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EDITORIAL

The changing faces of pediatric respiratory diseases

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Prior to 2000s, the diagnostic and therapeutic applications of pediatric pulmonary diseases had a relatively slow growth owing to a dearth of knowledge of the basic underpinnings and clinical applications of molecular biology.

Since then, a supernova of accelerated growth of knowledge of the molecular bases of disease has occurred leading to new diagnostic and therapeutic possibilities that we could only dream of previously. In particular, the expansion of genetics and epigenetics through advances in molecular biology has led to the identification of new pathological entities and to a better understanding of disease pathogenesis as in cystic fibrosis and asthma. Improved tests for the study of respiratory function and the use of immune biologic modulators of the genetic response have become readily available to health care providers.

The review by Parisi *et al*. (1) published in this issue of PRJ highlights some of these advances.

It is appropriate that clinic and hospital-based pediatricians, hospitals and all health care providers entrusted to the care of children and their families recognize the progress achieved so far as these scientific achievements continue to be translated to improved diagnosis and treatment of their respiratory diseases.

The design of reference centers to be developed and supported for the public will be the task of governmental health policy institutions and should take into consideration the progress that has been made by these scientific achievements. It will be the task of the **Pediatric Respiratory Journal** to disseminate this knowledge to the readership to assure that infants and children entrusted to their care have access to the best possible care.

References

1. Parisi GF, Papale M, Manti S, Presti S, Mollica F, Rotolo N, Leonardi S. New Trends in Pediatric Pulmonology: our experience. Pediatr Respir J. 2023;1(1):4-15. doi: 10.56164/PediatrRespirJ.2023.09.

PERSPECTIVE

New trends in pediatric pulmonology: our experience

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ABSTRACT

Until a few years ago, pediatric pulmonology was one of the most static branches of pediatrics. In recent years, the description of new pathological entities affecting the lung and airways, the identification of new disease biomarkers, the use of new respiratory function tests suitable not only for collaborating but also for non-collaborating children, the enhancement of respiratory endoscopy, the advent of new therapies such as biological drugs and genetic modulators have made pediatric pulmonology one of the most dynamic branches of pediatric medicine.

The objective of this article will be to explore the new fields of pediatric pulmonology by making a parallelism with the evolutions that our center had to follow in order to adapt to the new healthcare and research standards. These same standards of care are needed by patients, their families, and need to be known by primary care pediatricians. In addition, health policy must take into account these upgrades in pediatric pulmonology to provide the best quality of care to all patients and uniformly throughout the country.

IMPACT STATEMENT

Pediatric pulmonology today represents one of the most dynamic branches of pediatrics with many innovations that centers must adapt to in order to keep up with the times.

KEY WORDS
Pediatric pulmonology; children; lung; airway; new trends.

INTRODUCTION

Until a few years ago, pediatric pulmonology was one of the most static branches of pediatrics. In recent years, the description of new pathological entities affecting the lung and airways, the identification of new disease biomarkers, the use of new respiratory tests suitable not only for collaborating but non-collaborating children, the enhancement of respiratory endoscopy, the advent of new thera-

pies, such as biological drugs and genetic modulators, have made pediatric pulmonology one of the most dynamic branches of pediatric medicine (**Figure 1**). This concept goes well with the extremely current ones of precision medicine and tailor-made medicine and with those of disease endotypes and phenotypes (1). For all these reasons, the centers that deal with pediatric pulmonology have had to implement their activities to comply with the standards of care that are now necessary and have had to adapt the laboratory and respiratory function equipment according to the most recent acquisitions in these sectors (2).

The objective of this article will be to explore the new fields of pediatric pulmonology by making a parallelism with the evolutions that our center has had to follow in order to adapt to the new healthcare and research standards.

BIOMARKERS IN PEDIATRIC LUNG DISEASES

In recent years, scientific studies on processes involving metabolites have been increasing decisively, thus sealing the importance of metabolomics. The study of omics science is closely related to that of biomarker. One of the medical areas most affected by these innovations is certainly childhood respiratory disorders, including asthma and cystic fibrosis (CF) (3, 4). Asthma is a complex disease, mostly characterized by chronic airway inflammation and airway hyperresponsiveness and obstruction with a prominent role of T helper type 2 (Th2) cells that eventually lead to airway epithelial remodelling (5-9). Beyond the concept of phenotype, the last attempt to classify asthma is based on the so called endotypes, which are the different biological pathways involved in the disease. The individuation of different phenotypes and endotypes of asthma could be useful for predicting clinical responses to various asthma treatments and then improving long-term prognosis and patients' quality of life (10) (**figure 2**). In this sense, biomarkers could be advantageous for identifying medical treatments tailored to individual characteristics.

CF is the most common life-shortening autosomal recessive hereditary disease in Caucasians with a prevalence of 1 case per 2500 live births. CF is a multisystem disease caused by mutations in the cystic fibrosis trans-membrane conductance regulator (CFTR) gene (11, 12). The lung is the primary site of CF, to which most of the CF-associated patient morbidity and mortality is linked. CF occurs very early in infancy and is defined by the presence of infections and chronic inflammation leading to a deterioration in lung function, respiratory exacerbations, and bronchiectasis (13, 14).

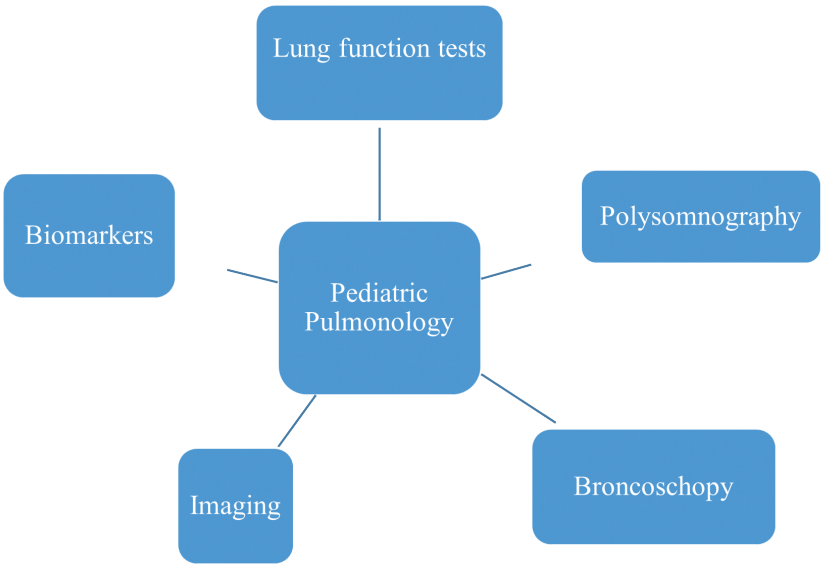

Figure 1. *Expanding fields in Pediatric Pulmonology.*

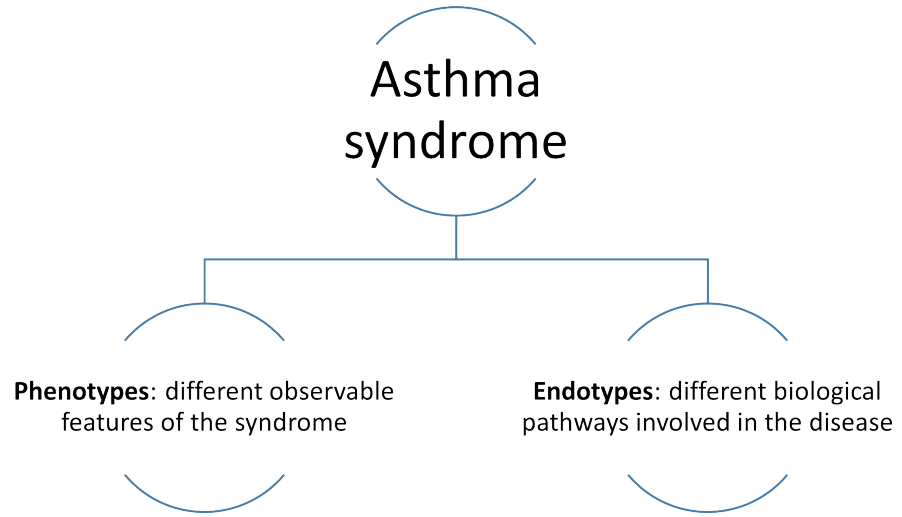

Figure 2. *Phenotypes and endotypes define asthma as a syndrome.*

Thus, identifying a biomarker for disease progression might be crucial for improving these patients' outcomes. Moreover, with therapeutic introduction of potentiators and correctors of the CFTR protein, the study of biomarkers could be a valuable tool for monitoring their effectiveness over time (15). Globally, therefore, the study of biomarkers could have a strategic role in CF management and also in asthma.

FRACTIONAL-EXHALED NITRIC OXIDE (FeNO)

Introduction

Fraction-exhaled nitric oxide (FeNO) is the main biomarker of study in the field of asthma (3).

Nitric oxide (NO) is produced through the action of the enzyme, nitric oxide synthase (NOS), and is responsible for regulating various biological functions of the organism. In lungs, NO production is stimulated by Th2-inflammation mediators in macrophages and airway epithelial cells. NO plays a crucial role in lung physiology as a bronchodilator and inflammatory mediator. Thus, FeNO is a quantitative indicator of airway NO production, and it also reflects eosinophilic inflammation (16, 17).

In 1997, Nelson *et al.* demonstrated that children with asthma had higher level of expired NO, which decreased during corticosteroid treatment as airflow obstruction improved but still remained higher than normal even after treatment (18). Over the years, the relationship of FeNO levels to bronchial hyper-responsiveness, to blood eosinophils count, and to IgE levels in children has been validated. FeNO evaluation is uncomplicated, secure, and well accepted by children, and it has been clearly standardized even in the paediatric field (19-22).

Our experience

In 2019, our research group published a review of the literature from 1990 to present about NO and its use in clinical practice (23). In this review, we discussed not only the role of FeNO but also that of nasal nitric oxide (nNO) and alveolar nitric oxide (CaNO). Each of them is produced at different levels of the respiratory tract and is involved in various diseases. nNO finds its use, principally, in the allergic rhinitis. In fact it can be used as a measure of therapeutic efficacy, but not for the evaluation of the severity. In primary ciliary dyskinesia (PCD), where high levels exclude the disease, and in chronic rhinosinusitis, is not currently used as a diagnostic or prognostic marker. FeNO has a greatest use in bronchial asthma, particularly, it is considered a non-invasive biomarker to identify and to monitor airway inflammation but currently, there is not a consensus on the use of the FeNO in the management of asthma treatment. Finally, CaNO is the least used in clinical practice because lack of standardization of measurement techniques.

VOLATILE ORGANIC COMPOUNDS (VOCs) AND EXHALED BREATH CONDENSATE (EBC)

Introduction

The analysis of volatile organic compounds (VOCs) in exhaled air is a novel metabolomics approach to assess airway inflammation in asthmatic patients (24). VOCs are gaseous molecules (benzenes, toluenes, xylenes, acetone) originating in the airways or bloodstream that spread from the pulmonary capillary bed in the alveoli (25). VOCs are measured with gas chromatography (GS) coupled with mass spectrometry (MS) or with the innovative electronic Nose (e-Nose) devices. The use of e-Nose devices is increasingly attracting attention in the field of medical diagnostics, thanks to its electronic sensors that allow it to identify and quantify a wide range of volatile substances in the air that may vary depending on the type of pathology in question (26, 27). In 2010, Dallinga *et al.* showed that a subgroup of VOCs in exhaled air could be used to differentiate children with asthma from healthy ones (28). Van Vliet *et al.* found that VOCs could help to discern children with persistently controlled asthma from those with uncontrolled asthma during all clinical visits (29). More recently, Brinkman *et al.* demonstrated that a loss of asthma control could be discriminated from clinically stable episodes by longitudinal monitoring of VOCs assessed with the eNose (30).

All of this evidence suggests that the study of VOCs might by a promising, non-invasive tool for monitoring children with respiratory diseases, but further studies are needed (3).

The exhaled breath condensate (EBC) study represents an innovative approach with great potential for understanding the biochemical and metabolic mechanisms of respiratory diseases. The condensate of exhaled air is a fluid obtained by cooling the exhaled air during the current volume breathing and collecting it in a condenser (31, 32).

A fundamental characteristic of the condensate is the possibility to add different doses of biomarkers and to investigate different and potential pathogenic processes involved in respiratory diseases. In fact, in the EBC, several mediators have already been dosed that have allowed to investigate physiopathological aspects related to various respiratory diseases (33). Among all, we mention the standard mediators of inflammation such as isoprostanes (a family of eicosanoids, indicators of oxidative stress from the non-enzymatic oxidation of tissue phospholipids) or leukotrienes (arachidonic acid metabolites) that play an active role in different inflammatory processes (34-36).

With this technique. patients with asthma have shown higher levels of 8-isoprostane, hydrogen peroxide, nitrites, and leukotrienes as signs of oxidative stress (37, 38).

Our experience

The literature shows that EBC analysis is a helpful tool in the management of asthma and CF patients. In fact, the composition of the EBC seems to reflect that of the airway lining fluid and several studied biomarkers appear to correlate with clinical severity and risk of exacerbations (31, 36).

We demonstrated that CF patients have low concentrations of antioxidant agents, particularly glutathione, and increased levels of 8-isoprostane in the exhaled breath suggesting an altered oxidizing environment in the airways of patients with CF (36). Recently, alongside the possibility of dosing individual mediators, the possibility to analyze the condensate through the metabolomic approach has been proposed, the use of which would allow us to change the perspective away from studying a single biomarker (which alone cannot reflect the complexity pathogenesis of a disease) toward one of studying biomarker profiles (39).

PERIOSTIN AND YKL-40

Introduction

Periostin is another emerging biomarker in the field of pediatric lung diseases. The name of this protein originates from the periosteum, the membrane that covers the outer surface of all bones, and it was supposed to be implicated in bone development and repair (40). Bronchial epithelial cells and fibroblasts secrete periostin in response to interleukins (IL)-4 and -13 in order to mediate collagen synthesis, fibrogenesis, and activation of beta growth factor (TGF-beta). In addition, periostin is produced by eosinophils once they have been stimulated by IL-4 and -13 and then secreted by lymphocytes, monocytes, and macrophages after exposure to an allergen (41).

In adult asthmatic patients, serum periostin levels are higher than in controls and are useful for predicting clinical responses to anti-IgE and anti-IL-13 treatments (42, 43).

Studies in children have demonstrated higher serum periostin levels in asthmatic patients than in healthy controls in addition to a significant relationship with blood eosinophils or IgE (44-46).

YKL-40 is a chitinase-like protein that seems to be important in homeostasis of many human systems. Chitinases are enzymes that catalyze the hydrolysis of chitin. Mammals do not have chitin, but they are able to express chitinases (47). YKL-40 is produced by neutrophils, macrophages, synovial cells, and cultured chondrocytes, in which it has a mitogenetic effect on cell proliferation regulation (48).

Konradsen *et al.* demonstrated that YKL-40 levels are incremented in children with severe, therapy-resistant asthma compared to healthy children. Moreover, YKL-40 levels correlated with FeNO, blood neutrophils, and bronchial wall thickening (49).

Our experience

These data were confirmed by Leonardi *et al.* in a cohort of 30 asthmatic children. Furthermore, higher values of YKL-40 were found even in children with intermittent asthma compared to healthy subjects suggesting its role as sensitive biomarker of disease in pediatric ages (50).

YKL-40 seems to also have a pathogenetic role in CF patients as demonstrated by higher levels of both serum and sputum YKL-40 in CF patients compared to healthy controls. Furthermore, sputum YKL-40 levels, rather than serum levels, seem to be more sensitive for lung damage in patients with chronic *Pseudomonas aeruginosa* airway colonization (51).

HIGH MOBILITY GROUP BOX 1 (HMGB-1)

Introduction

High mobility group box 1 (HMGB-1) is a protein belonging to the family of proteins that are subjects of growing scientific interest; it is involved in the processes of regulation of innate immunity, favoring chemotaxis and proinflammatory cytokine release in response to tissue damage (52-54). Several studies have demonstrated higher HMGB-1 levels in patients with allergic rhinitis, nasal polyposis, asthma, COPD, infectious diseases, and CF (55-62).

Our experience

Manti *et al.* enrolled 30 children with asthma and 44 healthy children demonstrating that HMGB1 is a sensitive biomarker of allergic asthma in children and demonstrating a significant correlation between the decrease of HMGB1 levels and a successful treatment with inhaled corticosteroid response (62).

NEW LUNG FUNCTION TESTS

Conventional spirometry has long been considered the primary test of respiratory function deficit in children and adults. However, performing forced breathing maneuvers is challenging in an uncooperative child. Furthermore, recent evidence suggests that conventional spirometry is not sensitive for the early detection of lung damage affecting the small airways or the evaluation of homogeneity of air ventilation (63-65). For these reasons, techniques such as gas dilutions and multiple-breath washout (MBW) have been implemented over the last few years because they allow for the early assessment of damage to small airways. These methods permit the identification of ventilatory inhomogeneity in the lungs by analyzing the clearance of an inert gas used as a tracer (**Figure 3**). The equipment consists of a mass spectrometer combined with a flow meter. For all tests, the lung clearance index (LCI) is the parameter that is most often used to evaluate ventilatory inhomogeneity. Because it is sufficient to breathe with a normal tidal volume during the examination, this examination is particularly suitable for studying respiratory function, even in uncooperative children (66).

The LCI is used to evaluate the homogeneity of lung ventilation. It is obtained using the multiple breath washout (MBW) technique. This parameter indicates how many lung turnovers are needed to expel an inert gas from the lungs by breathing (67). Serial LCI measurements are used for longitudinal lung function evaluation in obstructive diseases, such as CF (especially in mild disease), asthma, and primary ciliary dyskinesia (PCD) (68-70). MBW is carried out at rest,

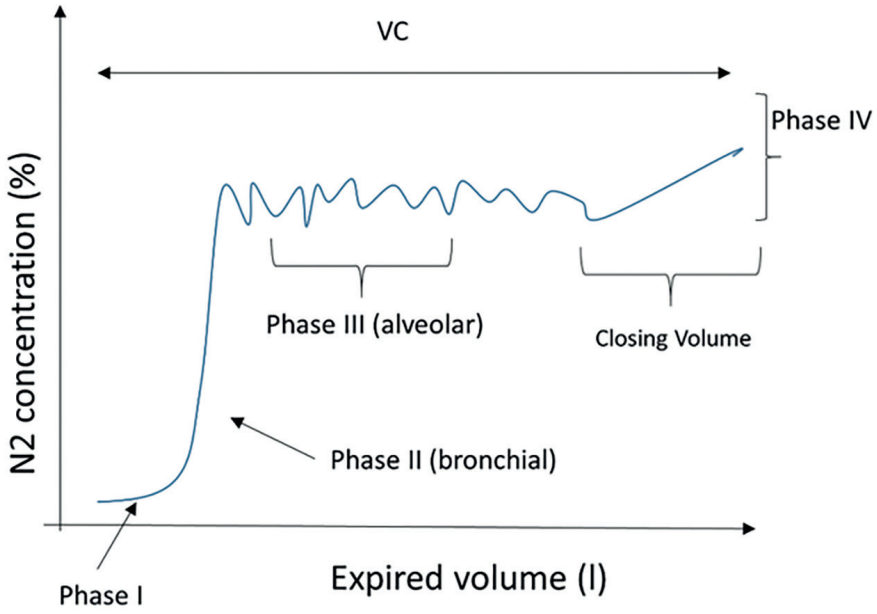

Figure 3. *Normal expirogram during breath washout. The N2 concentration movement in one breath. Four phases can be described: first phase (I) corresponds to the absolute dead space; second phase (II), called the bronchial or transitional phase, represents the alveolar gas arriving from the lung unit; third space (III) is also called alveolar space; fourth phase (IV) starts when an increase in nitrogen concentration is visible and ends when the residual volume is reached. Basal airway closure is a possible explanation of the meaning of phase IV. Volume between the start of phase IV and residual volume is defined as "closing volume." If closing volume occurs before the residual volume reaching, it means that peripheral airways are obstructed. In phase III, there is a slope, a sensitive index of peripherical obstruction. In obstructive diseases, there is an elevation of this inclination.*

and no collaboration is required. This detail is critical for children, particularly preschool-aged children. Those six years and older tend to be cooperative and can perform spirometry. In infants, pulmonary function tests are performed under sedation or during sleep (71). By contrast, preschool-aged children are too old to be sedated and too young to be cooperative. This third age group has been mostly ignored from the functional testing point of view. In 2007, the American Thoracic Society (ATS) published a statement concerning pulmonary function testing in preschool children; various techniques were described, including the multiple-breath inert gas wash-out test (72). Concerning the latter, the authors highlighted the necessity of standard criteria to establish procedures and medical staff education because only a few facilities have had experience with this technique (72).

Our experience

Over the last fifteen years, the LCI has been expanded in the pulmonology field because of its simplicity and sensitivity. Simplicity is crucial, especially for pediatric patients, because children are less inclined than adults to perform tests such as spirometry, a benchmark functional test (66). For this reason, at our center we evaluate LCI in patients with various diseases such as CF, asthma, or other lung diseases (for example, in children with a history of childhood cancer) (66, 73).

In 2020, we published the results of a clinical trial that aimed to study the trend of LCI in a cohort of patients at risk of lung damage, which were childhood cancer survivors. We showed that they maintained good respiratory function indices and regular ventilatory homogeneity. In addition, it became evident that LCI increased as years had passed since the last chemotherapy, evidenced that evolution toward pulmonary fibrosis that is typical of adults with a history of cancer (73).

More recently, we have confirmed the sensitivity of LCI as a marker of disease in CF and in monitoring response to treatment, particularly with DNase (74).

POLYSOMNOGRAPHY AND PEDIATRIC SLEEP DISORDERS

Sleep is defined as a reversible suspension of the individual's sensory-motor interaction with the external

environment. Pediatric sleep disorders create an interruption of the normal night sleep process and therefore a poor quality of sleep itself. The prevalence of these disorders in children is about 25% and they are divided into the following categories: insomnia, sleep breathing disorders, hyper-somnolence (narcolepsy), circadian sleep-wake rhythm disorders, para-somnias (pavor nocturnus, enuresis and somniloquio) and sleep-related movement disorders (75).

Among nocturnal breathing disorders, obstructive sleep apnea syndrome (OSA) is certainly the most frequent. OSA is a disease characterized by prolonged episodes of partial obstruction and/or complete intermittent obstruction (hypopnea or apnea, respectively) of the upper airways (75-77).

