managed with DHTs were asthma (51.2%) and chronic respiratory failure (19.5%). DHT use was prevalent for patient monitoring, with a substantial proportion of respondents utilizing these tools regularly, while others employed them occasionally based on symptoms, and 24% using them primarily for research purposes (**Figure 1**). Additionally, a notable group of participants reported having extensive experience, exceeding five years, with DHTs. The most frequently used digital technologies were included electronic health record systems, telemedicine, and home ventilation systems, followed by telemonitoring systems, mobile health apps, and home polysomnography (**Figure 2**).

Over 60% of participants identified key barriers to DHT adoption, including limited resources (78.3%), high costs (74.5%), and the complexity of use, which necessitates specialized training (60.4%). More than 80% of respondents suggested that increasing financial support and

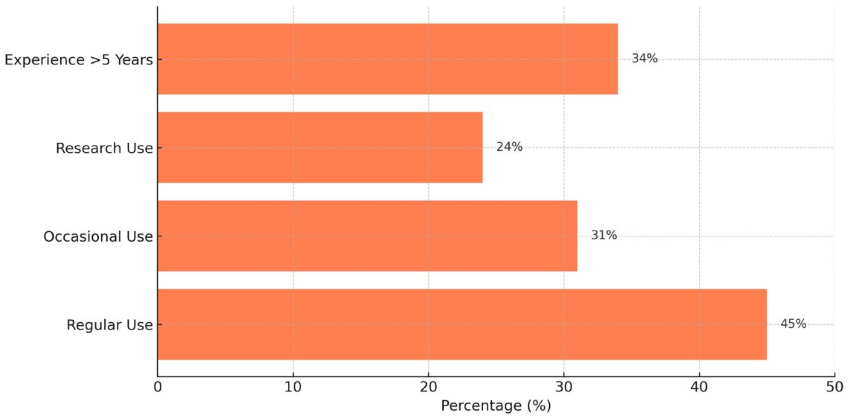

***Figure 1**. Preliminary results of the survey: DHT usage patterns among pediatric respiratory specialists in Italy.*

providing targeted training for healthcare staff would facilitate the wider implementation of DHTs, particularly in smaller hospitals and among general pediatricians.

DISCUSSION

The findings from this survey provide valuable insights into the current landscape of DHT use in pediatric respiratory care across Italy. A significant proportion of the respondents, primarily pediatricians working in tertiary hospitals, demonstrated a moderate to high level of engagement with DHTs, particularly in the management of asthma and chronic respiratory failure. However, the results also highlight a considerable gap in the widespread adoption of these technologies, underscoring important barriers that need to be addressed to fully realize the potential of DHTs in pediatric pulmonology.

The high percentage of respondents using DHTs for patient monitoring (45% regularly, 31% occasionally) is suggestive of a solid foundation for integrating digital tools into clinical practice. This is consistent with previous studies that have shown DHTs to be effective in improving patient outcomes, particularly through continuous monitoring and early detection of exacerbations in chronic conditions such as asthma (1); the fact that a quarter of participants use DHTs exclusively for research purposes reflects the uneven integration of these technologies into routine care. This could be partly due to the relatively high percentage (34%) of professionals with over five years of experience using DHTs, indicating that while a subset of clinicians is familiar with these tools, they may still be seen as niche solutions rather than standard practice.

≤The reported barriers to broader adoption of DHTs − primarily lack of resources, high costs, and the complexity of using these technologies − are consistent with those found in other studies on the challenges of integrating digital health solutions into clinical practice (3). This evidence underscores the importance of addressing both financial and educational hurdles to foster broader DHT adoption. The fact that over 80% of participants identified increased financial resources and staff training as key facilitators for DHT use suggests that targeted investments in these areas could significantly enhance the integration of digital tools into pediatric respiratory care.

Moreover, the emphasis on training reflects a critical need to equip healthcare professionals with the skills to navigate and optimize DHTs in clinical settings. Given the complexity of some technologies, such as telemonitoring systems and home polysomnography, providing specialized training could alleviate concerns regarding the usability and effectiveness of these tools. In addition, expanding the use of DHTs to smaller hospitals and general pediatricians, as suggested by survey respondents, could improve access to these technologies and promote a more standardized approach to care across different healthcare settings.

This study has some limitations that should be acknowledged. First, the survey sample, while representative of pediatric respiratory specialists within IPRS, may not fully capture the diversity of practices and experiences across Italy. Additionally, the self-reported nature of the data may introduce bias, as respondents with more favorable views on DHTs might have been more inclined to participate.