For this reason, most pediatric pulmonary centers are able to perform polysomnography to diagnose these disorders. Nocturnal polysomnography is the gold standard exam, recommended by the American Academy of Pediatrics, for the diagnostic setting and the definition of OSA severity in pediatric age (78). It allows an objective and quantitative assessment of the respiratory disorder and sleep pattern and an accurate stratification of patients in terms of severity. This helps determine which children may be most at risk of postoperative sequelae or complications or even have residual OSA after surgery and which ones could benefit from treatment with noninvasive ventilation. The polysomnography instrumentation provides for the recording of sleep patterns through the electroencephalogram and the evaluation of eye movements, muscle movements of the chin and legs, breathing and chest and abdomen movements; in addition, blood oxygenation is recorded with the pulse oximeter and heart rate with the electrocardiogram (79).

Our experience

In our center, we perform nocturnal cardio-respiratory polygraphy annually in patients with CF, as well as in children with suspected OSA. One of the major studies we have published on the topic has allowed us to correlate LCI with the severity of nocturnal hypoxemia in patients with mild to moderate CF by demonstrating that the finding of a pathological LCI shows high sensitivity in identifying a pattern of nocturnal hypoxemia (76).

PEDIATRIC BRONCHOSCOPY

Bronchoscopy has come a long way since the days of Gustav Killian, who in 1897 used a rigid tube and with the help of a lighthouse inspected the airways of corpses. It is also considerably advanced from the days of Chevalier Jackson, who introduced both the lighted endoscope and the practice of interventional endoscopy by removing foreign bodies from the esophagus and airways. It has also been a long way since 1967, when the flexible fiber optic bronchoscope was introduced in pulmonology for the adult population. At the time, no one thought that the technique could ever be applicable to children because, in addition to the lack of proper equipment, there were no pediatric pulmonologists to request and perform the procedure. In this sense, the history of flexible bronchoscopy in children mirrors the development of pediatric pulmonology itself. The introduction, in 1980, of a flexible fiber-optic bronchoscope small enough to allow for inspection of the airways of small children and infants by Robert E. Wood not only provided a new diagnostic tool, but also contributed to define a new sub-specialty of pediatrics: pediatric pulmonology. Nearly 30 years later, flexible bronchoscopy has become a major component of pediatric pulmonology education worldwide and an indispensable tool for clinical practice and research (80-83). Both diagnostic and therapeutic bronchoscopy represents a tool of fundamental importance in the management of the pediatric patient with complex respiratory pathology. It is also a safe test, well tolerated even by the youngest children and which does not require special precautions. The pediatrician who deals with pulmonology is a central figure in respiratory endoscopy as he is not only the material executor of the examination but the one who establishes the indications, knows how to interpret the images and, once the diagnosis has been made, knows the way to better patient management.

Our experience

One of the most interesting experiences we have gained in our center is about therapeutic bronchoscopy in patients with CF. For example, children or adults with persistent or massive atelectasis of part of the lung due to mucus plug formation can be effectively

subjected to such a procedure, which involves complete aspiration of all mucous secretions and instillation of various drugs such as acetylcysteine, antibiotics, dornase alfa, or surfactant. We describe the case of a 28-year-old girl (F508del/F508del) with recent decline in respiratory function and increased cough. On chest imaging there was the finding of persistent thickening at the right intercleidoylar site (**Figure 4**). Upon undergoing therapeutic BAL, the patient had an improvement in respiratory symptoms, and chest X-ray showed enhanced air entry into the lungs (**Figure 5**).

IMAGING IN PEDIATRIC PULMONOLOGY

The diagnosis of many respiratory diseases can be difficult in pediatric age and conventional imaging methods (chest X-ray and CT computed tomography) have always been the most used tools by the clinician for completing the diagnostic process (84, 85). Chest X-ray is still considered by many guidelines as a first-level examination in many clinical pictures, despite the fact that it requires expensive instruments, exposes the patient to radiation and is not without operator-dependent variability. The disadvantages of this method and the growing familiarity and knowledge of ultrasound have therefore prompted clinicians to seek new avenues for pulmonary diagnostics (84, 85). Pulmonary ultrasound (LUS) is easy to perform, it can be performed at the patient's bed as in a family pediatrician's office, it does not expose the patient to radiation and can therefore be repeated several times without side effects (86).

It has been shown that the ability to perform lung ultrasound scans can be easily acquired in a short time even by medical students without any ultrasound skills (87, 88). LUS is therefore an easy and quick learning method for anyone, it provides a lot of information without causing any harm to the patient and has an extremely low cost. This method could in the future not only replace the conventional chest X-ray as a first-line examination in many emergency rooms, but even become a daily use tool for the family pediatrician.

CONCLUSIONS

Pediatric pulmonology today represents one of the most dynamic branches of pediatrics. This has made this specialization attractive also for pediatric residents who see the possibility of growing in this field both from a clinical and a scientific point of view. Furthermore, progress will not stop here because we are at the peak of a scientific evolution that will lead to new acquisitions in the immediate future. Our pediatric pulmonology center now represents a referral point for children with respiratory diseases, which is the result of the center's growth in terms of facilities, equipment, skills, and expertise. This was necessary to bring us up to what are now expected standards of care for a medical center dealing with childhood respiratory diseases. These same standards of care are needed by patients,

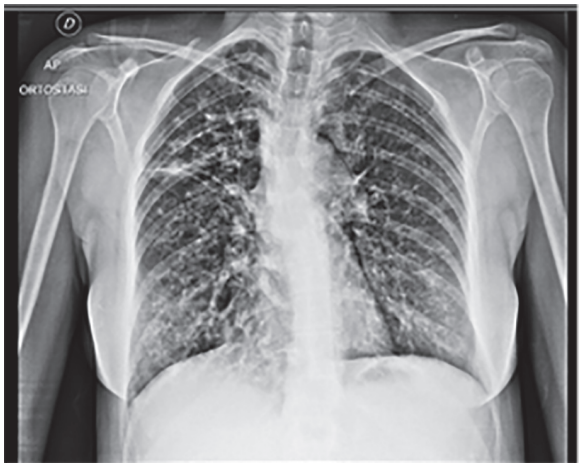

***Figure 4**. 28-year-old girl (F508del/F508del) with cystic fibrosis. Chest X ray before therapeutic bronchoscopy.*

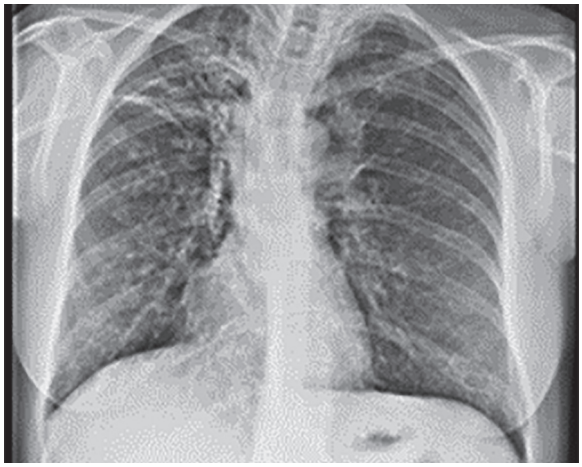

***Figure 5**. 28-year-old girl (F508del/F508del) with cystic fibrosis. Chest X ray after two months from therapeutic bronchoscopy.*

their families, and need to be known by primary care pediatricians. In addition, health policy must take into account these upgrades in pediatric pulmonology to provide the best quality of care to all patients and uniformly throughout the country. The **Table 1** summarizes the main topics covered in the article.

Biomarkers	- Fractional-Exhaled Nitric Oxide (FENO) - Volatile Organic Compounds (VOCS) - Exhaled Breath Condensate (EBC) - Periostin - YKL-40 - HMGB-1
Lung Function Tests	- Multiple breath washout (MBW)
Polysomnography	- Pediatric sleep disorders - Cystic fibrosis
Bronchoscopy	- Diagnostic - Therapeutic
Imaging	- Pulmonary ultrasound (LUS)

Table 1. *Summary of the main topics covered in the article.*

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests

The Authors have declared no conflict of interests.

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Authorship

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Author contributions

GFP, SL: conceptualization. MP, SM, SP: methodology. NR, FM: validation. GFP, SP: formal analysis. SM, MP: investigation. GFP: resources. FM, NR: data curation. GFP: writing-original draft preparation. SM, MP, SP, FM: writing-review and editing. NR: visualization. SL: supervision. All authors have read and agreed to the published version of the manuscript

Ethical approval

Human studies and subjects

N/A.

Animal studies

N/A.

Data sharing and data accessibility

N/A.

Publication ethics

Plagiarism

N/A.

Data falsification and fabrication

The contents of the article are original and any overlaps with other articles are by the Authors themselves and appropriately cited.

REFERENCES

1. Collaco JM, Abman SH. Evolving Challenges in Pediatric Pulmonary Medicine. New Opportunities to Reinvigorate the Field. Am J Respir Crit Care Med. 2018;198(6):724-9. doi: 10.1164/rccm.201709-1902PP.
2. Noah TL, Tolleson-Rinehart S, Esther CR, Peterson-Carmichael SL, Davis SD, Moore PE. The future of pediatric pulmonology: A survey of division directors, assessment of current research funding, and discussion of workforce trends (published online ahead of print, 2020 Dec 15). Pediatr Pulmonol. 2020;10.1002/ppul.25228. doi: 10.1002/ppul.25228.
3. Kruizinga MD, Essers E, Stuurman FE, Yavuz Y, de Kam ML, Zhuparris A, et al. Clinical validation of digital biomarkers for paediatric patients with asthma and cystic fibrosis: potential for clinical trials and clinical care. Eur Respir J. 2022;59(6):2100208. doi: 10.1183/13993003.00208-2021.
4. Vece TJ, Esther CR. Identifying Biomarkers in Pediatric Rare Lung Disease. chILD Grows Up. Am J Respir Crit Care Med. 2019;200(12):1458-9. doi: 10.1164/rccm.201908-1594ED.
5. Holgate ST, Wenzel S, Postma DS, Weiss ST, Renz H, Sly PD. Asthma. Nat Rev Dis Primers. 2015;1(1):15025. doi: 10.1038/nrdp.2015.25.
6. Corren J. Asthma phenotypes and endotypes: an evolving paradigm for classification. Discov Med. 2013;15:243-9. Available from: https://www.discoverymedicine.com/Jonathan-Corren/2013/04/26/asthma-phenotypes-and-endotypes-an-evolving-paradigm-for-classification/. Accessed: Jan 20, 2023.

7. Fitzpatrick AM. Biomarkers of asthma and allergic airway diseases. Ann Allergy Asthma Immunol. 2015;115:335-40. doi: 10.1016/j.anai.2015.09.003.
8. Zhang N, Xu J, Jiang C, Lu S. Neuro-Immune Regulation in Inflammation and Airway Remodeling of Allergic Asthma. Front Immunol. 2022;13:894047. doi: 10.3389/fimmu.2022.894047.
9. Martin J, Townshend J, Brodlie M. Diagnosis and management of asthma in children. BMJ Paediatr Open. 2022;6(1):e001277. doi: 10.1136/bmjpo-2021-001277.
10. Gautam Y, Johansson E, Mersha TB. Multi-Omics Profiling Approach to Asthma: An Evolving Paradigm. J Pers Med. 2022;12(1):66. doi: 10.3390/jpm12010066.
11. Shteinberg M, Haq IJ, Polineni D, Davies JC. Cystic fibrosis. Lancet. 2021;397(10290):2195-211. doi: 10.1016/S0140-6736(20)32542-3.
12. Available from: http://www.genet.sickkids.on.ca/Home.html. Accessed: Jan 20, 2023.
13. Turcios NL. Cystic Fibrosis Lung Disease: An Overview. Respir Care. 2020;65(2):233-51. doi: 10.4187/respcare.06697.
14. Petrocheilou A, Moudaki A, Kaditis AG. Inflammation and Infection in Cystic Fibrosis: Update for the Clinician. Children (Basel). 2022;9(12):1898. doi: 10.3390/children9121898.
15. Roda J, Pinto-Silva C, Silva IAI, Maia C, Almeida S, Ferreira R, et al. New drugs in cystic fibrosis: what has changed in the last decade? Ther Adv Chronic Dis. 2022;13:20406223221098136. doi: 10.1177/20406223221098136.
16. Dodig S, Richter D, Zrinski-Topic R. Inflammatory markers in childhood asthma. Clin Chem Lab Med. 2011;49:587-99. doi: 10.1515/CCLM.2011.094.
17. Smith AD, Cowan JO, Taylor DR. Exhaled nitric oxide levels in asthma: Personal best versus reference values. J Allergy Clin Immunol. 2009;124:714-8. doi: 10.1016/j.jaci.2009.07.020.
18. Nelson BV, Sears S, Woods J, Ling CY, Hunt J, Clapper LM, Gaston B. Expired nitric oxide as a marker for childhood asthma. J Pediatr. 1997;130:423-7. doi: 10.1016/s0022-3476(97)70204-x.
19. Covar RA, Szefler SJ, Martin RJ, Sundstrom DA, Silkoff PE, Murphy J, Young DA, et al. Relations between exhaled nitric oxide and measures of disease activity among children with mild-to-moderate asthma. J Pediatr. 2003;142:469-75. doi: 10.1067/mpd.2003.187.
20. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, et al. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J Respir Crit Care Med. 2011;184:602e615. doi: 10.1164/rccm.9120-11ST.
21. Miraglia Del Giudice M, Marseglia GL, Leonardi S, Tosca MA, Marseglia A, Perrone L, et al. Fractional exhaled nitric oxide measurements in rhinitis and asthma in children. Int J Immunopathol Pharmacol. 2011;24(4 Suppl):29-32. doi: 10.1177/03946320110240s407.
22. Ulrik CS, Lange P, Hilberg O. Fractional exhaled nitric oxide as a determinant for the clinical course of asthma: a systematic review. Eur Clin Respir J. 2021;8(1):1891725. doi: 10.1080/20018525.2021.1891725.
23. Pappalardo MG, Parisi GF, Tardino L, Savasta S, Brambilla I, Marseglia GL, et al. Measurement of nitric oxide and assessment of airway diseases in children: an update. Minerva Pediatr. 2019;71(6):524-32. doi: 10.23736/S0026-4946.19.05513-0.
24. Licari A, Castagnoli R, Brambilla I, Marseglia A, Tosca MA, Marseglia GL, et al. Asthma Endotyping and Biomarkers in Childhood Asthma. Pediatr Allergy Immunol Pulmonol. 2018;31:44-55. doi: 10.1089/ped.2018.0886.
25. Ferraro V, Carraro S, Bozzetto S, Zanconato S, Baraldi E. Exhaled biomarkers in childhood asthma: old and new approaches. Asthms Res Pract 2018;4:9. doi: 10.1186/s40733-018-0045-6.
26. Van der Schee MP, Paff T, Brinkman P, van Aalderen WMC, Haarman EG, Sterk PJ. Breathomics in lung disease. Chest. 2015;147:224-31. doi: 10.1378/chest.14-0781.
27. Fens N, van der Schee MP, Brinkman P, Sterk PJ. Exhaled breath analysis by electronic nose in airways disease. Established issues and key questions. Clin Exp Allergy. 2013;43:705-15. doi: 10.1111/cea.12052.
28. Dallinga JW, Robroeks CM, van Berkel JJ. 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.
29. Van Vliet D, Smolinska A, Jöbsis Q. Association between exhaled inflammatory markers and asthma control in children. J Breath Res. 2016;10(1):016014. doi: 10.1088/1752-7155/10/1/016014.
30. Brinkman P, van de Pol MA, Gerritsen MG. Exhaled breath profiles in the monitoring of loss of control and clinical recovery in asthma. Clin Exp Allergy. 2017;47(9):1159-69. doi: 10.1111/cea.12965.
31. Bannier MAGE, Rosias PPR, Jöbsis Q, Dompeling E. Exhaled Breath Condensate in Childhood Asthma: A Review and Current Perspective. Front Pediatr. 2019;7:150. doi: 10.3389/fped.2019.00150. doi:10.3389/fped.2019.00150.
32. Davis MD, Montpetit AJ. Exhaled Breath Condensate: An Update. Immunol Allergy Clin North Am. 2018;38(4):667-78. doi:10.1016/j.iac.2018.06.002.
33. Davis MD, Fowler SJ, Montpetit AJ. Exhaled breath testing - A tool for the clinician and researcher. Paediatr Respir Rev. 2019;29:37-41. doi:10.1016/j.prrv.2018.05.002.
34. Paredi P, Kharitonov SA, Barnes PJ. Analysis of expired air for oxidation products. Am. J. Respir. Crit. Care Med. 2002;166:S31-7. doi: 10.1164/rccm.2206012.
35. Montuschi P, Kharitonov SA, Ciabattoni G, Corradi M, van Rensen L, Geddes DM, et al. Exhaled 8-isoprostane as a new non-invasive biomarker of oxidative stress in cystic fibrosis. Thorax. 2000;55:205-9. doi: 10.1136/thorax.55.3.205.
36. Spicuzza L, Parisi GF, Tardino L, Ciancio N, Nenna R, Midulla F, et al. Exhaled markers of antioxidant activity and oxidative stress in stable cystic fibrosis

patients with moderate lung disease. J Breath Res. 2018;12(2):026010. doi:10.1088/1752-7163/aa9b39.

37. Teng Y, Sun P, Zhang J, Yu R, Bai J, Yao X, et al. Hydrogen peroxide in exhaled breath condensate in patients with asthma: a promising biomarker? Chest. 2011;140(1):108-16. doi: 10.1378/chest.10-2816.
38. Formanek W, Inci D, Lauener RP, Wildhaber JH, Frey U, Hall GL. Elevated nitrite in breath condensates of children with respiratory disease. Eur Respir J. 2002;19(3):487-91. doi: 10.1183/09031936.02.00101202.
39. Fuschillo S, Paris D, Tramice A, Ambrosino P, Palomba L, Maniscalco M, et al. Metabolomic Profiling of Exhaled Breath Condensate and Plasma/Serum in Chronic Obstructive Pulmonary Disease. Curr Med Chem. 2022;29(14):2385-98. doi: 10.2174/0929867328666210810122350.
40. Alzobaidi N, Rehman S, Naqvi M, Gulati K, Ray A. Periostin. A Potential Biomarker and Therapeutic Target in Pulmonary Diseases. J Pharm Pharm Sci. 2022;25:137-48. doi: 10.18433/jpps32306.
41. Sonnenberg-Riethmacher E, Miehe M, Riethmacher D. Periostin in Allergy and Inflammation. Front Immunol. 2021;12:722170. doi: 10.3389/fimmu.2021.722170.
42. Busse W, Spector S, Rosen K, et al. High eosinophil count: a potential biomarker for assessing successful omalizumab treatment effects. J Allergy Clin Immunol. 2013;132:485-486.e11. doi: 10.1016/j.jaci.2013.02.032.
43. Maxfield AZ, Landegger LD, Brook CD, Campbell AP, Bergmark RW, et al. Periostin as biomarker for nasal polyps in chronic rhinosinusitis. Otolaryngol Head Neck Surg. 2018;158:181-6. doi: 10.1177/0194599817737967.
44. Song JS, You JS, Jeong SI, Yang S, Hwang IT, Im YG, et al. Serum periostin levels correlate with airway hyperresponsiveness to methacholine and mannitol in children with asthma. Allergy. 2015;70:674-81. doi: 10.1111/all.12599.
45. Inoue T, Akashi K, Watanabe M, Ikeda Y, Ashizuka S, Motoki T, et al. Periostin ad a biomarker for the diagnosis of pediatric asthma. Pediatr Allergy Immunol. 2016;27:521-6. doi: 10.1111/pai.12575.
46. Sung M, Lee KS, Ha EG, Lee SJ, Kim MA, Lee SW, et al. An association of periostin levels with the severity and chronicity of atopic dermatitis in children. Pediatr Allergy Immunol. 2017;28:543-50. doi: 10.1111/pai.12744.
47. Jin Y, Song J, Xu F, Zhang D, He J, Zheng J, et al. Association between YKL-40 and asthma: a systematic meta-analysis. Sleep Breath. 2021;26(3):1011-22. doi: 10.1007/s11325-021-02495-w.
48. Zhao T, Su Z, Li Y, Zhang X, You Q. Chitinase-3 like-protein-1 function and its role in diseases. Signal Transduct Target Ther. 2020;5(1):201. doi: 10.1038/s41392-020-00303-7.
49. Konradsen JR, James A, Nordlund B, Reinius LE, Söderhäll C, Melén E, et al. J Allergy Clin Immunol. 2013;132(2):328-35.e5. doi: 10.1016/j.jaci.2013.03.003. Erratum in: J Allergy Clin Immunol. 2013 Nov;132(5):1259. Wheelock, Asa [corrected to Wheelock, Asa M].
50. Leonardi S, Filippelli M, Lanzafame A, Parisi G, Mistrello G, Musumeci M, et al. Serum ykl-40 in children with asthma. J Biol Regul Homeost Agents. 2015;29(2 Suppl 1):114-9. Available from: https://www.iris.unict.it/handle/20.500.11769/17742?-mode=full.462. Accessed: Jan 20, 2023.
51. Leonardi S, Parisi GF, Capizzi A, Manti S, Cuppari C, Scuderi MG, et al. YKL-40 as marker of severe lung disease in cystic fibrosis patients. J Cyst Fibros. 2016;15:583-6. doi: 10.1016/j.jcf.2015.12.020.
52. Vijayakumar EC, Bhatt LK, Prabhavalkar KS. High Mobility Group Box-1 (HMGB1): A Potential Target in Therapeutics. Curr Drug Targets. 2019;20(14):1474-85. doi: 10.2174/1389450120666190618125100.
53. Imbalzano E, Quartuccio S, Di Salvo E, Crea T, Casciaro M, Gangemi S. Association between HMGB1 and asthma: a literature review. Clin Mol Allergy. 2017;15:12. doi: 10.1186/s12948-017-0068-1.
54. Manti S, Leonardi S, Parisi GF, et al. Focus on Pleiotropic Role of HMGB1 in the Onset of Allergic and Non-Allergic Respiratory Diseases. Current Respiratory Medicine Reviews 2017;13:1-5. doi: 10.2174/1573398X13666170529113627.
55. Chirico V, Lacquaniti A, Salpietro V, Munafò C, Calabrò MP, Buemi M, et al. Highmobility group box 1 (HMGB1) in childhood: from bench to bedside. Eur J Pediatr. 2014;173(9):1123-36. doi: 10.1007/s00431-014-2327-1.
56. Salpietro C, Cuppari C, Grasso L, Tosca MA, Miraglia Del Giudice M, La Rosa M, et al. Nasal high-mobility group box-1 protein in children with allergic rhinitis. Int Arch Allergy Immunol. 2013;161(2):116-21. doi: 10.1159/000345246.
57. Cavone L, Cuppari C, Manti S, Grasso L, Arrigo T, Calamai L, et al. Increase in the Level of Proinflammatory Cytokine HMGB1 in Nasal Fluids of Patients With Rhinitis and its Sequestration by Glycyrrhizin Induces Eosinophil Cell Death. Clin Exp Otorhinolaryngol. 2015;8(2):123-8. doi: 10.3342/ceo.2015.8.2.123.
58. Ma L, Zeng J, Mo B, Wang C, Huang J, Sun Y, et al. High mobility group box 1: a novel mediator of Th2-type response-induced airway inflammation of acute allergic asthma. J Thorac Dis. 2015;7(10):1732-41. doi: 10.3978/j.issn.2072-1439.2015.10.18.
59. Rowe SM, Jackson PL, Liu G, Hardison M, Livraghi A, Solomon GM, et al. Potential role of high-mobility group box 1 in cystic fibrosis airway disease. Am J Respir Crit Care Med. 2008;178(8):822-31. doi: 10.1164/rccm.200712-1894OC.
60. Gaggar A, Rowe SM, Matthew H, Blalock JE. Proline-Glycine-Proline (PGP) and High Mobility Group Box Protein-1 (HMGB1): Potential Mediators of Cystic Fibrosis Airway Inflammation. Open Respir Med J. 2010;4:32-8. doi: 10.2174/1874306401004020032.
61. Cuppari C, Manti S, Chirico V, Caruso R, Salpietro V, Giacchi V, et al. Sputum high mobility group box-1 in asthmatic children: a noninvasive sensitive biomarker reflecting disease status. Ann Allergy Asthma Immunol. 2015;115(2):103-7. doi: 10.1016/j.anai.2015.06.008.

62. Manti S, Leonardi S, Parisi GF, De Vivo D, Salpietro A, Spinuzza A, et al. High mobility group box 1: biomarker of inhaled corticosteroid treatment response in children with moderate-severe asthma. Allergy Proc. 2017;38:197-203. doi: 10.2500/aap.2017.38.4047.

63. Fuchs SI, Gappa M. Lung clearance index: clinical and research applications in children. Paediatr Respir Rev. 2011;12(4):264-70. doi: 10.1016/j.prrv.2011.05.001.

64. Horsley AR, Gustafsson PM, Macleod KA, Saunders C, Greening AP, Porteous DJ, et al. Lung clearance index is a sensitive, repeatable and practical measure of airways disease in adults with cystic fibrosis. Thorax. 2008;63(2):135-40. doi: 10.1136/thx.2007.082628.

65. Usemann J, Yammine S, Singer F, Latzin P. Inert gas washout: background and application in various lung diseases. Swiss Med Wkly. 2017;147:w14483.

66. Parisi GF, Pignatone E, Papale M, Mulé E, Manti S, Leonardi S. Lung Clearance Index: A New Measure of Ventilation Inhomogeneity in Childhood Respiratory Diseases, Current Respiratory Medicine Reviews. 2021; 17(4). doi: 10.2174/1573398X17666211201092525.

67. Lombardi E, Gambazza S, Pradal U, Braggion C. Lung clearance index in subjects with cystic fibrosis in Italy. Ital J Pediatr. 2019;45(1):56. doi: 10.1186/s13052-019-0647-5.

68. Saunders C, Bayfield K, Irving S, Short C, Bush A, Davies JC. Developments in multiple breath washout testing in children with cystic fibrosis. Curr Med Res Opin. 2017;33(4):613-20. doi: 10.1080/03007995.2016.1268999.

69. Nuttall AGL, Velásquez W, Beardsmore CS, Gaillard EA. Lung clearance index: assessment and utility in children with asthma. Eur Respir Rev. 2019;28(154):190046. doi: 10.1183/16000617.0046-2019.

70. Irving S, Dixon M, Fassad MR, Frost E, Hayward J, Kilpin K, et al. Primary Ciliary Dyskinesia Due to Microtubular Defects is Associated with Worse Lung Clearance Index. Lung. 2018;196(2):231-8. doi: 10.1007/s00408-018-0086-x.

71. Vogt B, Falkenberg C, Weiler N, Frerichs I. Pulmonary function testing in children and infants. Physiol Meas. 2014;35(3):R59-90. doi: 10.1088/0967-3334/35/3/R59.

72. Beydon N, Davis SD, Lombardi E, Allen JL, Arets HG, Aurora P, et al. An official American Thoracic Society/European Respiratory Society statement: pulmonary function testing in preschool children. Am J Respir Crit Care Med. 2007;175(12):1304-45. doi: 10.1164/rccm.200605-642ST.

73. Parisi GF, Cannata E, Manti S, Papale M, Meli M, Russo G, et al. Lung clearance index: A new measure of late lung complications of cancer therapy in children. Pediatr Pulmonol. 2020;55(12):3450-6. doi:10.1002/ppul.25071.

74. Terlizzi V, Parisi GF, Ferrari B, Castellani C, Manti S, Leonardi S, et al. Effect of Dornase Alfa on the Lung Clearance Index in Children with Cystic Fibrosis: A Lesson from a Case Series. Children (Basel). 2022;9(11):1625. doi:10.3390/children9111625.

75. Arnardottir ES, Islind AS, Óskarsdóttir M, Ólafsdóttir KA, August E, Jónasdóttir L, et al. The Sleep Revolution project: the concept and objectives (published online ahead of print, 2022 Jun 30). J Sleep Res. 2022;e13630. doi: 10.1111/jsr.13630.

76. Papale M, Parisi GF, Spicuzza L, Licari A, Bongiovanni A, Mulè E, et al. Lung clearance index evaluation in detecting nocturnal hypoxemia in cystic fibrosis patients: Toward a new diagnostic tool. Respir Med. 2020;164:105906. doi: 10.1016/j.rmed.2020.105906.

77. Martinez LA, Constantinides SM. Sleep Assessment for Sleep Problems in Children. Nurs Clin North Am. 2021;56(2):299-309. doi: 10.1016/j.cnur.2021.02.008.

78. Trosman I. Childhood obstructive sleep apnea syndrome: a review of the 2012 American Academy of Pediatrics guidelines. Pediatr Ann. 2013;42(10):195-9. doi: 10.3928/00904481-20130924-09.

79. Eichelberger H, Nelson ALA. Nocturnal events in children: When and how to evaluate. Curr Probl Pediatr Adolesc Health Care. 2020;50(12):100893. doi: 10.1016/j.cppeds.2020.100893.

80. Piccione J, Hysinger EB, Vicencio AG. Pediatric advanced diagnostic and interventional bronchoscopy. Semin Pediatr Surg. 2021;30(3):151065. doi: 10.1016/j.sempedsurg.2021.151065.

81. Goussard P, Pohunek P, Eber E, Midulla F, Di Mattia G, Merven M, et al. Pediatric bronchoscopy: recent advances and clinical challenges. Expert Rev Respir Med. 2021;15(4):453-75. doi: 10.1080/17476348.2021.1882854.

82. Eber E, Antón-Pacheco JL, de Blic J, Doull I, Faro A, Nenna R, Nicolai T, et al. ERS statement: interventional bronchoscopy in children. Eur Respir J. 2017;50(6):1700901. doi: 10.1183/13993003.00901-2017.

83. Chantzaras AP, Panagiotou P, Karageorgos S, Douros K. A systematic review of using flexible bronchoscopy to remove foreign bodies from paediatric patients. Acta Paediatr. 2022;111(7):1301-12. doi: 10.1111/apa.16351.

84. Tiddens HAWM, Kuo W, van Straten M, Ciet P. Paediatric lung imaging: the times they are a-changin'. Eur Respir Rev. 2018;27(147):170097. doi: 10.1183/16000617.0097-2017

85. Ciet P, Bertolo S, Ros M, Casciaro R, Cipolli M, Colagrande S, et al. State-of-the-art review of lung imaging in cystic fibrosis with recommendations for pulmonologists and radiologists from the "iMAging managEment of cySTic fibROsis" (MAESTRO) consortium. Eur Respir Rev. 2022;31(163):210173. doi: 10.1183/16000617.0173-2021.

86. Bhalla D, Naranje P, Jana M, Bhalla AS. Pediatric lung ultrasonography: current perspectives (published online ahead of print, 2022 Jun 18). Pediatr Radiol. 2022;1-13. doi: 10.1007/s00247-022-05412-9.

87. Lim JS, Lee S, Do HH, Oh KH. Can Limited Education of Lung Ultrasound Be Conducted to Medical Students Properly? A Pilot Study. Biomed Res Int. 2017;2017:8147075. doi: 10.1155/2017/8147075

88. Heiberg J, Hansen LS, Wemmelund K, Sørensen AH, Ilkjaer C, Cloete E, et al. Point-of-Care Clinical Ultrasound for Medical Students. Ultrasound Int Open. 2015;1(2):E58-E66. doi: 10.1055/s-0035-1565173.

REVIEW

New threats for pediatric respiratory health: beware of vaping

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ABSTRACT

Electronic cigarette (EC) was proposed on the market about 15 years ago as a harmless alternative to traditional combustion cigarettes (CC). Since then, EC and other electronic devices that deliver nicotine by simulating traditional smoking without combustion have achieved unexpected success, with around 80 million users worldwide by 2023. Such devices are commonly felt to be safer than CC, especially among adolescents, who are also the main target for aggressive marketing from the tobacco industry. Increasing evidence shows that e-liquids and vape contain toxicants and irritants and that acute and chronic vaping causes airway inflammation and bronchoconstriction and reduces responses to infections. Moreover, some studies have shown that second- and third-hand smoke, as well as in utero exposure, may cause detrimental effects to the airways and to health in general. Notably, the recent E-cigarette or Vaping use-Associated Lung Injury (EVALI) epidemic in the USA has shown that EC has higher acute toxicity than CC, while long-term effects are still not known. Since adolescents and children are often completely unaware of the health risks associated with vaping or of the potential presence of nicotine in e-liquids, pediatricians play a crucial role in educating them, in order to prevent vaping, as well as smoking. Pediatricians should always consider the possibility of vaping as the cause of unusual respiratory diseases, especially in adolescents. This narrative review paper briefly outlines the most recent data on EC and their effects on the airways, focusing on childhood and adolescence.

IMPACT STATEMENT

The worldwide vaping epidemic among adolescents represents a serious threat to health and a new challenge for healthcare professionals. Pediatricians should screen and educate patients and parents on vaping.

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ABBREVIATIONS

ARDS: Acute Respiratory Distress Syndrome; CC: Combustion Cigarette; EC: Electronic Cigarette; EU: European Union; ENDS: Electronic Nicotine Delivery Systems; EVALI: E-cigarette or Vaping use-Associated Lung Injury; FDA: Food and Drug Administration; HTTP: Heated Tobacco Products; PG: Propylene Glycol; US/USA: United States of America; VG: Vegetable Glycerin; WHO: World Health Organization.

KEY WORDS

Adolescents; e-cigarette; nicotine; smoke; vape.

INTRODUCTION

In 2003, the Chinese pharmacist Hon Lik proposed the first modern model of electronic cigarette (EC) on the Chinese market as a harmless alternative to traditional combustion cigarettes (CC). About three years later, EC reached the European and American markets achieving unexpected success, with an exponential growth in sales and estimates referring to around 80 million users worldwide by 2023 (1). In the European Union, 1 citizen out of 7 (41% of 2020 EU citizens) had tried EC at least once, while only around 1 in 20 (6%) had tried heated tobacco products (HTP) in 2020 (2). In Italy, according to recent data from the National Institute of Health, there are 12.4 million current smokers (24.2% of the population) and 1.2 million habitual or occasional EC users (2.4% of the population) (3). EC and other electronic devices that deliver nicotine by simulating traditional smoking without combustion (Electronic Nicotine Delivery Systems - ENDS) (see Glossary, **Table 1**) will soon overtake CC in the market for several reasons: first of all, they were introduced as non-harmful alternatives to CC, which release more than 7000 compounds during combustion (at least 70 carcinogens), and so they are commonly felt to be safer (4). Secondly, due to the increasing restrictions on the sale of tobacco worldwide, together with people prematurely dying from smoking, the industry is using aggressive marketing to target youths, especially on social media, in order to recruit new long-lasting customers, taking advantage of the fact that restrictions on ENDS sales are lacking or patchily distributed (5). Last but not least, EC use is easily conceivable, the colorful packaging is captivating, and the availability of many sweet flavors attracts many customers. Unsurprisingly, the available data clearly show that vaping is spreading especially among the very young, who start vaping mostly because they are driven by curiosity and the desire to imitate their peers and are often completely unaware of the health risks associated with vaping or of the potential presence of nicotine in e-liquids (6, 7). In 2018, the US Surgeon General declared youth EC use as an epidemic in the USA, where these devices have become the most used tobacco product among adolescents since 2014. According to data from the National Youth Tobacco Survey in the US, 19.6% of high school students (3.02 million) and 4.7% of middle school students (550.000) reported current (within the preceding 30 days) EC use in 2020 (8). In Italy, 1.7% of adolescents already habitually use EC and 41.5% have tried EC at least once (9). Such data are particularly worrying considering that a recent systematic review including 9 prospective longitudinal studies on adolescents who never smoked tobacco CC with follow-up periods between 4 and 24 months, showed that EC use increases the risk of becoming a current CC smoker by 4-fold, serving as a gateway to cigarette smoking (10).

Finally, recently the phenomenon of stealth vaping (*i.e.*, the act of vaping in a discreet manner by using small quantities of vape or smaller devices or devices resembling different types of electronic devices) started to spread among adolescents, making it even more difficult to detect the addiction inside the family or school environment (11).

This narrative review paper briefly outlines the most recent data on EC and their potential detrimental effects on the airways, focusing on childhood and adolescence. For the purpose of this review, we will refer only to EC, while we will not include evidence on devices with different aerosolization mechanisms such as HTP.

E-CIGARETTES, E-LIQUIDS AND VAPE: WHAT ARE VAPERS INHALING?

The aerosol produced by EC and other ENDS is generally called *"vape"* and appears denser than that produced by CC. EC have undergone numerous evolutions over the years, but they are all basically equipped with three main components: 1) a power source (usually a rechargeable lithium battery); 2) an atomizer, equipped with a resistance that heats up as the current passes through it, allowing the solution (e-liquid) to be vaporized by heating it to high temperatures; 3) a liquid storage unit (12). EC may be activated by the user's inhalation or by manual activation through a button. The most modern devices allow the user to set the resistance and power, thus varying the temperature of the aerosol: with higher

Cloud-chasing	Vaping technique with the goal to create different types of aerosol plume, emerging as competition among adolescents.
Dabbing	The term refers to vaping marijuana by heating concentrated cannabis oil ("dabs").
Dripping	Vaping technique in which dense vape is generated manually by dripping e-liquids directly onto the device's heating coils.
Dual user	User of both EC and traditional combustible cigarettes.
E-Cigarette	Strictly speaking, "electronic cigarettes" are portable battery-powered electronic devices that simulate the act of smoking a traditional cigarette without burning tobacco, by delivering an inhalable liquid-based aerosol.
ENDS	"Electronic Nicotine Delivery Systems" (ENDS) is a generic term used to identify all the currently available electronic devices used to deliver nicotine by inhalation without tobacco combustion. ENDS include EC, e-pipes, e-cigars, e-hookahs, vape pens, personal vaporizers, and so on.
EVALI	This acronym was introduced in 2019 and stands for "E-*cigarette or Vaping use-Associated Lung Injury*". EVALI is a diagnosis of exclusion in patients presenting with respiratory distress and a recent history of vaping, abnormal chest CT, absence of signs of pulmonary infection or any other alternative plausible diagnoses. Some Authors recently proposed introducing also the term EVALD (*"E-cigarette or Vaping use-Associated Lung Disease"*) to underline that vaping may cause different types of lung disease and not only acute injury.
Heat-not-burn devices/Heated tobacco products	Electronic devices generating aerosol by heating up tobacco, without burning it.
Pod-mods	Miniaturized ENDS usually resembling a USB flash drive and delivering high concentration of nicotine by using nicotine salts which do not cause a sensation of harshness or irritation on the airways during inhalation.
Puff	The term refers to disposable, cheap and highly concealable EC resembling pod-mods, the use of which is spreading among adolescents.
Stealth Vaping	The act of vaping in a discreet manner by using small quantities of vape or particularly small devices. ENDS resembling other devices such as car keys, teapots, credit card holders, asthma inhalers and so on are also available on the web.
Smoker	Combustible cigarette user.
Vape	The aerosol produced by ENDS, which usually appears denser than that produced by traditional cigarettes. The act of inhaling and exhaling the vapor produced by ENDS is known as "vaping".
Vaper	Electronic cigarette user.

Table 1. *Vaping glossary.*

temperatures, a stronger "hit" (sensation felt in the throat during inhalation) is generated (13, 14). As for e-liquids, they consist of a solution mostly composed (80-95%) of solvents such as propylene glycol (PG) or vegetable glycerin (VG, also known as glycerol); the remaining components are represented by one or more flavoring additives which make it possible to obtain a vapor with a distinctive flavor, and by nicotine which may be absent in EC but, when present, can reach high concentrations, up to more than 50 mg/mL (in the EU a limit has been set at 20 mg/mL). There are more than 15,000 different types of EC flavors on the market, ranging from the aroma of tobacco to food (fruit, sweets, candies) or stimulating drinks (coffee, alcohol). All these compounds are generally recognized as safe by the Food and Drug Administration (FDA) as they are widely used in the food and cosmetic industries, but it should be noted that their effects when chronically inhaled are still only partially known. Moreover, the e-liquid composition declared by manufacturing companies is not always truthful, both regarding the levels of nicotine (15, 16) and the

presence of other substances, including toxic and/or irritative ones, such as tobacco alkaloids and nitrosamines, volatile organic compounds, ethanol, metals, formaldehyde, acetaldehyde and acrolein, which have been found in e-liquids and vape, at different concentrations depending on the temperatures to which the liquid had been heated (17-19). In general, vapers probably inhale fewer toxic substances than CC smokers, but it is not easy to elucidate this issue because research studies are performed on precise quantities of vape produced and inhaled under standardized conditions, while in real life the exposure depends on the methods of EC use (voltage, heat of the liquid) and on the habits of the vaper (20).

Notably, among the currently available four generations of EC, the devices belonging to the fourth are those that give rise to more concern: such devices are the so-called "pod-mods" which look like USB pens and are very popular among teenagers because it is easy to hide their use (21). Pod-mods are particularly dangerous as they use a formulation of nicotine derived from nicotine salts with benzoic acid, delivering high concentrations without causing a sensation of harshness or irritation on the airways, thus increasing the amount consumed in a short time (22). Recently, disposable, and cheaper EC have become available and have gained immediate success, with an increase in sales from 2019 to 2020 in the US of 1000% in high school users and 400% in middle school users (8, 23).

EFFECTS OF VAPING ON RESPIRATORY HEALTH

The first case report describing respiratory distress caused by vaping dates back to 2012 (24), and since then, the number of published papers on the subject has increased exponentially (25). Increasingly available evidence shows that vaping exerts negative effects on airway biology and, consequently, on respiratory health, and this was largely expected, as there were previous reports of respiratory symptoms caused by inhalation of substances later found in e-liquids and vape. Apart from the detrimental effects of inhalation of toxicants and irritants, it has been reported that inhalation of PG and VG causes acute dry cough and wheezing and long-term respiratory impairment in theater and cinema workers exposed to stage fog produced by heating these solvents, which have hygroscopic properties that cause hyperosmotic stress and the subsequent release of inflammation mediators, ciliary function alteration and bronchoconstriction (26). Furthermore, the inhalation of diacetyl (2,3-butanedione), one of the most used chemical compounds in e-liquids, and especially in buttery or sweet aromas, has been shown to cause bronchiolitis obliterans in microwave popcorn factory workers ("*The Popcorn Worker's Lung*") (27-29). Moreover, some flavors contain known allergens such as cinnamaldehyde, eugenol and benzaldehyde (30-31).

To date, the effects of vaping reported both in vitro and in vivo on the respiratory system can be summarized in: a) pro-inflammatory effect, b) stimulation of bronchial hyperreactivity and c) increased susceptibility to infections (32-34) (**Figure 1**).

In 2022, an analysis of longitudinal data from the US PATH (Population Assessment of Tobacco and Health) study clearly demonstrated that vaping is an independent risk factor for the development of respiratory symptoms, such as cough and wheezing in otherwise healthy young adults, including those who have never smoked CC (35). A recent review and meta-analysis of epidemiological studies, both cross-sectional and longitudinal, has shown a significant association of EC use with asthma (pooled adjusted odds ratio 1.39 (95% CI 1.28-1.51)) and COPD (pooled adjusted odds ratio 1.49 (95% CI 1.36-1.65)), controlling for cigarette smoking and other covariates. Among the 15 selected studies on asthma, 11 included adolescents and were school-based data collections, mainly on high school students, of which 6 were carried out in the US, 4 in Asia and 1 in Canada (36). Nevertheless, evidence on vaping effects in childhood and adolescence, and also in asthmatics, is still scarce and mainly based on cross-sectional studies on adolescents, showing that EC use increases by about 2-fold the risk of developing self-reported symptoms attributable to chronic bronchitis and/or asthma, such as chronic cough, phlegm, dyspnea (37). It should be noted that adolescents with asthma smoke as much as their non-asthmatic peers and seem to use EC more than their peers due to the belief that vaping is safer than CC (38-41).