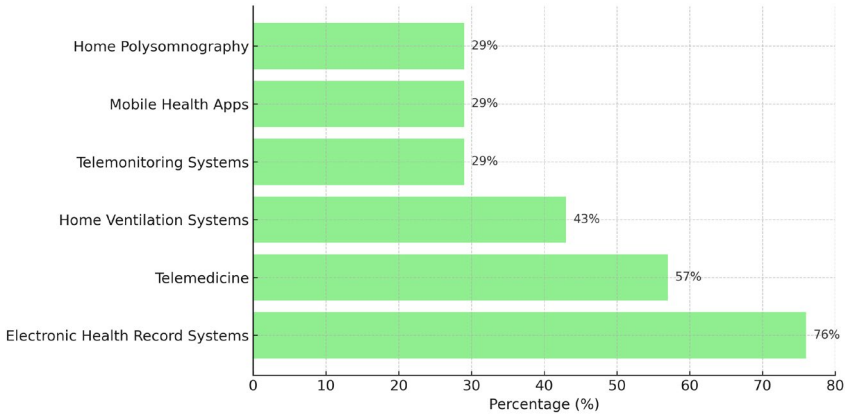

***Figure 2**. Preliminary results of the survey: DHT usage statistics among pediatric respiratory specialists in Italy.*

CONCLUSIONS

The results of this survey provide a clear snapshot of the current use and perception of DHTs among pediatric respiratory specialists in Italy. While there is a promising level of engagement with DHTs, particularly for patient monitoring and telemedicine, the integration of more advanced digital tools remains limited. Key barriers such as resource constraints, high costs, and the complexity of these technologies continue to hinder their wider adoption. However, the strong consensus among respondents on the need for increased financial resources and targeted training underscores the significant potential for expanding DHT use in pediatric respiratory care. By addressing these barriers, particularly through strategic investments and education, healthcare systems can better equip pediatric specialists to utilize DHTs, ultimately improving the quality of care and outcomes for children with respiratory diseases. Further research and action are needed to facilitate this digital transformation, ensuring that both tertiary and community healthcare settings can fully benefit from these innovative tools.

ACKNOWLEDGMENTS

We would like to sincerely thank the Italian Pediatric Respiratory Society for their support in facilitating this study. We also extend our deep appreciation to the pediatric respiratory specialists who participated in the survey, sharing their valuable insights and experiences.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests

The Authors have declared no conflict of interests.

Financial support

There was no institutional or private funding for this article.

Author contributions

Conceptualization and study design: GF, SLG, VM and AL; methodology development, data collection and analysis: AP; drafting of the manuscript: GR, BA, CC, VAF, SM, MN, RN, AO, GFP, MP, LV and AL; Supervision of the project, and final approval of the version to be submitted: GF, SLG, VM, and AL. All Authors have read and approved the final manuscript and agree to be accountable for all aspects of the work.

Ethical approval

Human studies and subjects

This study was conducted in accordance with the ethical standards set forth by the Italian Pediatric Respiratory Society. All participants were informed about the purpose of the study, and their consent was obtained before participation. The data collected were anonymized to ensure confidentiality and privacy.

Data sharing and data accessibility

The survey data supporting the findings of this study are available upon reasonable request from the corresponding author, subject to ethical considerations and the approval of the Italian Pediatric Respiratory Society to ensure the confidentiality and privacy of participants.

Publication ethics

Plagiarism

Authors declare no potentially overlapping publications with the content of this manuscript and all original studies are cited as appropriate.

Data falsification and fabrication

All the data corresponds to the real.

REFERENCES

1. Mosnaim G, Safioti G, Brown R, DePietro M, Szefler SJ, Lang DM, et al. Digital Health Technology in Asthma: A Comprehensive Scoping Review. J Allergy Clin Immunol Pract. 2021;9(6):2377-98. doi: 10.1016/j.jaip.2021.02.028.
2. Greiwe J. Telemedicine Lessons Learned During the COVID-19 Pandemic. Curr Allergy Asthma Rep. 2022;22(1):1-5. doi: 10.1007/s11882-022-01026-1.
3. Borges do Nascimento IJ, Abdulazeem H, Vasanthan LT, Martinez EZ, Zucoloto ML, Østengaard L, et al. Barriers and facilitators to utilizing digital health technologies by healthcare professionals. NPJ Digit Med. 2023;6(1):161. doi: 10.1038/s41746-023-00899-4.

www.pediatric-respiratory-journal.com