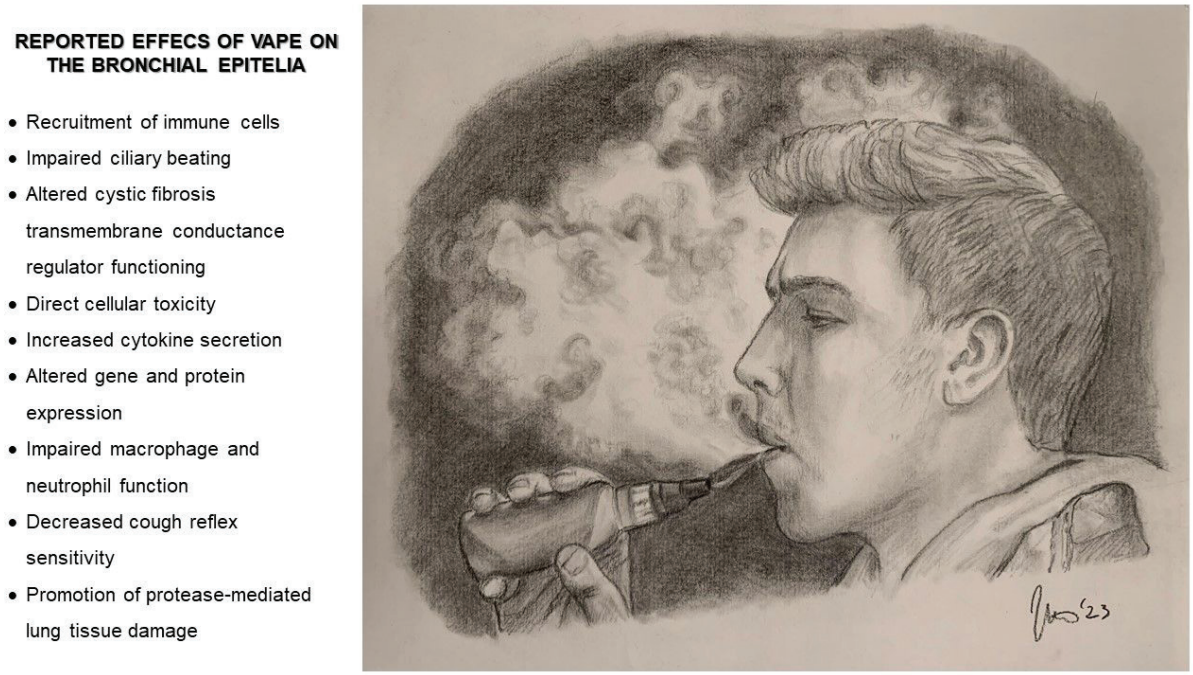

Figure 1. *Main effects of vaping on the airways. The picture summarizes the currently reported effects of vape exposure both in vitro and in vivo on the airway's mucosa.*

Finally, the EVALI (*E-cigarette or Vaping use-Associated Lung Injury*) epidemic that occurred in the US in 2019 demonstrated that vaping is associated with severe acute lung toxicity patterns which have never been described in CC smokers (42). This condition has been linked to the use of e-liquids containing tetrahydrocannabinol and/or vitamin E acetate and affects mainly young adults and adolescents showing respiratory symptoms such as shortness of breath, cough and chest pain, sometimes evolving into ARDS with the need for mechanical ventilation, and gastrointestinal symptoms (nausea, vomiting, diarrhea, abdominal pain) (43-44). All patients also show one or more constitutional symptoms (mostly fever) and CT scans of the thorax typically demonstrate ground-glass opacities with perilobular and peribronchial distribution and subpleural sparing, consistent with an organizing pneumonia pattern of lung injury resulting from toxic inhalation (45-46). In these patients, systemic glucocorticosteroid treatment seems to be effective while antibiotics do not improve lung function (47-48). To date, a few EVALI cases have been reported in other countries, including a recent ARDS case report in a 15-year-old girl in Italy (49).

It should be noted that while vitamin E acetate is safe as food supplementation or in cosmetics, it interacts with phosphatidylcholine when inhaled, thus altering the surfactant, which is no longer able to warrant the surface tension and the related normal functioning of the alveoli, subsequently giving rise to inflammation (50-51). As far as the long-term consequences of vaping are concerned, the presence of known carcinogens in vape and e-liquids suggests caution, but as yet no studies are available in this regard except for some studies on mice reporting an increased risk of lung adenocarcinoma (52). As regards second-hand smoke, in 2022 Islam et al. showed for the first time an association between exposure to passive smoke from EC containing nicotine and an increased risk of developing respiratory symptoms such as wheezing or bronchitis in more than 2,000 adolescents and young adults (53). This finding is not surprising, since the presence of toxic compounds in the indoor air of EC users' houses, such as $PM_{2.5}$ PM_{10}, nicotine and volatile organic compounds, had already been reported. This evidence advises against using EC indoors, especially in the presence of children and adolescents. There is currently no available data on third-hand va-

ping exposure, but traces of nicotine and particulate matter have been detected on surfaces exposed to vaping (54-56). With respect to pregnancy, data relating to the birth of low-birth-weight newborns from mothers who had vaped during pregnancy are starting to emerge (57, 58): as a result, the World Health Organization (WHO) has declared the use of ENDS to be unsafe in pregnancy (59).

THE ROLE OF PEDIATRICIANS

Pediatricians play a crucial role in fighting smoking, as more than half of current smokers started smoking before the age of 18. In such a scenario, the vaping epidemic represents both a new challenge for healthcare providers and an insidious threat for young people, and adolescents in particular, whose brain is particularly at risk of becoming nicotine addicted. Since adolescents who begin vaping or smoking early are less likely to stop using tobacco products (60-63), they represent the main target of advertising campaigns by EC brands, who are continuously trying to recruit new customers to replace those quitting or prematurely dying from smoking. Therefore, pediatricians should be updated on the subject and on health effects of vaping, in order to adequately educate and warn their patients and their parents, including all the possible ways of exposure (64) (**Figure 2**). Their commitment and involvement are particularly important when considering that knowledge of EC is usually inadequate in both adolescents and parents, as shown in a recent single-site prospective questionnaire analysis of 300 adolescents and their parents (65). The education of parents should also be targeted to their smoking and vaping habits since, similarly to what happens with CC, parents using ENDS predicts a higher probability of their children using them, even after controlling for parent past month CC use (66). The same is true regarding exposure to vaping imagery in television or film, which was found to be associated with a significantly increased risk of vaping uptake among young people (67). Last but not least, pediatricians should warn families about the risks of device exposure causing burns and wounds (68-70), as well as of an increasing number of reports on poisoning due to accidental or intentional ingestion of e-liquid in childhood and adolescence (71).

In the last few years, several scientific societies have become aware of the tremendous implications of vaping epidemic in adolescence as well as vape's potential health effects throughout life (72) and started to take a position, recommending that environments where children and adolescents live should be free of ENDS and other tobacco products. In 2015, The American Academy of Pediatrics have released recommendations on vaping, suggesting universal screening and prevention counseling on tobacco use and other substance use in adolescence, including EC, both for parents and children (63). In 2018 the Forum of International Respiratory Societies (FIRS) released a position statement including, among other recommendations, that i) EC be regulated as tobacco products and included in smoke-free policies, ii) sale of EC to youths should be banned worldwide, iii) advertising accessible by youths and young adults should be banned, and iv) flavoring should be prohibited in EC (73). The European Respiratory Society (ERS) Tobacco Control Committee in 2021 warned about the potential toxicity of prolonged exposure to vape, until proved otherwise (74). The European Academy of Paediatrics (EAP) recently released ten recommendations regarding EC, underlining that since there is incontrovertible evidence that the acute toxicity of EC is greater than that of CC and even if the chronic toxicity of EC is still unknown, it cannot be assumed that EC are safer than CC (75).

Lastly, it should be noted that EC are currently not recognized as smoking cessation aids by both the FDA and WHO due to inconclusive evidence, with some studies demonstrating their effectiveness (76-77) and others showing that they may perpetuate addiction or even encourage dual use (78). Both these institutions have produced educational material warning against potential health effects of ENDS, especially in children, adolescents, and young adults, which is freely available on the Internet (79-80).

CONCLUSIONS

It is staggering how the use of ENDS is rapidly spreading among youth and adolescents and represents a worrisome source of new threats to respiratory health and to health in general in the short and probably also

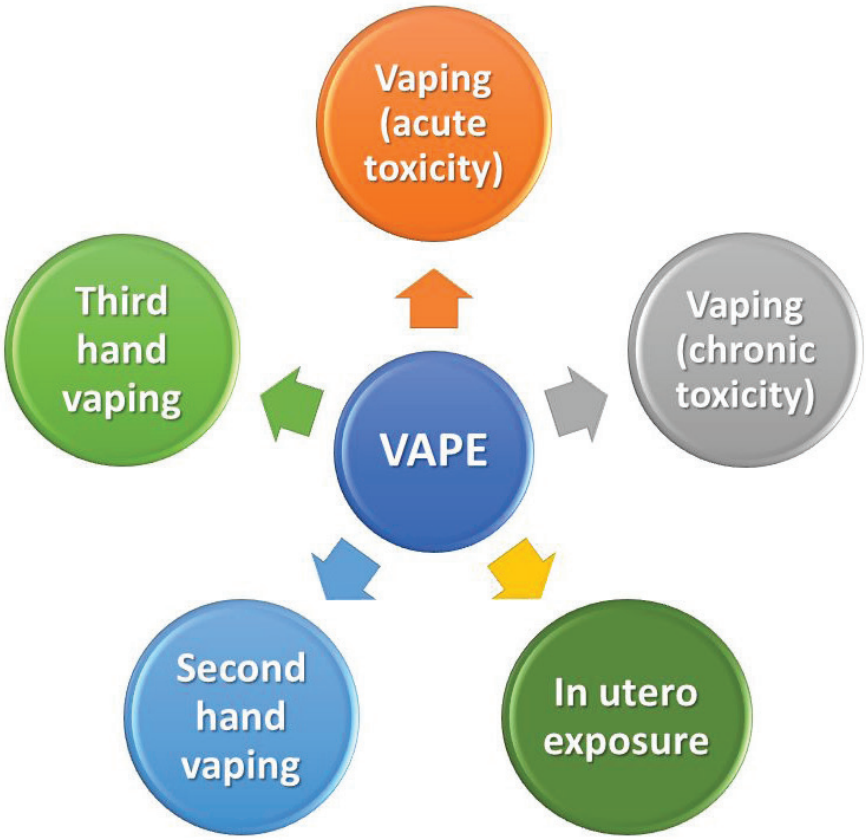

Figure 2. *Harmful routes of vaping exposure. Available evidence shows that vaping exerts detrimental effects both in the short and long term in first-hand vapers, but data are becoming available on second and third-hand vaping as well as on in utero exposure.*

in the long term. Data on the detrimental effects of different ways of vaping exposure, including in utero, are rapidly becoming available.

COMPLIANCE WITH ETHICAL STANDARDS

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Author contributions

MDC and AB: conceptualized the study, drafted the initial manuscript, reviewed the literature and critically revised the final manuscript. MS, VR, DP: contributed to the review of the literature and data collection. They also actively participated in manuscript drafting, critically reviewing it. VR: performed the artwork, too. All Authors read and approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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The data underlying this article are available in the article.

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REFERENCES

1. Jerzyński T, Stimson GV, Shapiro H, Król G. Estimation of the global number of e-cigarette users in 2020. Harm Reduct J. 2021;18(1):109. doi: 10.1186/s12954-021-00556-7.
2. EU Directorate General for Health and Food safety - Special Eurobarometer Report Attitudes of Europeans towards tobacco and electronic cigarettes, February 2021. Available from: https://op.europa.eu/it/publication-detail/-/publication/c070c04c-6788-11eb-aeb5-01aa75ed71a1. Accessed: Jan 20, 2023.
3. Italian National Institute of Health - 30th May 2022 Press Release. Available from: https://www.iss.it/web/guest//comunicati-stampa/-/asset_publisher/fjTKmjJgSgdK/content/id/7146126. Accessed: Jan 20, 2023.
4. Goniewicz ML, Knysak J, Gawron M, Kosmider L, Sobczak A, Kurek J, et al. Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. Tob Control. 2014;23(2):133-9. doi: 10.1136/tobaccocontrol-2012-050859.
5. Chatziparasidis G, Kantar A. Vaping in Asthmatic Adolescents: Time to Deal with the Elephant in the Room. Children (Basel). 2022;9(3):311. doi: 10.3390/-children9030311.
6. GorukantiA,DelucchiK,LingP,Fisher-TravisR,Halpern-Felsher B. Adolescents' attitudes towards e-cigarette ingredients, safety, addictive properties, social norms, and regulation. Prev Med. 2017;94:65-71. doi: 10.1016/j ypmed.2016.10.019.
7. Bernat D, Gasquet N, Wilson KO, Porter L, Choi K. Electronic Cigarette Harm and Benefit Perceptions and Use Among Youth. Am J Prev Med. 2018;55(3):361–7. doi:10.1016/j.amepre.2018.04.043.
8. Wang TW, Neff LJ, Park-Lee E, Ren C, Cullen KA, King BA. E-cigarette Use Among Middle and High School Students - United States, 2020. MMWR Morb Mortal Wkly Rep. 2020;69(37):1310-12. doi: 10.15585/mmwr.mm6937e1.
9. Palmi I, Mortali C, Mastrobattista L. Il tabagismo in Italia in età evolutiva. Pneumologia Pediatrica. 2021;21(83):29-34. Available from: https://simri.it/_getFile.php?idFile=1928. Accessed: Mar 3, 2023.
10. O'Brien D, Long J, Quigley J, Lee C, McCarthy A, Kavanagh P. Association between electronic cigarette use and tobacco cigarette smoking initiation in adolescents: a systematic review and meta-analysis. BMC Public Health. 2021;21(1):954. doi: 10.1186/s12889-021-10935-1.
11. Ramamurthi D, Chau C, Jackler RK. JUUL and other stealth vaporisers: hiding the habit from parents and teachers. Tob Control. 2018: tobaccocontrol-2018-054455. doi: 10.1136/tobaccocontrol-2018-054455.
12. Di Cicco M, Sepich M, Ragazzo V, Peroni DG, Comberiati P. Potential effects of E-cigarettes and vaping on pediatric asthma. Minerva Pediatr. 2020;72(5):372-82. doi: 10.23736/S0026-4946.20.05973-3.
13. Choi H, Lin Y, Race E, Macmurdo MG. Electronic Cigarettes and Alternative Methods of Vaping. Ann Am Thorac Soc. 2021;18(2):191-9. doi: 10.1513/AnnalsATS.202005-511CME.
14. Clapp PW, Jaspers I. Electronic Cigarettes: Their Constituents and Potential Links to Asthma. Curr Allergy Asthma Rep. 2017;17(11):79. doi: 10.1007/s11882-017-0747-5.
15. Dinakar C, O'Connor GT. The Health Effects of Electronic Cigarettes. N Engl J Med. 2016;375(14): 1372-81. doi: 10.1056/NEJMra1502466.
16. Goniewicz ML, Hajek P, McRobbie H. Nicotine Content of Electronic Cigarettes, Its Release in Vapour and Its Consistency Across Batches: Regulatory Implications. Addiction. 2014;109(3):500-7. doi: 10.1111/add.12410.
17. Wang P, Chen W, Liao J, Matsuo T, Ito K, Fowles J et al. A Device-Independent Evaluation of Carbonyl Emissions From Heated Electronic Cigarette Solvents. PLoS One. 2017;12(1):e0169811. doi: 10.1371/journal.pone.016981.
18. Rubinstein ML, Delucchi K, Benowitz NL, Ramo DE. Adolescent Exposure to Toxic Volatile Organic Chemicals From E-Cigarettes. Pediatrics. 2018;141(4): e20173557. doi: 10.1542/peds.2017-3557.
19. Bhave SY, Chadi N. E-cigarettes and Vaping: A Global Risk for Adolescents. Indian Pediatr. 2021;58(4): 315-9.
20. Korzun T, Lazurko M, Munhenzva I, Barsanti KC, Huang Y, Jensen RP et al. E-Cigarette Airflow Rate Modulates Toxicant Profiles and Can Lead to Concerning Levels of Solvent Consumption. ACS Omega. 2018;3(1): 30-6. doi: 10.1021/acsomega.7b01521.
21. Owotomo O, Walley S. The youth e-cigarette epidemic: updates and review of devices, epidemiology and regulation. Curr Probl Pediatr Adolesc Health Care. 2022;52(6):101200. doi: 10.1016/j.cppeds.2022.101200.
22. Fadus MC, Smith TT, Squeglia LM. The rise of e-cigarettes, pod mod devices, and JUUL among youth: Factors influencing use, health implications, and downstream effects. Drug Alcohol Depend. 2019;201:85-93. doi: 10.1016/j.drugalcdep.2019.04.011.
23. Williams R. The rise of disposable JUUL-type e-cigarette devices. Tob Control. 2020; 29(e1): e134-5. doi: 10.1136/tobaccocontrol-2019-055379.
24. McCauley L, Markin C, Hosmer D. An unexpected consequence of electronic cigarette use. Chest. 2012;141(4): 1110-3. doi: 10.1378/chest.11-1334.
25. Landman ST, Dhaliwal I, Mackenzie CA, Martinu T, Steele A, Bosma KJ. Life-threatening bronchiolitis related to electronic cigarette use in a Canadian youth. CMAJ. 2019;191(48):E1321-331. doi: 10.1503/cmaj.191402.
26. Varughese S, Teschke K, Brauer M, Chow Y, van Netten C, Kennedy SM. Effects of theatrical smokes and fogs on respiratory health in the entertainment industry. Am J Ind Med. 2005;47(5):411-8. doi: 10.1002/ajim.20151.
27. Gwinn WM, Flake GP, Bousquet RW, Taylor GJ, Morgan DL. Airway injury in an in vitro human epithelium-fibroblast model of diacetyl vapor exposure: diacetyl-induced basal/suprabasal spongiosis. Inhal Toxicol. 2017;29(7): 310-21. doi: 10.1080/08958378.2017.1369604.
28. Kreiss K, Gomaa A, Kullman G, Fedan K, Simoes EJ, Enright PL. Clinical bronchiolitis obliterans in workers at a microwave-popcorn plant. N Engl J Med. 2002;347(5):330-8. doi: 10.1056/NEJMoa020300.
29. van Rooy FG, Rooyackers JM, Prokop M, Houba R, Smit LA, Heederik DJ. Bronchiolitis obliterans syndrome in

chemical workers producing diacetyl for food flavorings. Am J Respir Crit Care Med. 200;176(5): 498-504. doi: 10.1164/rccm.200611-1620OC.

30. Costigan S, Belmonte JL. An Approach to Allergy Risk Assessments for E-Liquid Ingredients. Regul Toxicol Pharmacol. 2017;87:1-8. doi: 10.1016/j.yrtph.2017.04.003.
31. Kosmider L, Sobczak A, Prokopowicz A, Kurek J, Zaciera M, Knysak J et al. Cherry-flavoured electronic cigarettes expose users to the inhalation irritant, benzaldehyde. Thorax. 2016;71(4):376-7. doi: 10.1136/thoraxjnl-2015-207895.
32. Bals R, Boyd J, Esposito S, Foronjy R, Hiemstra PS, Jiménez-Ruiz CA et al. Electronic cigarettes: a task force report from the European Respiratory Society. Eur Respir J. 2019;53(2):1801151. doi: 10.1183/13993003.01151-2018.
33. Gotts JE, Jordt SE, McConnell R, Tarran R. What Are the Respiratory Effects of E-Cigarettes? BMJ. 2019;366:l5275. doi: 10.1136/bmj.l5275.
34. Hickman E, Jaspers I. Current E-Cigarette Research in the Context of Asthma. Curr Allergy Asthma Rep. 2020;20(10):62. doi: 10.1007/s11882-020-00952-2.
35. Xie W, Tackett AP, Berlowitz JB, Harlow AF, Kathuria H, Galiatsatos P et al. Association of Electronic Cigarette Use with Respiratory Symptom Development among U.S. Young Adults. Am J Respir Crit Care Med. 2022;205(11):1320-9. doi: 10.1164/rccm.202107-1718OC.
36. Wills TA, Soneji SS, Choi K, Jaspers I, Tam EK. E-cigarette use and respiratory disorders: an integrative review of converging evidence from epidemiological and laboratory studies. Eur Respir J. 2021;57(1):1901815. doi: 10.1183/13993003.01815-2019.
37. Di Cicco M, Sepich M, Beni A, Comberiati P, Peroni DG. How E-cigarettes and vaping can affect asthma in children and adolescents. Curr Opin Allergy Clin Immunol. 2022;22(2):86-94. doi: 10.1097/ACI.0000000000000807.
38. Lee A, Lee SY, Lee KS. The Use of Heated Tobacco Products is Associated with Asthma, Allergic Rhinitis, and Atopic Dermatitis in Korean Adolescents. Sci Rep. 2019;9(1):17699. doi: 10.1038/s41598-019-54102-4.
39. Fedele DA, Barnett TE, Dekevich D, Gibson-Young LM, Martinasek M, Jagger MA. Prevalence of and beliefs about electronic cigarettes and hookah among high school students with asthma. Ann Epidemiol. 2016;26(12):865-9. doi: 10.1016/j.annepidem.2016.10.004.
40. Turner E, Fedele DA, Thompson L, Salloum RG. Patterns of electronic cigarette use in youth with asthma: Results from a nationally representative sample. Ann Allergy Asthma Immunol. 2018;120(2):220-2. doi: 10.1016/j.anai.2017.11.020.
41. Schweitzer RJ, Wills TA, Tam E, Pagano I, Choi K. E-cigarette use and asthma in a multiethnic sample of adolescents. Prev Med. 2017;105:226-231. doi: 10.1016/j.ypmed.2017.09.023.
42. Layden JE, Ghinai I, Pray I, Kimball A, Layer M, Tenforde MW et al. Pulmonary Illness Related to E-Cigarette Use in Illinois and Wisconsin - Final Report. N Engl J Med. 2020;382(10):903-16. doi: 10.1056/NEJMoa1911614.
43. Rebuli ME, Rose JJ, Noël A, Croft DP, Benowitz NL, Cohen AH et al. The E-cigarette or Vaping Product Use-Associated Lung Injury Epidemic: Pathogenesis, Management, and Future Directions: An Official American Thoracic Society Workshop Report. Ann Am Thorac Soc. 2023;20(1):1-17. doi: 10.1513/AnnalsATS.202209-796ST.
44. Bhatt JM, Ramphul M, Bush A. An update on controversies in e-cigarettes. Paediatr Respir Rev. 2020; 36:75-86. doi: 10.1016/j.prrv.2020.09.003.
45. Thakrar PD, Boyd KP, Swanson CP, Wideburg E, Kumbhar SS. E-cigarette, or vaping, product use-associated lung injury in adolescents: a review of imaging features. Pediatr Radiol. 2020; 50(3):338-44. doi: 10.1007/s00247-019-04572-5.
46. Friedman J, Schooler GR, Kwon JK, Artunduaga M. Pediatric electronic cigarette or vaping product use-associated lung injury (EVALI): updates in the coronavirus disease 2019 (COVID-19) pandemic era. Pediatr Radiol. 2022;52(10):2009-16. doi: 10.1007/s00247-022-05454-z.
47. Rao DR, Maple KL, Dettori A, Afolabi F, Francis JKR, Artunduaga M, et al. Clinical Features of E-cigarette, or Vaping, Product Use-Associated Lung Injury in Teenagers. Pediatrics. 2020;146(1):e20194104. doi: 10.1542/peds.2019-4104.
48. Reddy A, Jenssen BP, Chidambaram A, Yehya N, Lindell RB. Characterizing e-cigarette vaping-associated lung injury in the pediatric intensive care unit. Pediatr Pulmonol. 2021;56(1):162-70. doi: 10.1002/ppul.25086.
49. Casamento Tumeo C, Schiavino A, Paglietti MG, Petreschi F, Ottavianelli A, Onofri A, et al. E-cigarette or Vaping product use Associated Lung Injury (EVALI) in a 15 year old female patient - case report. Ital J Pediatr. 2022;48(1):119. doi: 10.1186/s13052-022-01314-6.
50. Blount BC, Karwowski MP, Shields PG, Morel-Espinosa M, Valentin-Blasini L, Gardner M, et al. Vitamin E Acetate in Bronchoalveolar-Lavage Fluid Associated with EVALI. N Engl J Med. 2020;382(8):697-705. doi: 10.1056/NEJMoa1916433.
51. Farber HJ, Conrado Pacheco Gallego M, Galiatsatos P, Folan P, Lamphere T, Pakhale S, et al. Harms of Electronic Cigarettes: What the Healthcare Provider Needs to Know. Ann Am Thorac Soc. 2021;18(4):567-72. doi: 10.1513/AnnalsATS.202009-1113CME.
52. Tang MS, Wu XR, Lee HW, Xia Y, Deng FM, Moreira AL, et al. Electronic-cigarette smoke induces lung adenocarcinoma and bladder urothelial hyperplasia in mice. Proc Natl Acad Sci U S A. 2019;116(43):21727-31. doi: 10.1073/pnas.1911321116.
53. Islam T, Braymiller J, Eckel SP, Liu F, Tackett AP, Rebuli ME, et al. Secondhand nicotine vaping at home and respiratory symptoms in young adults. Thorax. 2022;77(7):663-8. doi: 10.1136/thoraxjnl-2021-217041.
54. Collaco JM, Drummond MB, McGrath-Morrow SA. Electronic cigarette use and exposure in the pediatric population. JAMA Pediatr. 2015;169(2):177-82. doi: 10.1001/jamapediatrics.2014.2898.
55. Davis ES, Sassano MF, Goodell H, Tarran R. E-Liquid Autofluorescence can be used as a Marker of Vaping

Deposition and Third-Hand Vape Exposure. Sci Rep. 2017;7(1): 7459. doi: 10.1038/s41598-017-07862-w.

56. Tzortzi A, Teloniatis S, Matiampa G, Bakelas G, Tzavara C, Vyzikidou VK, et al. Passive exposure of non-smokers to E-Cigarette aerosols: sensory irritation, timing and association with volatile organic compounds. Environ Res. 2020;182:108963. doi: 10.1016/j.envres.2019.108963.
57. Cardenas VM, Ali MM, Fischbach LA, Nembhard WN. Dual use of cigarettes and electronic nicotine delivery systems during pregnancy and the risk of small for gestational age neonates. Ann Epidemiol. 2020; 52:86-92. e2. doi: 10.1016/j.annepidem.2020.08.002.
58. Kim S, Oancea SC. Electronic cigarettes may not be a "safer alternative" of conventional cigarettes during pregnancy: evidence from the nationally representative PRAMS data. BMC Pregnancy Childbirth. 2020;20(1): 557. doi: 10.1186/s12884-020-03247-6.
59. Mescolo F, Ferrante G, La Grutta S. Effects of E-Cigarette Exposure on Prenatal Life and Childhood Respiratory Health: A Review of Current Evidence. Front Pediatr. 2021;9:711573. doi: 10.3389/fped.2021.711573.
60. Leventhal AM, Strong DR, Kirkpatrick MG, Unger JB, Sussman S, Riggs NR, et al. Association of Electronic Cigarette Use With Initiation of Combustible Tobacco Product Smoking in Early Adolescence. JAMA. 2015; 314(7):700-7. doi: 10.1001/jama.2015.8950.
61. Berry KM, Fetterman JL, Benjamin EJ, Bhatnagar A, Barrington-Trimis JL, Leventhal AM, et al. Association of Electronic Cigarette Use With Subsequent Initiation of Tobacco Cigarettes in US Youths. JAMA Netw Open. 2019; 2(2): e187794. doi: 10.1001/jamanetworkopen.2018.7794.
62. Siqueira LM, AAP Committee on Substance use and Prevention. Nicotine and Tobacco as Substances of Abuse in Children and Adolescents. Pediatrics. 2017; 139(1): e20163436. doi: 10.1542/peds.2016-3436.
63. Farber HJ, Walley SC, Groner JA, Nelson KE; Section on Tobacco Control. Clinical Practice Policy to Protect Children From Tobacco, Nicotine, and Tobacco Smoke. Pediatrics. 2015;136(5):1008-17. doi: 10.1542/peds.2015-3108. PMID: 26504137.
64. Becker TD, Rice TR. Youth vaping: a review and update on global epidemiology, physical and behavioral health risks, and clinical considerations. Eur J Pediatr. 2022;181(2):453-62. doi: 10.1007/s00431-021-04220-x.
65. Bailey JA, Epstein M, Kosterman R. Parent ENDS use predicts adolescent and young adult offspring ENDS use above and beyond parent cigarette use. Addict Behav. 2022;125:107157. doi: 10.1016/j.addbeh.2021.107157.
66. Hassanein ZM, Barker AB, Murray RL, Britton J, Agrawal S, Leonardi-Bee J. Impact of Smoking and Vaping in Films on Smoking and Vaping Uptake in Adolescents: Systematic Review and Meta-Analysis. Health Educ Behav. 2022;49(6):1004-13. doi: 10.1177/10901981221086944.
67. Allem JP, Van Valkenburgh SP, Donaldson SI, Dormanesh A, Kelley TC, Rosenthal EL. E-cigarette imagery in Netflix scripted television and movies popular among young adults: A content analysis. Addict Behav Rep. 2022;16:100444. doi: 10.1016/j.abrep.2022.100444.
68. Brownson EG, Thompson CM, Goldsberry S, Chong HJ, Friedrich JB, Pham TN, et al. Explosion Injuries from E-Cigarettes. N Engl J Med. 2016; 375(14):1400-2. doi: 10.1056/NEJMc1608478.
69. Katz MG, Russell KW. Injury from E-Cigarette Explosion. N Engl J Med. 2019;380(25):2460. doi: 10.1056/NEJMicm1813769.
70. Russell KW, Katz MG, Phillips RC, Kelley-Quon LI, Acker SN, Shahi N, et al. Adolescent Vaping-Associated Trauma in the Western United States. J Surg Res. 2022;276:251-5. doi: 10.1016/j.jss.2022.02.026.
71. Ang E, Tuthill D, Thompson J. E-cigarette liquid ingestion: a fast growing accidental issue in children. Arch Dis Child. 2018;103(11):1091. doi: 10.1136/archdischild-2018-314886.
72. Hajat C, Stein E, Shantikumar S, Niaura R, Ferrara P, Polosa R. A scoping review of studies on the health impact of electronic nicotine delivery systems. Intern Emerg Med. 2022;17(1):241-68. doi: 10.1007/s11739-021-02835-4.
73. Ferkol TW, Farber HJ, La Grutta S, Leone FT, Marshall HM, Neptune E, et al. Electronic cigarette use in youths: a position statement of the Forum of International Respiratory Societies. Eur Respir J. 2018;51(5):1800278. doi: 10.1183/13993003.00278-2018.
74. Grigg J. Tobacco control and the ERS: new problems and old foes. Eur Respir J. 2021;57(1):2003499. doi: 10.1183/13993003.03499-2020.
75. Bush A, Lintowska A, Mazur A, Hadjipanayis A, Grossman Z, Del Torso S, et al. E-Cigarettes as a Growing Threat for Children and Adolescents: Position Statement From the European Academy of Paediatrics. Front Pediatr. 2021;9:698613. doi: 10.3389/fped.2021.698613.
76. Hajek P, Phillips-Waller A, Przulj D, Pesola F, Myers Smith K, Bisal N, et al. A Randomized Trial of E-Cigarettes versus Nicotine-Replacement Therapy. N Engl J Med. 2019;380(7):629-37. doi: 10.1056/NEJMoa1808779.
77. Chan GCK, Stjepanović D, Lim C, Sun T, Shanmuga Anandan A, Connor JP, et al. A systematic review of randomized controlled trials and network meta-analysis of e-cigarettes for smoking cessation. Addict Behav. 2021;119:106912. doi: 10.1016/j.addbeh.2021.106912.
78. Hussain S, Shahid Z, Foroozesh MB, Sofi UF. E-cigarettes: A novel therapy or a looming catastrophe. Ann Thorac Med. 2021;16(1):73-80. doi: 10.4103/atm.ATM_190_20.
79. US Food and Drug Administration. E-Cigarettes, Vapes, and other Electronic Nicotine Delivery Systems (ENDS). Available from: https://www.fda.gov/tobacco-products/products-ingredients-components/e-cigarettes-vapes-and-other-electronic-nicotine-delivery-systems-ends. Accessed: Jan 20, 2023.
80. World Health Organization. Tobacco: E-cigarettes Q&A. Available from: https://www.who.int/news-room/questions-and-answers/item/tobacco-e-cigarettes. Accessed: Jan 20, 2023.

REVIEW

Air pollution and children's health

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ABSTRACT

Air pollution cannot be considered just a regional problem but is a global issue. In the past 70 years billions of tons of carbon dioxide and millions of metric tons of methane, the two key greenhouse gases, have been emitted annually into the atmosphere from production and burning of fossil fuels for energy and transportation. Gases and Particulate Matters have many adverse effects on human health as a consequence of oxidative stress at the cellular level with alteration of the intracellular redox balance stimulating the production of pro inflammatory cytokines and chemokines.

Children are more susceptible to air pollution than adults and the effects of atmospheric pollutant have been demonstrated on the foetus and pre-school child. Ultrafine particles generated by traffic emissions have been suggested to have particularly bad effects on the airways due to high level of pulmonary deposition and their ability to induce inflammation and oxidative stress.

To improve air quality and reduce air pollution the WHO, supported by 109 scientific Societies, Scientific Associations and Patients' Associations, issued new guidelines to reduce atmospheric pollutants in the world. It is important that all pediatricians continue to advocate for measures to protect the foetus and the child from atmospheric pollution as well as treating the consequences.

IMPACT STATEMENT

Air pollution has had a huge increase in the last 70 years. Gases and Particulate Matter directly irritate the airways but can damage all organs of our body carried by the bloodstream. Proactive initiatives are necessary to protect the fetus and child.

KEY WORDS
Air pollution; childrens' health.

INTRODUCTION

From 5 December to 9 December 1952 a heavy smog (dense polluting fog) affected London causing thousands of deaths from respiratory and heart problems. The fog was so thick that people walking the streets could not even see their feet.

When some chemicals mix with water and air, they may be converted into acids which irritate the upper and lower airways and skin, as well as corroding buildings. That winter had been particularly cold with heavy snowfall in the region and people had burned very large quantities of coal in their houses to keep warm (1).

This “heavy fog” was caused by thermal inversion, the causes of which were unknown at the time. Under normal conditions, smoke rises into the atmosphere and is dispersed. However, if an anticyclone forms it pushes the air down, heating it, thus creating a thermal inversion with the air near the ground colder than the air above it. So, when hot smoke comes out of the chimneys it remains trapped at ground level. Furthermore, during the day the sun’s rays are blocked by the thick fog and thus the air at the ground level remains cold. This favors the condensation of water around solid particles creating a thick toxic fog. In the case of London in those days more polluted air had been carried by the winds from the industrial area towards the city further increasing pollution.

The chronicle of the time does not report how many children suffered from this serious pollution, but this and other episodes that occurred previously paved the way for studies on the effects of pollution on the health of the environment and those that live there.

ATMOSPHERIC POLLUTANTS

Worldwide, in the past 70 years, billions of tons of carbon dioxide and more than 120 million metric tons of methane, the two key greenhouse gases, have been emitted annually into the atmosphere from the production and burning of fossil fuels for energy and transportation. The consumption of energy from fossil fuels in 2020 (oil and derivatives, natural gas, and coal) represents 79%, while the energy deriving from non-fossil fuels [nuclear energy, biological fuels, hydroelectric energy, wind energy and other renewable sources] comprises the remaining 21% (2). Among the biofuels, which represent about 3% of this output, part comes from burning wood used in homes for fireplaces.

Atmospheric pollutants are defined as substances present in the air that can harm animals, plants, materials, and humans (**Table 1**) (3)**.**

Primary pollutants include sulfur dioxide, nitrogen oxides (especially nitric oxide and NO2), reactive hydrocarbons [which also include Volatile Organic Compounds (VOCs) present among indoor pollutants, that are not reported in this paper] and carbon monoxide (CO).

Secondary pollutants include ozone (O3) (which is derived from the interaction in the atmosphere between NO2 and hydrocarbons), sulfuric acid which derives from interaction between atmospheric sulfur and ammonium nitrate. This latter is synthesized from atmospheric nitrogen oxides formed in the atmosphere from the primary pollutants.

The corpuscular part of atmospheric pollutants is defined as Particulate Matter (PM) which is named on the basis of the aerodynamic diameter PM-10 (when particles <10 μm in diameter), in PM-2.5 (particles with diameter <2 .5 μm), and the ultra-active particles (UFP) PM-0.1 (diameter <0.1 μm). All PM-2.5 and PM-0.1 are included in PM-10.

All PM are invisible to the naked eye unlike larger particles which are seen as dust under adequate lighting.

The largest PMs can cause ill effects especially in the upper airways, causing coughing and tearing, PM-2.5 easily reach the terminal bronchioles and alveoli and UFPs enter the alveolar capillaries and then systematically to all the cells of the human body where they may cause significant damage (4, 5).

EFFECTS ON HUMAN HEALTH

Air pollution causes almost the same number of deaths worldwide as tobacco smoke (about 7 million a year) and 70% of mortality attributed to non-communicable diseases (6).

The effects of atmospheric pollutants are manifold (**Table 2**):

Immunological: effects on innate and adaptive immunity with inhibition of interferon-gamma synthesis, stimulation of Th2 and Th17 immunity, increased IgE-mediated allergy**,** and eosinophilic inflammation.

On the upper airways: reduction of mucociliary clearance and reduction of antioxidants in the airway lumen.

Cardiovascular: elevation of inflammatory markers and dysregulation of the autonomic system.

Pulmonary: Production of reactive oxygen species (ROS), alteration of phagocytosis.

Primary pollutants:
sulfur dioxide
nitric oxide
nitrogen dioxide
reactive hydrocarbons (including VOCs)
Secondary pollutants:
ozone (O3)
sulfuric acid
Particulate Matter:
PM10
PM2.5
PM2.5

Table 1. *Air pollutants.*

Epigenetics: epigenetic regulation of physiology and susceptibility via DNA methylation, histone acetylation, micro-RNA and other RNA expression, leading to silencing of some genes and expression of other proinflammatory genes (7).

Not all but most of these effects depend on oxidative stress at the cellular level with alteration of the intracellular red-ox balance and stimulation or blockage of nuclear factor erythroid 2–related factor 2 (Nrf2), which is an emerging regulator of cellular resistance to oxidative stress. Nrf2 controls the basal and induced expression of an array of approximately 200 antioxidant response element–dependent genes to regulate the physiological and pathophysiological outcomes of oxidant exposure (8). The basic sequence of events supporting the dynamic redox equilibrium is, therefore, the following: pollutant (or signal); increase in oxidants and/or electrophiles; signal transduction by a redox-sensitive functional shift in the target; feedback activation of a response switching off the signal; re-establishment of homeostasis. The failure, therefore, to restore the redox steady state constitutes a condition of altered homeostasis and is seen as decreased health condition; it paves the way for the stimulation of another nuclear activator (NfKb) which exerts its proinflammatory activity on the nucleus by stimulating numerous genes to produce proinflammatory cytokines and interleukins (9). There is also genetic regulation of inflammation through receptors and mediators including toll-like receptor 4, TNF-alpha and -beta.

EFFECTS ON THE CHILD

The child is more susceptible to air pollution than the adult because, although the diameter of the pediatric airways is smaller and the tidal volume is less than that of the adult, the respiratory rate is much higher (27 ± 4 breaths per minute in the first 3 years of life, compared with 15 breaths per minute at 18 year of age) and therefore the amount of air ventilated in one minute when related to body weight is much greater than that of adults. For these reasons, pre-school and school-age children inhale more pollutants per kilogram of body weight than adults (10). Furthermore, the respiratory system contains only 20% of the alveoli at birth which increase in number through linear growth.

Finally, the child's immunological system is relatively immature with greater susceptibility to respiratory infections, which worsen the effects of atmospheric pollution and vice versa.

Effects of atmospheric pollutant exposure in pregnancy on the offspring

The studies carried out on pregnant women exposed to environmental pollution, measured through surveys of motoring stations distributed throughout relevant country, have given worrying results about the effects on the fetus and newborn baby.

A review of 13,775 pregnancies in Scotland, using scans performed in the 1st, 2nd, 3rd trimester of pregnancy found that exposure to higher levels of PM-2.5, PM-10, and NO2 were associated with lower infant head size during pregnancy and at birth (11).

A multicenter study in Canada, utilizing scans across all trimesters of pregnancy, reported that the risk of intrauterine fetal growth retardation was increased among women exposed to relatively low levels of am-

Immunological: inhibition of interferon-gamma synthesis, stimulation of Th2 and Th17 immunity.
Upper airways: < mucociliary clearance, < antioxidant
Cardiovascular: dysregulation of automatic system.
Pulmonary: production of ROS.
Epigenetics: DNA methylation, hystone acetylation,micro-RNA.

Table 2. *Main effects of air pollutant on human health.*

bient air pollutants (CO, NO2, and PM-2.5) in urban areas during pregnancy (12).

A meta-analysis that including nearly 3 million births across 14 centers from nine developed countries found that air pollution was associated with a higher risk of low-birth-weight infants. (13).

In a multicenter study performed in Spain on 1295 pregnant women, a correlation was demonstrated between levels of environmental exposure to benzene and NO2 in pregnancy and the airflow obstruction in the offspring in the preschool age (14).

Traffic pollution and child health

Ultrafine particles generated by traffic emissions have been suggested to have particularly bad effects in the airways due to a high level of pulmonary deposition and their ability to induce inflammation and oxidative stress (15).

A questionnaire study involving the parents was carried out on 2490 children aged 3 to 6 in the kindergartens of Changsha, capital of the province of Hunan in central-southern China, with a population of 7.22 million inhabitants, to investigate doctor-diagnosed asthma in preschool children and its relationship with exposure to ambient air pollution in pregnancy and during the first year of life. The result of this study was that the exposure to SO2 (as proxy of industrial air pollution), NO2 (as proxy of traffic pollution) and PM10 in utero and during the first year of life was associated with a higher risk of asthma attacks at 3-6 years of age compared with subjects living in low-exposure areas (16).

A Danish study examined the effect of exposure to air pollution on wheezing symptoms in children under the age of 3 years with genetic susceptibility to asthma. Significant positive associations were found between concentrations of PM-10, NO2, NO(x), CO and wheezing symptoms in infants (aged 0-1 year). Only the traffic-related gases [NO2, NO(x)] showed significant effects throughout the 3 years of life, albeit reducing after the age of 1 year (17).

Between 1999 and 2016 on the metropolitan region Utah's Wasatch Front 146,397 subjects with acute lower respiratory tract infections (ALRI) were studied. In the same period PM-2.5 air pollution concentrations were measured using community-based air quality monitors. The authors found that approximately 77% (n = 112,467) of subjects were 0-2 years of age. The odds of a health care encounter for ALRI for these young children increased within 1 week of elevated PM-2.5 and peaked after 3 weeks. This study demonstrated in a large sample of patients that short-term exposure to elevated PM-2.5 air pollution was associated with greater healthcare utilization for ALRI in young children, older children, and adults (18).

The distance of children's homes from traffic-intensive roads and the risk of wheezing in children has also been investigated.

In one paper it was found that the risk of wheezing episodes is greatest in children who live within 50 meters of a main road in the city (19). In another study, the risk was reportedly higher in children who live within 200 meters of a very busy road (*e.g.*, large ring roads) especially in children who had lived there for more than 2 years (20). Of course, these studies may be confounded by socio-economics status, the wealthy rarely live near major roads!

A multicenter study performed in Atlanta, Georgia, between 1993-2010 had demonstrated a close correlation between concentrations of air pollutants caused by traffic, ozone and PM-2.5 and visits to the Emergency Department for upper respiratory tract infections and pneumonia in children between 0-4 years of age (21).

In a prospective study, 1759 children (average age, 10 years) from schools in 12 southern California communities were recruited and their lung function measured annually for eight years. Over this period, deficits in the rate of increase of FEV1 were associated with exposure to NO2, acid vapor, PM-2.5, and elemental carbon (22).

The same authors subsequently studied three cohorts of children in 3 separate 4-year periods and found an increase in the rate of growth of spirometry in successive 4-year periods which correlated with the reduction of NO2 and of PM-2.5 and less than PM-10, associated with the implementation of air quality-control policies. Significant improvements in lung-function development were observed in both boys and girls and in children with and without an asthma diagnosis (23).

In a meta-analysis of 87 studies, other authors found that exposure, even for a short time, to atmospheric pollutants (O3, CO, NO2, SO2, PM-10 and PM-2.5)

resulted in an increased risk of emergency room visits and hospitalizations in asthmatic subjects. The risk was the same in children and older adults (24).

In a recent official ATS workshop on "Outdoor Air Pollution and New-Onset Airway Disease", the Epidemiology Group found that long-term exposure to air pollution, especially components of traffic-related air pollution such as nitrogen dioxide and black carbon, is associated with onset of childhood asthma. (25)

PROACTIVE INITIATIVES TO REDUCE THE RISKS FROM AIR POLLUTION

To improve air quality and reduce air pollution, which cannot be considered just a regional problem, but a global issue, the WHO issued guidelines in 2005 to reduce atmospheric pollutants and establish risk thresholds for the health of individuals.

In 2005 the Air Quality Guidelines (AQG) recommended not to exceed the concentration of PM-2.5 of 10 µg/m^3, and of NO2 of 40 µg/m^3, in built-up areas, giving no indication for ozone (O3).

After the lockdown of the COVID-19 pandemic period with the resumption of full-time industrial activities, road, air and sea traffic and the war that broke out on February 24TH, 2022 in Eastern Europe, which also caused, among other things, a huge dispersion of methane gas in the Baltic Sea (rupture of the Nord-Stream gas pipelines), the air pollution problem has returned beyond pre-lockdown levels.

Anyway in 2021, the WHO updated the previous guidelines recommending a further reduction of the previously recommended thresholds, indicating as an objective the average annual concentration of PM-2.5 not exceeding 5 µg/m^3, NO2 not exceeding 10 µg/m^3, and the peak seasonal average for 8 h of ozone not exceeding 60 µg/m^3 (**Table 3**) (26).

The reason for this drastic reduction of the thresholds for NO2, O3 and PM-2.5 is consequent on recent studies showing that the adverse effects of air pollution are not only limited to high exposures; harmful health effects can be observed at very low concentration levels, with no observable thresholds below which exposure can be considered safe (27).

These new guidelines have been signed by the Presidents of 109 Scientific Societies, Scientific Associations and Patients' Associations from all over the world.

While the guidelines are not legally binding, we hope that they will influence air quality policy across the globe for many years to come.

It is important that all pediatricians continue to advocate for measures to protect the fetus and child from atmospheric pollution, as well as treating the consequences.

Average annual concentration of PM-2.5 not exceeding 5 µg/m^3.
NO2 not exceeding 10 µg/m^3.
Seasonal average for 8 h of ozone (O3) not exceeding 60 µg/m^3.

Table 3. *2021 WHO Air Quality Guidelines Recommendations.*

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests

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Authorship

Professors Angelo Barbato, Andrew Bush.

Author contributions

ABa and ABu check the international medical databases (PUBMED, EMBASE, COCHRANE), wrote the paper and made the tables.

Ethical approval

Human studies and subjects

N/A.

Animal studies

N/A.

Data sharing and data accessibility

The data underlying this article are available in the article.

Publication ethics

Plagiarism

N/A.

Data falsification and fabrication

All the data correspond to the real.

REFERENCES

1. The Great Smog of 1952 - Met Office. Available from: https://www.metoffice.gov.uk › weather › case-studies.
2. Perera F, Nadeau K. Climate Change, Fossil-Fuel Pollution, and Children's Health. N Engl J Med. 2022;386(24):2303-2314. doi: 10.1056/NEJMra2117706.
3. Kampa M, Castanas E. Human health effects of air pollution. Environ Pollut. 2008;151(2):362-7. doi: 10.1016/j.envpol.2007.06.012.
4. Schraufnagel DE, Balmes RJ, Cowl CT, De Matteis S, Jung SH, Mortimer K, et al. Air pollution and noncommunicable diseases. Chest. 2019;155:409-16. doi: 10.1016/j.chest.2018.10.042.
5. Thurston GD, Kipen H, Annesi-Maesano I, Balmes J, Brook RD, Cromar K, et al. A Joint ERS/ATS Policy Statement: what Constitutes an Adverse Health Effect of Air Pollution? An Analytical Framework. Eur Respir J. 2017;49(1):1600419. doi: 10.1183/13993003.00419-2016.
6. Landrigan PJ. Air pollution and health. Lancet Public Health. 2017;2(1):e4-e5. doi: 10.1016/S2468-2667(16)30023-8. Erratum in: Lancet Public Health. 2017 Feb;2(2):e73. PMID: 29249479.
7. Keswani A, Akselrod H, Anenberg S. Health and clinical impacts of air pollution and linkages with climate change. New Engl J Med Evid. 2022;1(7) doi: 10.1056/EVIDra2200068.
8. Qiang Ma. Role of Nrf2 in Oxidative Stress and Toxicity. Annu Rev Pharmacol Toxicol. 2013;53:401-26. doi: 10.1146/annurev-pharmtox-011112-140320.
9. Sies H., Ursini F. Homeostatic control of redox status and health. IUBMB Life. 2022;74:24-28. doi: 10.1002/iub.2519.
10. Phalen RF, Oldham MJ, Beaucage CB, Crocker TT, Mortensen JD. Postnatal enlargment of human tracheobronchial airways and implications for particle deposition. Anat Rec. 1985;212:368-80. doi: 10.1002/ar.1092120408.
11. Clemens T, Turner S, Dibbenet C. Maternal exposure to ambient air pollution and fetal growth in North-East Scotland: A population-based study using routine ultrasound scans.Environ Int. 2017;107:216-26. doi: 10.1016/j.envint.2017.07.018.
12. Liu S, Krewski D, Shiet Y, Chen Y, Burnett RT. Association between maternal exposure to ambient air pollutants during pregnancy and fetal growth restriction. J Expo Sci Environ Epidemiol. 2007;17(5):426-32. doi: 10.1038/sj.jes.7500503.
13. Dadvand P, Parker J, Michelle L, Bell L, Bonzini M, Brauer M, Darrow LA, et al. Maternal Exposure to Particulate Air Pollution and Term Birth Weight: A Multi-Country Evaluation of Effect and Heterogeneity. Environ Health Perspect. 2013;121:267-373. doi: 10.1289/ehp.1205575.
14. Morales E, Garcia-Esteban R, Asensio de la Cruz O. Intrauterine and early postnatal exposure to outdoor air pollution and lung function at preschool age. Thorax. 2015;70:64-73. doi: 10.1136/thoraxjnl-2014-205413.
15. Schlesinger RB, Kunzli N, Hidy GM, Gotschi T, Jerrettet M. The health relevance of ambient particulate matter characteristics: coherence of toxicological and epidemiological inferences. Inhal Toxicol. 2006;18:95-125. doi: 10.1080/08958370500306016.
16. Deng Q, Lu C, Norbäck D. Early life exposure to ambient air pollution and childhood asthma in China. Environ Res. 2015;143(Pt A):83-92. doi: 10.1016/j.envres.2015.09.032.
17. Andersen ZJ, Loft S, Ketzel M, Stage M, Scheike T, Hermansen MN, et al. Ambient air pollution triggers wheezing symptoms in infants. Thorax. 2008;63(8):710-6. doi: 10.1136/thx.2007.085480.
18. Horne BD, Joy EA, Hofmann MG, Gesteland PH, Cannon JB, Lefler JS, et al. Short-Term Elevation of Fine Particulate Matter Air Pollution and Acute Lower Respiratory Infection. Am J Respir Crit Care Med. 2018;198(6):759-66. doi: 10.1164/rccm.201709-1883OC.
19. Venn AJ, Lewis SA, Cooper M, Hubbard R, Brittonet J. Living near a main road and the risk of wheezing illness in children. Am J Respir Crit Care Med. 2001;164:2177-80. doi: 10.1164/ajrccm.164.12.2106126.
20. McConnell R, Berhane K, Yao L, Jerrett M, Lurmann F, Gilliland F, et al. Traffic, susceptibility, and childhood asthma. Environ Health Perspect. 2006;114(5):766-72. doi: 10.1289/ehp.8594.
21. Darrow LA, Klein M, Flanders WD, Mulholland JA, Tolbert PE, Strickland MJ. Air pollution and acute respiratory infections among children 0-4 years of age: an 18-year time-series study. Am J Epidemiol. 2014;180(10):968-77. doi: 10.1093/aje/kwu234.
22. Gauderman WJ, Avol E, Gilliland F, Vora H, Thomas D, Berhane K, et al. The effect of air pollution on lung development from 10 to 18 years of age. N Engl J Med. 2004;351(11):1057-67. doi: 10.1056/NEJMoa040610. Erratum in: N Engl J Med. 2005 Mar 24;352(12):1276.
23. Gauderman WJ, Urman R, Avol E, Berhane K, McConnell R, Rappaport E, et al. Association of improved air quality with lung development in children. N Engl J Med. 2015;372(10):905-13. doi: 10.1056/NEJMoa1414123.
24. Zheng XY, Ding H, Jiang LN, Chen SW, Zheng JP, Qiu M, et al. Association between Air Pollutants and Asthma Emergency Room Visits and Hospital Admissions in Time Series Studies: A Systematic Review and Meta-Analysis. PLoS One. 2015;10(9):e0138146. doi: 10.1371/journal.pone.0138146.
25. Thurston GD, Balmes JR, Garcia E, Gilliland FD, Rice MB, Schikowski T, et al. Outdoor Air Pollution and New-Onset Airway Disease. An Official American Thoracic Society Workshop Report. Ann Am Thorac Soc. 2020;17(4):387-98. doi: 10.1513/AnnalsATS.202001-046ST.
26. Hoffmann B, Boogaard H, de Nazelle A, Andersen ZJ, Abramson M, Brauer M, et al. WHO Air Quality Guidelines 2021-Aiming for Healthier Air for all: A Joint Statement by Medical, Public Health, Scientific Societies and Patient Representative Organisations. Int J Public Health. 2021;66:1604465. doi: 10.3389/ijph.2021.1604465.
27. 27. Brunekreef B, Strak M, Chen J, Andersen ZJ, Atkinson R, Bauwelinck M, et al. Mortality and Morbidity Effects of Long-Term Exposure to Low-Level PM2.5, BC, NO2, and O3: An Analysis of European Cohorts in the ELAPSE Project. Res Rep Health Eff Inst. 2021;(208):1-127.

CASE REPORT

Ladd syndrome: a case report of uncommon respiratory findings

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ABSTRACT

Lacrimo-auriculo-dento-digital (LADD) syndrome is a rare genetic disorder caused by mutations in fibroblast growth factor (FGF) or FGF receptors. Specifically, FGF10 regulates multiple stages of structural lung morphogenesis, cellular differentiation, and response to lung injury. In case of its dysfunction, an abnormal pulmonary development with alveolar disruption can occur. Here we report the clinical case of a patient with LADD syndrome with pulmonary impairment and history of spontaneous pneumothorax.

IMPACT STATEMENT

A single-center experience in the diagnosis and management of respiratory disease in a patient with LADD syndrome, with the aim to review the pathogenic hypotheses and suggest a practical algorithm for standardized clinical management.

INTRODUCTION

LADD syndrome is a rare, autosomal dominant disease secondary to genetic alterations in FGF. Typical findings include hypoplasia and aplasia of salivary and lacrimal glands, digital and dental abnormalities, and hearing loss. Although there are few reports of lung disease in individuals with LADD syndrome, there is some evidence that genetic pathways are involved in lung development, leading to variable defects in lung structure and function.

CASE REPORT

Here we report the clinical case of a boy, born at term after cesarian section because of fetal distress. A single left kidney was discovered before birth, but no other perinatal problems were reported. At birth he was small for gestational age (2660 g) and had regular adaptation to extrauterine life. He grew up on the

KEY WORDS

LADD syndrome; pneumothorax; childhood interstitial lung disease; chest CT; fibroblast growth factor.

lower centiles for weight and height, with normal neurological development. Several physical features were highlighted over the years: reduced lacrimation, lack of saliva, and abnormalities of the fingers and ears. Despite the strong suspicion of a genetic origin, the genetic test was performed when he was five years old. At that age, he also came for the first time to our outpatient clinic. Respiratory complaints were mainly episodic wheezing associated with atopic substratum (oculorhinitis, eczema, polysensitization to grass and tree pollens, ragweed, house dust mites, dog and cat, and food allergy). Meanwhile, genetic results from NGS (Next Generation Sequencing) identified a mutation in the FGF10 (Fibroblast Growth Factor 10) gene consistent with the diagnosis of LADD syndrome. Inhalation therapy was initiated and modulated (inhaled corticosteroids + long-acting beta-agonists, ICS+LABA) over the years, maintaining good control of respiratory symptoms and lung function. He remained clinically stable until adolescence when he was hospitalized at the age of 15 years old after the sudden onset of thoracic pain and cough. No recent trauma or infection was referred. On physical examination, there was no cardiopulmonary impairment, oxygen saturation was normal with eupnea; on thoracic auscultation, a decrease in normal breath sounds was found in the left upper lung field with no other audible breath sounds. Hence, appropriate investigations and treatment were initiated. Blood chemistry tests showed normal blood count with negative C-reactive protein, the nasal swab was negative for viruses (including SARS-CoV-2), and there were no gas exchange abnormalities on blood gas analysis. Considering his previous clinical history, a chest X-ray was performed, which revealed an apical pneumothorax with a maximum size of 18 mm (**Figure 1**). He was hospitalized for clinical observation, along with antibiotic prophylaxis and bronchodilator. Chest X-ray after 48 hours showed an unchanged size of the pneumothorax, although there was no worsening of the boy's clinical condition. At this point, a more sensitive and specific investigation of the lungs was needed, and a chest CT was organized. Several interstitial anomalies were described, specifically: "*Bilateral reticular pattern alteration of the lung parenchyma at several levels (especially at mantle territories and the ventral segments of both upper lobes); interstitial thickening; pseudocysts in the paramediastinal subpleural and along the ventral territories of the upper lobes (largest identified in the left apical, with a transverse diameter of about 18 mm); aspects of retraction associated with the elements adjacent to the alterations described, particularly on the broncho-vascular structures, in the presence of some bronchiectasis; bilaterally, some nodules of dimensions (<1 cm), as well as a focal filling of the small airways"* (**Figure 2**).

Finally, the left pneumothorax was confirmed as unchanged with a thickness of up to 20 mm in the apical area and 23 mm in the antero-basal area. Based on CT findings, the lung abnormalities oriented toward a "childhood interstitial lung disease" (chILD). According to the diagnostic flowchart established by Bush *et al*. (1), specific blood tests (genetic tests for Cystic Fibrosis (CF), surfactant deficiency, and alpha-1-antitrypsin) were performed, with negative results. The boy's stable condition allowed for invasive investigations such as bronchoscopy lung biopsy avoided, and a wait-and-see policy was followed. Lung ultrasound monitored a progressive reduction of the pneumothorax field with total regression on chest radiography performed after one month. The patient was discharged from the hospital and presented to our outpatient clinic for clinical follow-up after one week, with complete resolution of respiratory signs and symptoms. Home therapy was based on inhaled corticosteroids (fluticasone propio-

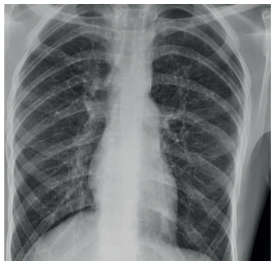

Figure 1. *Left apical pneumothorax.*

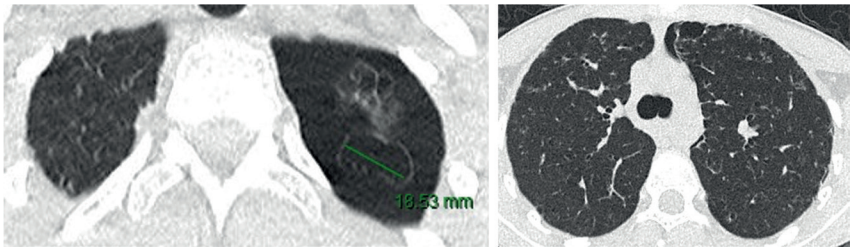

Figure 2. *HRCT showing pseudocysts (on the left image) and reticular pattern with bronchiectasis and nodules (on the right image).*

nate) and long-acting beta-agonists (formoterol) twice daily, as well as avoidance of exercise and air travel because of pneumothorax. Recently, the patient resumed regular physical activity and no longer complained of other respiratory symptoms. Genetic workup was completed with the exclusion of inherited or de-novo defects associated with congenital collagenopathies or folliculin dysfunction.

DISCUSSION

LADD syndrome, also known as lacrimo-auriculo-radio-dental syndrome (LARD) or Levy-Hollister syndrome, is a rare condition secondary to haploinsufficiency of FGF, particularly FGF10, FGF receptor (FGFR)2 and FGFR3 (2). In addition to some typical features involving the salivary and lacrimal glands, ears, teeth, and fingers (**Table 1**), several pulmonary conditions have also been reported anecdotally in individuals with LADD syndrome (3-5), possibly due precisely to FGF10 dysfunction. Indeed, FGF10 has been shown to be a key regulator of lung structure and function in both mouse and human models. Its pleiotropic functions range from morphogenesis of airway branching in utero to proliferation and differentiation of postnatal lung cells, including epithelial-mesenchymal crosstalk (6) and recovery of lung injury after various noxious stimuli (7) (**Figure 3**).
As a result, individuals with LADD syndrome could be affected by a highly heterogeneous spectrum of pneumopathies, from lethal congenital conditions to mild predispositions to chronic lung disease, manifesting after years of environmental “threats” and breathing. Although most of the evidence comes from animal models, in situ hybridization and RNA sequencing techniques in human fetal lung cells have confirmed stable expression of FGF10 during the later stages of human lung development (8), especially during the pseudo glandular and canalicular phase. As a result, many recent works have hypothesized a role of FGF10 signaling alterations in human lung pathologies, such as sudden infant death syndrome (SIDS), early-onset severe interstitial lung disease, bronchopulmonary dysplasia (BPD), lethal lung developmental disorders (LLDD), interstitial pulmonary fibrosis (IPF) or chronic obstructive pulmonary disease (COPD) (9, 10). Recently, an intriguing role has been proposed for some specific modifier genes in regulatory loci, which may epistatically interfere with FGF10 phenotypic expression, triggering or amplifying lung diseases (11). In this regard, Karolak *et al*. recently demonstrated the presence of heterozygous copy-number variant deletions or single-nucleotide variants (SNVs) involving TBX4 or FGF10 in children with pulmonary hypoplasias. Most of them had pulmonary acinar dysplasia (PAD), a rare congenital lung malformation secondary to arrested lung development at the pseudo-glandular stage, in which the presence of a few narrower bronchioles without alveoli leads to early and fatal respiratory failure (11). In this paper, they highlighted the central role of TBX4-FGF10-FGFR2 epithelial-mesenchymal signaling in human lung organogenesis and the potentially lethal effects on lung growth in case of genetic anomalies. Growing awareness of the importance of noncoding variants in developmental lung defects has led some authors to propose diagnostic algorithms that include whole genome sequencing (WGS) to address severe respiratory distress in the early-onset infant, especially if it is progressive and refractory (5). Over the years, this could lead to targeting FGF10 to prevent and treat such severe re-

Hypo/aplasia of parotid and salivary glands	Poor salivary flow, xerostomia, dental caries, absence of Stensen duct
Hypo/aplasia of lacrimal glands and lacrimal duct agenesis	Epiphora, recurrent ocular infections, xeropthalmia, nasolacrimal obstruction, dacryocystocele
Dental anomalies	Microdontia, hypodontia, enamel dysplasia, peg-shaped incisors
Digital anomalies	Hypoplastic, accessory or triphalangeal thumb, clinodactyly, syndactyly
Ear anomalies	Low-set ears, cryptotia, cupped auricles, cochlear hypoplasia, incus and stapes anomalies, hearing loss
Kidney anomalies (rare)	Unilateral renal agenesis
Oral cavity anomalies (rare)	Bifid uvula, bald tongue

Table 1. *Clinical key features of LADD syndrome, with related signs and symptoms.*

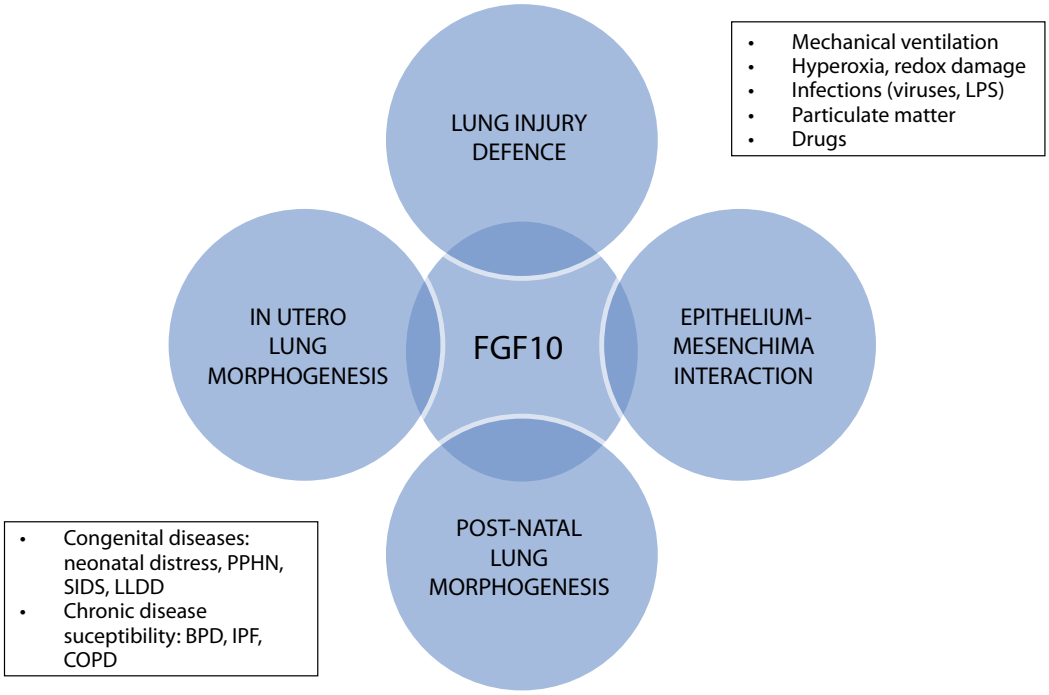

Figure 3. *Role of FGF10 signaling in pulmonary physiology and pathology.*
Redox: oxydative-reductive; LPS: lipopolysaccharides; PPHN: persistent pulmonary hypertension of the newborn; SIDS: sudden infant death syndrome; LLDD: lethal lung developmental disorders; BPD: bronchopulmonary dysplasia; IPF: interstitial pulmonary fibrosis; COPD: chronic obstructive pulmonary disease.

spiratory diseases. However, there are still preclinical concerns about its long-term tumorigenic potential and some evidence of cystic adenomatoid malformations (12) in mouse models overexpressing FGF10. To our knowledge, this is the first report of spontaneous pneumothorax in a child with LADD syndrome. Furthermore, no other cases of emphysematous interstitial lung disease in human children with LADD have been reported. However, De Langhe *et al*. described a similar "emphysema-like" pattern in mouse lungs as a consequence of premature arrest of terminal airway development (13). Whether the pneumothorax depends directly on FGF10 defects or is simply accidental needs further investigation. In any case, emphysematous interstitial areas resulting from altered alveolarization may predispose these subjects to recurrent episodes of pneumothorax and pneumomediastinum. Because of its rarity, specific guidelines for managing LADD syndrome are still lacking. However, the pulmonary disease can seriously impact the health and quality of life of these individuals throughout their lives. In light of this, we suggest a comprehensive 10-step pulmonary workup in the management of individuals with LADD disease:

1. At the first physical examination, perform a complete blood count, biochemistry, c-reactive protein, and capillary blood gases.
2. Test for immunoglobulins, immunoglobulin G (IgG) subclasses, and serum alpha1-antitrypsin, especially in cases of recurrent respiratory infections. Schedule a sweat test and first-line genetic testing for cystic fibrosis transmembrane conductance regulator (CFTR) mutations.
3. Assess lung structure and function with two-projection chest radiography and spirometry with bronchodilator response testing. Perform plethysmography if indicated and DLCO.
4. High-resolution chest CT should be scheduled based on the results of spirometry or chest radiography or in the presence of persistent respiratory symptoms.
5. If there are signs and symptoms of pulmonary hypertension or exertional dyspnea, a complete cardiologic evaluation and possibly an exercise test should be arranged.
6. Reassess the patient clinically twice a year and repeat pulmonary function tests at least once a year.

7. Recommend all vaccinations, especially the annual flu vaccine, and suggest strict avoidance of active and passive smoking, including e-cigarette smoking.
8. If susceptibility to pneumothorax is proven over time, avoid strenuous exercise, contact sports, and diving.
9. Institute respiratory physiotherapy programs and aggressive early treatment of respiratory infections. Assess the need for antibiotic prophylaxis.
10. Share management decisions with geneticists, otolaryngologists, ophthalmologists, dentists, and nephrologists. Suggest genetic counseling before pregnancy and early screening in utero for LLDD.

In conclusion, in this particular clinical case, lung disease could be a manifestation of LADD syndrome or a chance finding. Analyzing the chILD classification system, there is an "unknown" category for cases undiagnosed by biopsy, with inconclusive diagnosis, or with some missing information. Severe lung disease with features of LADD syndrome could represent a distinct syndrome belonging to the chILD group with a yet undetermined genetic etiology. NGS will allow us to discover new entities and better classify childhood disorders. Further studies are needed to define the involvement of pulmonary and bronchial structures in children with LADD syndrome to guide appropriate therapy and management.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests

The Authors have declared no conflict of interests.

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Authorship

Drs. Giulia Roberto, Beatrice Andrenacci, Maria De Filippo, Martina Votto, Amelia Licari, Gian Luigi Marseglia.

Author contributions

GR: conceptualization, writing - original draft. BA: conceptualization, writing - original draft. MDF: writing, review and editing. MV: writing, review and editing. AL: conceptualization, formal analysis, and supervision. GLM: supervision.

Ethical approval

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The Authors confirm that the patient has provided his consent to the anonymous publication of clinical information.

Animal studies

N/A.

Data sharing and data accessibility

N/A.

Publications ethics

Plagiarism

N/A.

Data falsification and fabrication

All the data correspond to the real.

REFERENCES

1. Bush A, Cunningham S, de Blic J, Barbato A, Clement A, Epaud R, et al. European protocols for the diagnosis and initial treatment of interstitial lung disease in children. Thorax. 2015;70:1078-84. doi: 10.1136/thoraxjnl-2015-207349.
2. Rohman E, Brunner HG, Kayserili H, Uyguner O, Nürnberg G, Lew ED, et al. Mutations in different components of FGF signaling in LADD syndrome. Nat Genet. 2006;38(4):414-7. doi: 10.1038/ng1757.
3. Wade EM, Parthasarathy P, Mi J, Morgan T, Wollnik B, Robertson SP, et al. Deletion of the last two exons of FGF10 in a family with LADD syndrome and pulmonary acinar hypoplasia. Eur J Hum Genet. 2022;30(4):480-4. doi: 10.1038/s41431-021-00902-0.
4. Prince LS. FGF10 and Human Lung Disease Across the Life Spectrum. Front Genet. 2018;9:517. doi: 10.3389/fgene.2018.00517.
5. Vincent M, Karolak JA, Deutsch G, Gambin T, Popek E, Isidor B, et al. Clinical, Histopathological, and Molecular Diagnostics in Lethal Lung Developmental Disorders. Am J Respir Crit Care Med. 2019;200(9):1093-1101. doi: 10.1164/rccm.201903-0495TR.

6. Yuan T, Volckaert T, Chanda D, Thannickal VJ, De Langhe SP. Fgf10 Signaling in Lung Development, Homeostasis, Disease, and Repair After Injury. Front Genet. 2018;9:418. doi: 10.3389/fgene.2018.00418.
7. Shi Q, Wang Q, Liu L, Chen J, Wang B, Bellusci S, et al. FGF10 protects against particulate matter (PM)-induced lung injury via regulation of endoplasmic reticulum stress. Int Immunopharmacol. 2022;105:108552. doi: 10.1016/j.intimp.2022.
8. Danopoulos S, Thornton ME, Grubbs BH, Frey MR, Warburton D, Bellusci S, et al. Discordant roles for FGF ligands in lung branching morphogenesis between human and mouse. J Pathol. 2019;247(2):254-65. doi: 10.1002/path.5188.
9. Reitnauer P, Black H, Leigh M, Aylsworth AS. A three generation family with features of lacrimo-auriculo-dento-digital syndrome (LADD) and severe chronic lung disease † 485. Pediatr Res. 1996;39(Suppl 4):83. doi: 10.1203/00006450-199604001-00505.
10. Bzdega K, Karolak JA. Phenotypic spectrum of *FGF10*-related disorders: a systematic review. Peer J. 2022;14;10:e14003. doi: 10.7717/peerj.14003.
11. Karolak JA, Vincent M, Deutsch G, Gambin T, Cogné B, Pichon O, et al. Complex Compound Inheritance of Lethal Lung Developmental Disorders Due to Disruption of the TBX-FGF Pathway. Am J Hum Genet. 2019;104(2):213-28. doi: 10.1016/j.ajhg.2018.12.010.
12. Gonzaga S, Henriques-Coelho T, Davey M, Zoltick PW, Leite-Moreira AF, Correia-Pinto J, et al. Cystic adenomatoid malformations are induced by localized FGF10 overexpression in fetal rat lung. Am J Respir Cell Mol Biol. 2008;39(3):346-55. doi: 10.1165/rcmb.2007-0290OC.
13. De Langhe SP, Carraro G, Warburton D, Hajihosseini MK, Bellusci S. Levels of mesenchymal FGFR2 signaling modulate smooth muscle progenitor cell commitment in the lung. Dev Biol. 2006;299(1):52-62. doi: 10.1016/j.ydbio.2006.07.001.

RESEARCH ARTICLE

Genetic and environmental influences on infant anthropometry at birth and four months of life: evidence from singleton and twin data in the HEALS and earlyFOOD projects

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ABSTRACT

Prenatal and postnatal developmental outcomes are associated with newborn survival and respiratory health and are determined by complex interactions between genes and the environment. However, the contribution of genetic dominance has been scarcely investigated. We aimed to investigate the genetic and environmental influences on infant weight, length, and head circumference in singleton and twin infants at birth and four months of life, using both traditional and behavioral genetics approaches accounting for genetic dominance.

A total of 173 newborns (65 singletons and 54 twin pairs) were consecutively recruited within the HEALS and earlyFOOD projects. At birth and four months of life, developmental outcomes were expressed as standard deviation scores (z-scores), and information about maternal and family factors was collected using questionnaires. We first considered singletons and a randomly selected twin for each pair and run linear regression models at birth and four months of life for each outcome. Then, we considered the twin pairs and estimated behavioral genetic models to disentangle the contribution of additive genetic effects (A), genetic dominance (D), shared (C) and unique (E) environmental influences.

In regression analyses, twin births were significantly associated with lower outcomes at birth ($p < 0.05$) and fertility treatment was significantly associated with higher birth length ($\beta = 0.58$, $p = 0.026$). ACDE models highlighted significant percentages of variance explained by additive genetic factors (23 to 29%). Significant percentages of variance explained by shared environmental factors were observed at four months of life for weight (43%, $p = 0.029$) and head circumference (50%, $p = 0.004$). A significant percentage of variance explained by dominance genetic factors was observed for length at birth (37%, $p = 0.037$).

KEY WORDS
Respiratory-related developmental outcomes; exposome; genome; infants; twins.

The joint assessment of additive and non-additive genetic effects, together with shared and unique environmental influences, provides new insights into the study of the determinants of respiratory-related developmental outcomes such as infant weight, length, and head circumference.

HIGHLIGHTS BOX

What is already known about this topic? Prenatal and postnatal developmental outcomes are associated with newborn survival and respiratory health and are determined by complex interactions between genes and the environment. In this regard, the contribution of genetic dominance has been scarcely investigated. **What does this article add to our knowledge?** We investigated the genetic and environmental influences on infant weight, length, and head circumference, considering singleton and twin data, and using both traditional models and behavioral genetics approaches also accounting for genetic dominance. **How does this study impact current management guidelines?** The joint assessment of additive/non-additive genetic effects, together with shared/unique environmental influences, represents an added value to the study of the determinants of respiratory-related developmental outcomes such as infant weight, length, and head circumference.

INTRODUCTION

Prenatal and postnatal developmental outcomes are important predictors of newborn survival and respiratory health and are determined by complex interactions between maternal and fetal genomes (1), along with intra-uterine and extra-uterine environments (2).

Using traditional approaches (correlation and regression), singleton studies identified potentially modifiable factors associated with birth weight such as maternal age and body mass index (BMI), parity, economic condition, education, and smoking (3, 4). Moreover, genome-wide association studies (GWAS) identified SNPs at several loci of the fetal genotype that were associated with offspring birth weight (5). Also, GWAS detected substantial correlations among birth weight, length, and head circumference that were ascribed to a substantial contribution of shared genetic effects (1). Twins share several genes, as well as the prenatal and postnatal environment, and provide a unique opportunity to disentangle the relative contributions of the genome and the exposome (*i.e.*, the totality of the exposures experienced by an individual throughout life) (6) in explaining inter-individual differences in developmental outcomes (7). Indeed, previous studies have reported considerable geographical differences in the relative contribution of environmental factors, mainly due to marked geographical differences in the inter-individual variation of maternal dietary habits and other family factors (8). Moreover, due to identifiability concerns, comprehensive behavioral genetics approaches accounting for non-additive genetic effects (*i.e.*, dominance) have been scarcely adopted in previous twin studies (9).

In the framework of the HEALS ("Health and Environment-wide Associations based on Large population Surveys") (10) and the earlyFOOD ("Long-term impact of gestational and early-life dietary habits on infant gut immunity and disease risk") (11) projects, we aimed to investigate the genetic and environmental influences on infant weight, length, and head circumference in singleton and twin infants at birth and four months of life, using both traditional and behavioral genetics approaches accounting for genetic dominance.

MATERIALS AND METHODS

Study design

Within the HEALS and earlyFOOD projects, a cohort of singleton and twin infants' mothers was consecutively recruited between May 2018 and September 2021, at the Obstetrics and Gynecology unit of the Buccheri La Ferla Hospital, Palermo, Italy. Mothers and related infants were excluded in the case of heterologous fertilization and lack of written informed consent.

The study was approved by the local Institutional Ethics Committee (Palermo 1, Italy, No. 07/2017). All the participant mothers were informed about all aspects of the research and provided their written consent before study entry.

Procedures

Pregnant mothers were invited to participate in the study upon arrival to the hospital. After birth, a neonatologist recorded weight, length, and head circumference of the infants. Before discharge, a structured questionnaire interview was administered to the enrolled mothers by a trained obstetrician, aiming to collect information about dietary habits, environmental exposures, and characteristics of the pregnancy. Mother-child pairs were also invited to participate in a follow-up visit four months after birth including children's anthropometric measurements and questionnaire evaluation.

Anthropometric measurements

Infant's weight, length, and head circumference were measured within 1 hour from delivery in a supine position by a trained neonatologist. Weight was measured using an electronic baby scale (Seca, Hamburg, Germany), length and head circumferences were measured with an inelastic tape.

The three anthropometric measurements at birth were expressed as standard deviation (SD) scores (z-scores) in relation to gestational age, gender, and primiparous status, using the Italian Neonatal Study (INeS) growth charts (12). Z-scores of the measurements at four months of life were calculated using the sex- and age-specific reference values of the World Health Organization (WHO) (13).

Questionnaire data

The questionnaire at birth included questions about the socio-demographic characteristics of the infants and their mothers, characteristics of the pregnancy, family context, health, lifestyle, and dietary habits of the mother in the four months before delivery. Variables included in the current analysis were gestational age (<37 weeks: preterm birth; ≥37 weeks: full-term birth), zygosity (monozygotic, MZ, and dizygotic DZ: only for twins), delivery mode (C-section or vaginal), primiparous status, maternal age (<35 years or ≥35 years), maternal body mass index (BMI, kg/m^2) before pregnancy (BMI < 25 or BMI ≥ 25), maternal education (graduated or not), maternal smoking during pregnancy, fertility treatment (*in vitro* fertilization (IVF), sperm fertilization), maternal health problems during pregnancy (vomiting, abortion threats, hypertension, gestational diabetes), and 40 maternal dietary habits in the four months before delivery (intake of alcohol, intake of coffee, consumption of 5 portions/day of fruits and vegetables, and consumption of other 37 food categories at least once a week). Included follow-up variables were breastfeeding duration (months) and maternal/paternal smoking.

Statistical analysis

Statistical analyses were carried out using R version 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria). Characteristics of the study participants were summarized through means and SD for quantitative variables and numbers (n) and percentages (%) for categorical variables. Group comparisons were carried out using the Kruskal-Wallis test (continuous variables) and Fisher's exact test (categorical variables). Statistical significance was set at $p < 0.05$.

To summarize the dietary patterns of the mothers during pregnancy, the answers to the 40 questions about the dietary habits were used as input for a clustering algorithm. Gower's general dissimilarity coefficient was used to compute a distance matrix (14), and the partitioning around medoids algorithm was applied (15). The optimal number of clusters, from one to five, was determined using the Silhouette statistic (the larger, the better) (16).

Genetic and environmental determinants of weight, length, and head circumference at birth and four months of life were investigated using two approaches: a conventional regression approach and a behavioral genetics approach (ACDE models).

In the first approach, singletons and only one randomly selected twin for each pair were included in order to address the issue of non-independence between paired observations (using mixed-models may not be advisable since mother-level random effects would overlap with model residuals in singletons). Then, for each outcome, we run linear regression models at birth and four months of life. Factors included in models at both visits were twin birth, maternal age ≥35 years, mother's graduation, and fertility treatment. Factors included only in models at birth (*i.e.*, the factors that were more temporally related with the contextual outcomes) were maternal dietary patterns during pregnancy (identified through the aforementioned cluster analysis), maternal smoking during pregnancy, maternal health problems during pregnancy, and maternal BMI ≥ 25 kg/m² before pregnancy. Factors included only in models at four months of life were breastfeeding duration, maternal/paternal smoking, C-Section, and preterm birth.

In the second approach, twin pairs were included (singletons were excluded) and ACDE models were run (A: additive genes; C: common environment; D: genetic dominance; E: erratic effects). ACDE models are structural equation models for twin data aiming to disentangle the contribution of the human genome and exposome in explaining the variance of a given outcome (7) and can be presented as:

$$\begin{cases} Y_1=\mu_z+aA_1+cC_1+dD_1+E_1 \\ Y_2=\mu_z+aA_2+cC_2+dD_2+E_2 \end{cases}$$

In the above equations, Y_1 and Y_2 are the outcomes in twins 1 and 2, respectively, and parameter μ_z is an intercept dependent on the zygosity status (z = DZ, MZ). A_1 and A_2 are latent random variables accounting for additive genetic influences, C_1 and C_2 are latent random variables accounting for common (or shared) environmental influences (*e.g.*, the intra-uterine environment), D_1 and D_2 are latent random variables accounting for dominance genetic influences. These random variables are assumed mutually uncorrelated within the same twin, and to follow a standard normal distribution. E_1 and E_2 are model residuals accounting for erratic (or unique) environmental factors (*e.g.*, the fetal intra-uterine position); they are assumed uncorrelated with latent variables, and to follow a normal distribution with variance equal to e^2. Consequently, a^2 is the outcome variance explained by additive genetic factors, c^2 is the variance explained by shared environmental factors, d^2 is the variance explained by dominance genetic factors, and e^2 is the variance explained by unique environmental factors (including measurement error).

The correlation between A_1 and A_2 is assumed 1 in MZ twins and 0.5 in DZ twins. The correlation between C_1 and C_2 is assumed 1 both in MZ and in DZ twins. The correlation between D_1 and D_2 is assumed 1 in MZ twins and 0.25 in DZ twins. All other pairs of latent variables (among which E_1 and E_2) are assumed uncorrelated with each other (**Figure 1**) (7). Identifiability concerns were addressed using a previously proposed estimation method (9), and the results were expressed as percentages of the total variance ($a^2 + c^2 + d^2 + e^2$) explained by each factor.

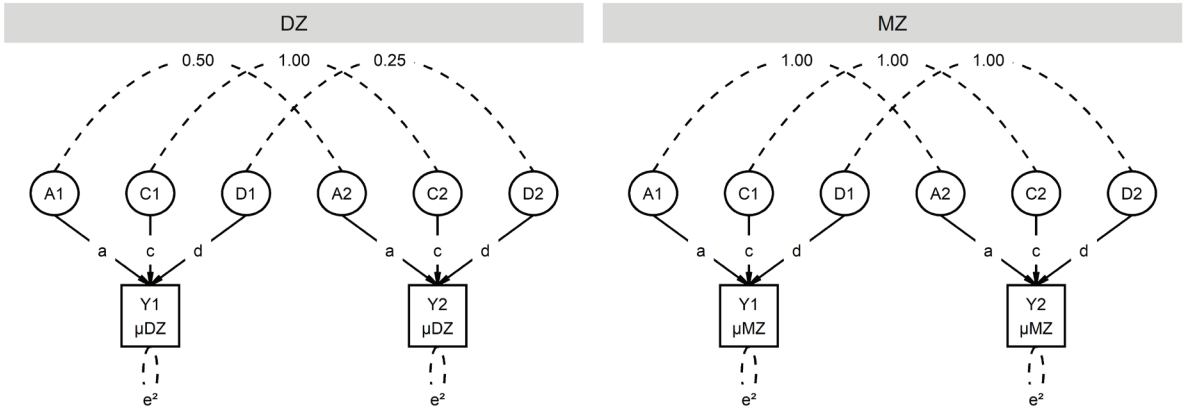

Figure 1. *Path diagram of the ACDE structural equation model for twin data. DZ: dizygotic. MZ: monozygotic. Squares represent observed variables (Y) with their intercepts (μ_{DZ} and μ_{MZ}). Circles represent random variables (A: additive genetic factors, C: shared environmental factors, D: dominance genetic factor), and solid lines refer to their effects (a, c, d). Dashed lines represent non-zero correlations among the random variables, and the variance of unique environmental factors (e^2).*

RESULTS

A total of 173 newborns (65 singletons and 54 twin pairs, 20 MZ, 34 DZ) were included in this study (Table 1). Compared to singletons, in twins (one twin selected at random from each pair) we observed significantly higher percentages of preterm births, C-sections, fertility treatments, maternal problems during pregnancy, and lower outcome z-scores at birth. A total of 93 newborns (29 singletons and 32 twin pairs, 12 MZ, 20 DZ) underwent the follow-up visit after 4 months. Compared to singletons, in twins a significantly lower duration of breastfeeding, higher frequency of maternal/paternal smoking, and lower outcome z-scores were observed (**Table 1**).
Two maternal dietary patterns (9 distinctive dietary habits) were identified by the clustering algorithm (**Figure 2**).

Dietary pattern 1 (hypocaloric diet, 65/119 mothers) was characterized by significantly higher frequency of coffee (83% *vs*. 59%) and fruit/vegetables consumption (91% *vs*. 76%) with respect to dietary pattern 2. Dietary pattern 2 (hypercaloric diet, 54/119 mothers) was characterized by significantly higher frequency of alcohol (17% *vs*. 3%), pasta (78% *vs*. 28%), appetizers (30% *vs*. 5%), sweet pastries (96% *vs*. 14%), cakes (93% *vs*. 22%), chocolate bars (44% *vs*. 20%), and beef consumption (48% *vs*. 28%) with respect to dietary pattern 1.
Conventional linear regression models showed that twin births were significantly associated with lower z-scores for all the outcomes at birth: weight (β = -0.74, $p < 0.001$), length (β = -0.6, $p = 0.001$), and head circumference (β = -0.62, $p = 0.005$) (**Table 2**). Fertility treatment was significantly associated with higher

	Overall	Twins	Randomly selected twins	Singletons	P-value⁺
Birth	n = 173	n = 108	n = 54	n = 65	
Female gender	74 (43)	51 (47)	23 (43)	23 (35)	0.454
Preterm birth (<37 weeks)	73 (42)	72 (67)	36 (67)	1 (2)	**<0.001**
C-Section	119 (69)	94 (87)	47 (87)	25 (38)	**<0.001**
Primiparous	93 (54)	62 (57)	31 (57)	31 (48)	0.357
Maternal age ≥35 years	73 (42)	52 (48)	26 (48)	21 (32)	0.092
Maternal BMI ≥25 kg/m² before pregnancy	47 (27)	34 (31)	17 (31)	13 (20)	0.203
Graduated mother	70 (40)	38 (35)	19 (35)	32 (49)	0.140
Maternal smoking during pregnancy	16 (9)	14 (13)	7 (13)	2 (3)	0.077
Maternal hypercaloric diet during pregnancy	77 (45)	46 (43)	23 (43)	31 (48)	0.586
Fertility treatment	28 (16)	24 (22)	12 (22)	4 (6)	**0.014**
Maternal health problems during pregnancy	56 (32)	46 (43)	23 (43)	10 (15)	**0.002**
Weight z-score	-0.43 (0.98)	-0.69 (0.84)	-0.66 (0.87)	0.00 (1.04)	**<0.001**
Length z-score	-0.54 (0.97)	-0.76 (0.99)	-0.69 (1.00)	-0.16 (0.82)	**0.002**
Head circumference z-score	-0.10 (1.05)	-0.27 (0.92)	-0.27 (0.95)	0.18 (1.19)	**0.024**
Four months of life	n = 93	n = 64	n = 32	n = 29	
Breastfeeding duration, months	2.4 (1.6)	1.9 (1.6)	1.9 (1.6)	3.6 (0.9)	**<0.001**
Maternal/paternal smoking	20 (22)	18 (28)	9 (28)	2 (7)	**0.046**
Weight z-score	-0.49 (1.02)	-0.67 (1.01)	-0.62 (1.05)	-0.09 (0.96)	**0.024**
Length z-score	-0.66 (1.34)	-1.05 (1.12)	-0.86 (1.06)	0.18 (1.39)	**0.002**
Head circumference z-score	-0.55 (1.23)	-0.85 (1.20)	-0.74 (1.07)	0.10 (1.04)	**0.004**

⁺ Randomly selected twins vs. singletons.

Table 1. *Characteristics of the study participants at birth and four months of life, by birth type. Data are reported as n (column %) for categorical variables and mean (SD) for quantitative variables. Significant p-values are in bold.*

	Weight z-score		Length z-score		Head circumference z-score	
Birth (n = 119)	β (p-value)	95% CI	β (p-value)	95% CI	β (p-value)	95% CI
Intercept	-0.19 (0.318)	(-0.56, 0.18)	-0.37 (0.036)	(-0.71, -0.02)	0.08 (0.701)	(-0.33, 0.49)
Twin	**-0.74 (<0.001)**	**(-1.13, -0.34)**	**-0.60 (0.001)**	**(-0.97, -0.24)**	**-0.62 (0.005)**	**(-1.05, -0.19)**
Maternal age ≥35 years	0.14 (0.464)	(-0.24, 0.52)	0.27 (0.131)	(-0.08, 0.62)	0.50 (0.018)	(0.09, 0.92)
Graduated mother	-0.14 (0.465)	(-0.51, 0.23)	-0.01 (0.946)	(-0.36, 0.33)	-0.30 (0.148)	(-0.71, 0.11)
Fertility treatment	0.36 (0.192)	(-0.19, 0.91)	**0.58 (0.026)**	**(0.07, 1.09)**	0.58 (0.060)	(-0.02, 1.19)
Maternal hypercaloric diet during pregnancy	0.32 (0.081)	(-0.04, 0.67)	0.22 (0.185)	(-0.11, 0.55)	0.13 (0.503)	(-0.26, 0.53)
Maternal smoking during pregnancy	-0.01 (0.983)	(-0.69, 0.68)	-0.04 (0.890)	(-0.68, 0.59)	0.12 (0.751)	(-0.63, 0.88)
Maternal health problems during pregnancy	-0.16 (0.452)	(-0.57, 0.26)	-0.23 (0.235)	(-0.61, 0.15)	-0.22 (0.337)	(-0.68, 0.23)
Maternal BMI ≥25 kg/m^2 before pregnancy	0.30 (0.177)	(-0.14, 0.73)	0.11 (0.584)	(-0.29, 0.51)	0.08 (0.749)	(-0.40, 0.56)
Four months of life (n = 61)	β (p-value)	95% CI	β (p-value)	95% CI	β (p-value)	95% CI
Intercept	-0.49 (0.311)	(-1.46, 0.47)	-0.11 (0.850)	(-1.26, 1.04)	-0.41 (0.420)	(-1.41, 0.60)
Twin	-0.46 (0.253)	(-1.25, 0.34)	-0.66 (0.165)	(-1.61, 0.28)	-0.55 (0.192)	(-1.37, 0.28)
Maternal age ≥35 years	0.26 (0.352)	(-0.3, 0.81)	0.36 (0.282)	(-0.30, 1.02)	0.29 (0.318)	(-0.29, 0.87)
Graduated mother	0.34 (0.278)	(-0.28, 0.97)	0.22 (0.551)	(-0.52, 0.97)	0.35 (0.287)	(-0.30, 1.00)
Fertility treatment	0.37 (0.331)	(-0.39, 1.14)	0.51 (0.263)	(-0.40, 1.42)	0.13 (0.742)	(-0.66, 0.93)
Breastfeeding duration (1 month increase)	0.03 (0.788)	(-0.2, 0.26)	0.03 (0.832)	(-0.24, 0.30)	0.06 (0.602)	(-0.17, 0.30)
Maternal/paternal smoking	0.08 (0.843)	(-0.72, 0.88)	0.10 (0.829)	(-0.85, 1.06)	0.06 (0.884)	(-0.77, 0.90)
C-Section	-0.19 (0.594)	(-0.89, 0.52)	-0.42 (0.316)	(-1.26, 0.42)	-0.19 (0.605)	(-0.92, 0.54)
Preterm birth (<37 weeks)	0.01 (0.984)	(-0.85, 0.87)	-0.47 (0.361)	(-1.50, 0.56)	-0.16 (0.725)	(-1.06, 0.74)

Table 2. *Regression models for developmental outcomes at birth and four months of life. Significant effects are in bold.*

	A		C		D		E	
Birth (54 twin pairs, 20 MZ, 34 DZ)	% (p-value)	95% CI	% (p-value)	95% CI	% (p-value)	95% CI	% (p-value)	95% CI
Weight z-score	**27 (<0.001)**	**(13, 40)**	0 (1.000)	(0, 46)	40 (0.055)	(0, 81)	**33 (0.002)**	**(12, 55)**
Length z-score	**29 (<0.001)**	**(18, 39)**	11 (0.594)	(0, 53)	**37 (0.037)**	**(2, 72)**	**23 (0.003)**	**(8, 38)**
Head circumference z-score	**23 (<0.001)**	**(11, 35)**	24 (0.239)	(0, 63)	23 (0.199)	(0, 58)	**30 (0.003)**	**(10, 50)**
Four months of life (32 twin pairs, 12 MZ, 20 DZ)	% (p-value)	95% CI	% (p-value)	95% CI	% (p-value)	95% CI	% (p-value)	95% CI
Weight z-score	**25 (<0.001)**	**(15, 34)**	**43 (0.029)**	**(4, 81)**	15 (0.349)	(0, 48)	**17 (0.026)**	**(2, 33)**
Length z-score	**26 (<0.001)**	**(15, 37)**	33 (0.135)	(0, 77)	22 (0.235)	(0, 59)	**19 (0.025)**	**(2, 35)**
Head circumference z-score	**23 (<0.001)**	**(14, 32)**	**50 (0.004)**	**(16, 85)**	9 (0.533)	(0, 38)	**17 (0.026)**	**(2, 33)**

A: additive genetic factors; C: shared environmental factors; D: dominance genetic factors; E: unique environmental factors.

Table 3. *ACDE models: percentage contributions of genetic/environmental factors to the inter-individual variation in developmental outcomes. Significant effects are in bold.*

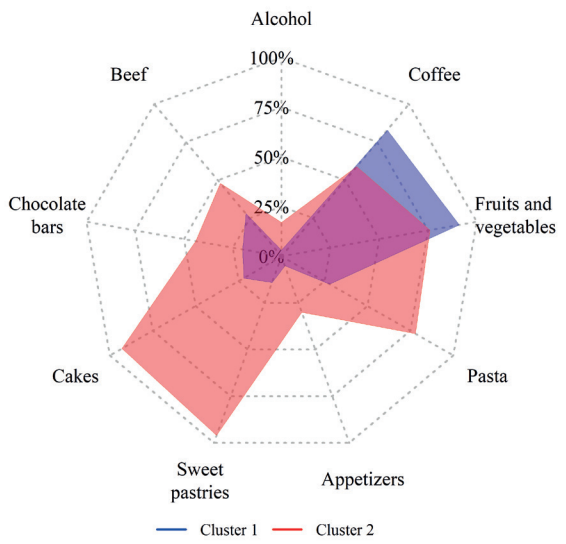

Figure 2. *Percentage distribution of distinctive dietary habits across the two dietary patterns.*

z-scores of length at birth (β = 0.58, p = 0.026). No significant associations were found at four months of life.

For all the outcomes, at both visits, ACDE models (**Table 3**) highlighted significant percentages of variance explained by additive genetic factors (23 to 29%). Significant percentages of variance explained by shared environmental factors were observed at four months of life for weight (43%, p = 0.029) and head circumference (50%, p = 0.004). A significant percentage of variance explained by dominance genetic factors was observed for length at birth (37%, p = 0.037). All the percentages of variance explained by unique environmental factors were significant (23 to 33% at birth, 17 to 19% at four months of life). No significant differences were observed between mean outcomes (model intercepts) in MZ and DZ (data not shown).

DISCUSSION

This study provides new insights into the genetic and environmental influences on infant anthropometric characteristics at birth and four months of life, combining singleton and twin data, and using a comprehensive behavioral genetics approach also accounting for dominance genetic influences. Through the application of conventional regression models, we found lower z-scores in twins and higher birth length following fertilization treatment of the parents. Through the application of ACDE models, we found persistent additive genetic effects through the study follow-up window, dominance genetic effects for length at birth, and significant influences of the shared environment at four months of life.

When considering the pool of individual data referring to singletons and a random representative for each twin pair, we found significantly lower mean z-scores at birth in twins than in singletons. This result is consistent with previous findings and may be ascribed to several aspects of the intra-uterine environment, such as uterine size and placental structure (17, 18).

Length z-scores at birth were significantly higher in infants born to parents who underwent fertilization treatment. Although some studies reported no association (19) or increased risk of adverse birth outcomes associated with IVF (20, 21), others highlighted patterns of increased length during gestation (22), preschool (23), and school age (24) associated with IVF, compared to spontaneous conception. Whether high levels of growth promoting hormones and epigenetic imprinting may play a role needs to be further explored.

For all the respiratory-related developmental outcomes, the analysis of twin data through the ACDE model highlighted a persistent effect of additive genetic factors through the study follow-up window (23 to 29%) and significant, prevalent effects of shared environmental factors only at four months of life. Whereas similar patterns were observed in several studies (25-33), opposite patterns have been observed in other studies where the shared environment was the most influential factor at birth (34-41). Such different findings may be ascribed to population differences in the inter-individual variation of maternal and family factors (8), gene-environment interaction processes (33, 41, 42), or potential confounding effects of the maternal genotype, which may jointly influence the genotype and the shared (*e.g.*, uterine) environment of the infants (43-45). Interestingly, fertility treatment (a component of the shared environment) was associated with birth length in regression models: such association may actually involve genetic mechanisms in the underlying causal pattern, consistently with higher insulin-like growth factor (IGF-I and IGF-II) levels found in IVF children (46).

We found substantial effects of dominance genetic factors at birth, even if the effect was statistically significant only for length. At four months of life, dominance effects were lower and were not statistically significant. Only a few previous studies included the evaluation of dominance effects in their analysis. Some Authors found that the heritability of BMI was about 64%, with a genetic component that

was predominantly non-additive (31). Other Authors included dominance effects in their analysis, but C (shared environmental effects) and D (dominance effect) contributions were estimated separately (using ACE or ADE models) (28, 40). Although the estimated contributions of the D component were consistent with those reported in the current study, proper comparisons are not feasible in the absence of a combined evaluation of C and D.

The simultaneous estimation of A, C, D and E contributions to the inter-individual variation in developmental outcomes represents the main novelty and strength of this study. Another strength is to have considered both singleton and twin data, using traditional and modern approaches at the same time. Indeed, whereas singleton studies are suitable for studying the effects of individual environmental exposures (using conventional regression approaches) or individual genes (using GWAS) on developmental outcomes separately, twin studies allow studying the relative contributions of the whole genome and exposome (7).

The main drawback of this study is the low sample size, especially for the follow-up data, which reduced the statistical power to detect significant effects. Therefore, larger studies are warranted for confirming and strengthening the study results.

CONCLUSIONS

The combined assessment of additive and non-additive genetic effects, together with shared and unique environmental influences, provides new insights into the study of the determinants of respiratory-related developmental outcomes, such as infant weight, length, and head circumference.

A thorough knowledge about the complex interplay between genetic and environmental factors may be useful in order to suggest clinical interventions for altered fetal growth and body composition, which might lead to primary prevention of future health risks.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests

The Authors have declared no conflict of interests.

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Authorship

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Author contributions

SF contributed to the conception of the work, data analysis, and drafting the work; LM, VM, RG, MRD, and ST contributed to data acquisition and revising the work critically for intellectual content; SB, SM, IAM, GV, and SLG contributed to the conception and design of the work, data interpretation, and revising the work critically for intellectual content.

Ethical approval

Human studies and subjects

The study was approved by the local Institutional Ethics Committee (Palermo 1, Italy, No. 07/2017). All the participants were informed about all aspects of the research and provided their written consent before study entry.

Animal studies

N/A.

Data sharing and data accessibility

The data that support the findings of this study may be available from the Corresponding Author upon reasonable request.

Publication ethics

Plagiarism

N/A.

Data falsification and fabrication

All the data correspond to the real.

REFERENCES

1. Beck JJ, Pool R, van de Weijer M, Chen X, Krapohl E, Gordon SD, et al. Genetic meta-analysis of twin birth weight shows high genetic correlation with singleton birth weight. Hum Mol Genet. 2021;30(19):1894-905. doi: 10.1093/hmg/ddab121.
2. Al-Gubory KH. Multiple exposures to environmental pollutants and oxidative stress: is there a sex specific risk of developmental complications for fetuses? Birth Defects Res Part C Embryo Today Rev. 2016;108(4):351-64. doi: 10.1002/bdrc.21142.
3. Mondal B. Risk factors for low birth weight in Nepali infants. Indian J Pediatr. 2000;67(7):477-82. doi: 10.1007/BF02760471.
4. Tavares M, Rodrigues T, Cardoso F, Barros H, Leite LP. Independent effect of maternal birth weight on infant birth weight. J Perinat Med. 1996;24(4):391-6. doi: 10.1515/jpme.1996.24.4.391.
5. Horikoshi M, Beaumont RN, Day FR, Warrington NM, Kooijman MN, Fernandez-Tajes J, et al. Genome-wide associations for birth weight and correlations with adult disease. Nature. 2016;538(7624):248-52. doi: 10.1038/nature19806.
6. Ferrante G, Fasola S, Cilluffo G, Piacentini G, Viegi G, La Grutta S. Addressing Exposome: An Innovative Approach to Environmental Determinants in Pediatric Respiratory Health. Front Public Health. 2022;10:871140. doi: 10.3389/fpubh.2022.871140.
7. Fasola S, Montalbano L, Cilluffo G, Cuer B, Malizia V, Ferrante G, et al. A Critical Review of Statistical Methods for Twin Studies Relating Exposure to Early Life Health Conditions. Int J Environ Res Public Health. 2021;18(23):12696. doi: 10.3390/ijerph182312696.
8. Yokoyama Y, Jelenkovic A, Hur YM, Sund R, Fagnani C, Stazi MA, et al. Genetic and environmental factors affecting birth size variation: a pooled individual-based analysis of secular trends and global geographical differences using 26 twin cohorts. Int J Epidemiol. 2018;47(4):1195-206. doi: 10.1093/ije/dyy081.
9. Jöreskog KG. Classical models for twin data. Struct Equ Model Multidiscip J. 2021;28(1):121-6. doi: 10.1080/10705511.2020.1789465.
10. Health and Environment-wide Associations based on Large population Surveys (HEALS) website. Available from: https://cordis.europa.eu/project/id/603946. Accessed: Oct 4, 2022.
11. Long-term impact of gestational and early-life dietary habits on infant gut immunity and disease risk (early-FOOD) website. Available from: https://www.healthydietforhealthylife.eu. Accessed: 4 Oct, 2022.
12. Bertino E, Spada E, Occhi L, Coscia A, Giuliani F, Gagliardi L, et al. Neonatal anthropometric charts: the Italian neonatal study compared with other European studies. J Pediatr Gastroenterol Nutr. 2010;51(3):353-61. doi: 10.1097/MPG.0b013e3181da213e.
13. Myatt M, Guevarra E. zscorer: Child Anthropometry z-Score Calculator, R package version 0.3.1 [Internet]. 2019. Available at https://CRAN.R-project.org/package=zscorer.
14. Gower JC. A general coefficient of similarity and some of its properties. Biometrics. 1971;27(4)857-71. doi: 10.2307/2528823.
15. Kaufman L, Rousseeuw PJ. Finding groups in data: an introduction to cluster analysis. Hoboken, New Jersey, USA: John Wiley & Sons; 2009.
16. Rousseeuw PJ. Silhouettes: a graphical aid to the interpretation and validation of cluster analysis. J Comput Appl Math. 1987;20:53-65. doi: 10.1016/0377-0427(87)90125-7.
17. Loos RJF, Derom C, Derom R, Vlietinck R. Determinants of birthweight and intrauterine growth in liveborn twins. Paediatr Perinat Epidemiol. 2005;19 Suppl 1:15-22. doi: 10.1111/j.1365-3016.2005.00611.x.
18. Fellman J, Eriksson AW. Birth weight among single and multiple births on the Åland islands. Twin Res Hum Genet Off J Int Soc Twin Stud. 2013;16(3):739-42. doi: 10.1017/thg.2013.14.
19. Xuan NM, Chau LTM, Tuan VM. Compare weight and height of in vitro fertilization-conceived children and spontaneous conceived children. Med Sci. 2020;24(104):2180-5.
20. Yu H, Liang Z, Cai R, Jin S, Xia T, Wang C, et al. Association of adverse birth outcomes with in vitro fertilization after controlling infertility factors based on a singleton live birth cohort. Sci Rep. 2022;12(1):1-10. doi: 10.1038/s41598-022-08707-x.
21. Schieve LA, Meikle SF, Ferre C, Peterson HB, Jeng G, Wilcox LS. Low and very low birth weight in infants conceived with use of assisted reproductive technology. N Engl J Med. 2002;346(10):731-7. doi: 10.1056/NEJMoa010806.
22. Turner S, Maclean E, Dick S, Aucott L, Maheshwari A. Is conception by in vitro fertilization associated with altered antenatal and postnatal growth trajectories? Fertil Steril. 2020;114(6):1216-24. doi: 10.1016/j.fertnstert.2020.06.039.
23. Zhang Y, Hong X, Gao W, Lv J, Yu C, Wang S, et al. A Comparison of Preterm Birth Rate and Growth from Birth to 18 Years Old between in Vitro Fertilization and Spontaneous Conception of Twins. Twin Res Hum Genet. 2021;24(4):228-33. doi: 10.1017/thg.2021.33.
24. Makhoul I, Tamir A, Bader D, Rotschild A, Weintraub Z, Yurman S, et al. In vitro fertilisation and use of ovulation enhancers may both influence childhood height in very low birthweight infants. Arch Dis Child-Fetal Neonatal Ed. 2009;94(5):F355-9. doi: 10.1136/adc.2008.144402.
25. Fujisawa KK, Ozaki K, Suzuki K, Yamagata S, Kawahashi I, Ando J. Genetic and environmental relationships between head circumference growth in the first year of life and sociocognitive development in the second year:

a longitudinal twin study. Dev Sci. 2012;15(1):99-112. doi: 10.1111/j.1467-7687.2011.01097.x.

26. Gielen M, Lindsey PJ, Derom C, Loos RJF, Derom R, Nijhuis JG, et al. Twin birth weight standards. Neonatology. 2007;92(3):164-73. doi: 10.1159/000102055.
27. Ijzerman RG, Stehouwer CD, van Weissenbruch MM, de Geus EJ, Boomsma DI. Intra-uterine and genetic influences on the relationship between size at birth and height in later life: analysis in twins. Twin Res Off J Int Soc Twin Stud. 2001;4(5):337-43. doi: 10.1375/1369052012687.
28. Jahanfar S. Birth weight and anthropometric measurements of twins. Ann Hum Biol. 2018;45(5):395-400. doi: 10.1080/03014460.2018.1526320.
29. Llewellyn CH, van Jaarsveld CH, Plomin R, Fisher A, Wardle J. Inherited behavioral susceptibility to adiposity in infancy: a multivariate genetic analysis of appetite and weight in the Gemini birth cohort. Am J Clin Nutr. 2012;95(3):633-9. doi: 10.3945/ajcn.111.023671.
30. Lunde A, Melve KK, Gjessing HK, Skjaerven R, Irgens LM. Genetic and environmental influences on birth weight, birth length, head circumference, and gestational age by use of population-based parent-offspring data. Am J Epidemiol. 2007;165(7):734-41. doi: 10.1093/aje/kwk107.
31. Segal N, Feng R, McGuire S, Allison D, Miller S. Genetic and environmental contributions to body mass index: comparative analysis of monozygotic twins, dizygotic twins and same-age unrelated siblings. Int J Obes. 2009;33(1):37-41. doi: 10.1038/ijo.2008.228.
32. Vlietinck R, Derom R, Neale MC, Maes H, van Loon H, Derom C, et al. Genetic and environmental variation in the birth weight of twins. Behav Genet. 1989;19(1):151-61. doi: 10.1007/BF01065890.
33. Clausson B, Lichtenstein P, Cnattingius S. Genetic influence on birthweight and gestational length determined by studies in offspring of twins. BJOG Int J Obstet Gynaecol. 2000;107(3):375-81. doi: 10.1111/j.1471-0528.2000.tb13234.x.
34. Chen CJ, Yu MW, Wang CJ, Tong SL, Tien M, Lee TY, et al. Chronological changes in genetic variance and heritability of anthropometric characteristics among Chinese twin infants. Acta Genet Med Gemellol (Roma). 1990;39(4):479-84. doi: 10.1017/s0001566000003706.
35. Mook-Kanamori DO, van Beijsterveldt CEM, Steegers EAP, Aulchenko YS, Raat H, Hofman A, et al. Heritability estimates of body size in fetal life and early childhood. PloS One. 2012;7(7):e39901. doi: 10.1371/journal.pone.0039901.
36. Pietiläinen KH, Kaprio J, Räsänen M, Rissanen A, Rose RJ. Genetic and environmental influences on the tracking of body size from birth to early adulthood. Obes Res. 2002;10(9):875-84. doi: 10.1038/oby.2002.120.
37. Silventoinen K, Pietiläinen KH, Tynelius P, Sørensen TIA, Kaprio J, Rasmussen F. Genetic and environmental factors in relative weight from birth to age 18: the Swedish young male twins study. Int J Obes 2005. 2007;31(4):615-21. doi: 10.1038/sj.ijo.0803577.
38. Silventoinen K, Karvonen M, Sugimoto M, Kaprio J, Dunkel L, Yokoyama Y. Genetics of head circumference in infancy: a longitudinal study of Japanese twins. Am J Hum Biol Off J Hum Biol Counc. 2011;23(5):630-4. doi: 10.1002/ajhb.21190.
39. Smit DJA, Luciano M, Bartels M, van Beijsterveldt CEM, Wright MJ, Hansell NK, et al. Heritability of head size in Dutch and Australian twin families at ages 0-50 years. Twin Res Hum Genet Off J Int Soc Twin Stud. 2010;13(4):370-80. doi: 10.1375/twin.13.4.370.
40. Whitfield JB, Treloar SA, Zhu G, Martin NG. Genetic and non-genetic factors affecting birth-weight and adult body mass index. Twin Res Hum Genet. 2001;4(5):365-70. doi: 10.1375/1369052012533.
41. Dubois L, Ohm Kyvik K, Girard M, Tatone-Tokuda F, Pérusse D, Hjelmborg J, et al. Genetic and environmental contributions to weight, height, and BMI from birth to 19 years of age: an international study of over 12,000 twin pairs. PLOS One. 2012;7(2):e30153. doi: 10.1371/journal.pone.0030153.
42. Dubois L, Girard M, Girard A, Tremblay R, Boivin M, Pérusse D. Genetic and environmental influences on body size in early childhood: a twin birth-cohort study. Twin Res Hum Genet. 2007;10(3):479-85. doi: 10.1375/twin.10.3.479.
43. Hur YM, Luciano M, Martin NG, Boomsma DI, Iacono WG, McGue M, et al. A comparison of twin birthweight data from Australia, the Netherlands, the United States, Japan, and South Korea: are genetic and environmental variations in birthweight similar in Caucasians and East Asians? Twin Res Hum Genet. 2005;8(6):638-48. doi: 10.1375/183242705774860169.
44. van Baal CG, Boomsma DI. Etiology of individual differences in birth weight of twins as a function of maternal smoking during pregnancy. Twin Res Off J Int Soc Twin Stud. 1998;1(3):123-30. doi: 10.1375/136905298320566258.
45. Warrington NM, Beaumont RN, Horikoshi M, Day FR, Helgeland Ø, Laurin C, et al. Maternal and fetal genetic effects on birth weight and their relevance to cardio-metabolic risk factors. Nat Genet. 2019;51(5):804-14. doi: 10.1038/s41588-019-0403-1.
46. Miles HL, Hofman PL, Peek J, Harris M, Wilson D, Robinson EM, et al. In vitro fertilization improves childhood growth and metabolism. J Clin Endocrinol Metab. 2007;92(9):3441-5. doi: 10.1210/jc.2006-2465.

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