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).

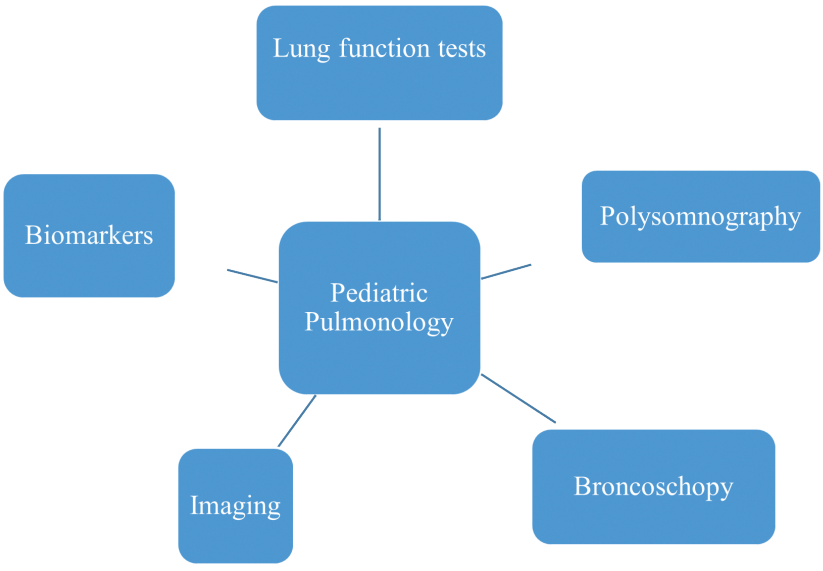

Figure 1. *Expanding fields in Pediatric Pulmonology.*

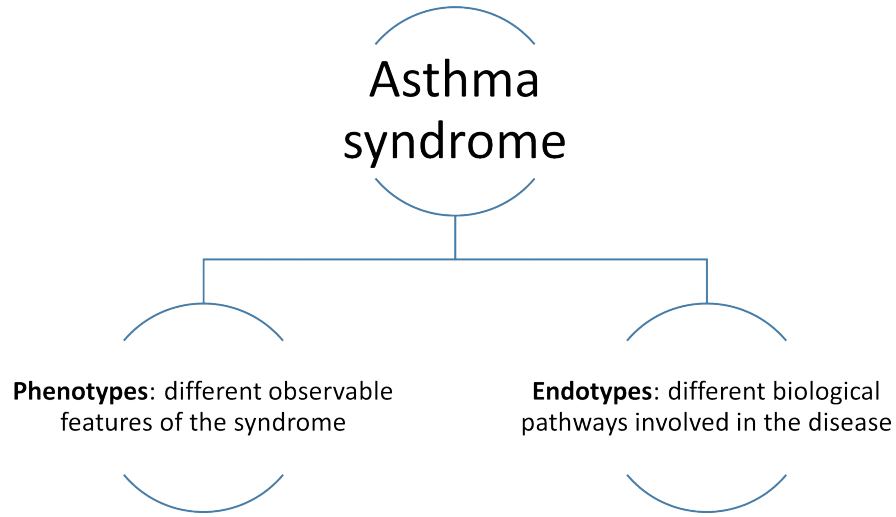

Figure 2. *Phenotypes and endotypes define asthma as a syndrome.*

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,

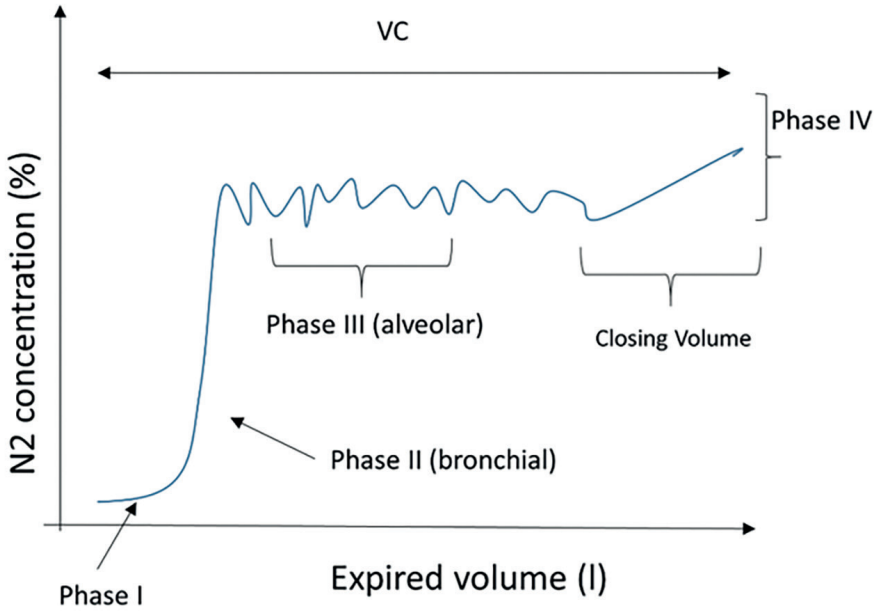

Figure 3. *Normal expirogram during breath washout. The N2 concentration movement in one breath. Four phases can be described: first phase (I) corresponds to the absolute dead space; second phase (II), called the bronchial or transitional phase, represents the alveolar gas arriving from the lung unit; 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,

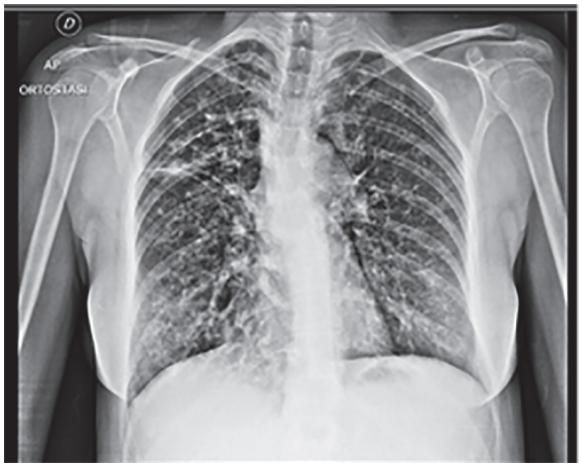

***Figure 4**. 28-year-old girl (F508del/F508del) with cystic fibrosis. Chest X ray before therapeutic bronchoscopy.*

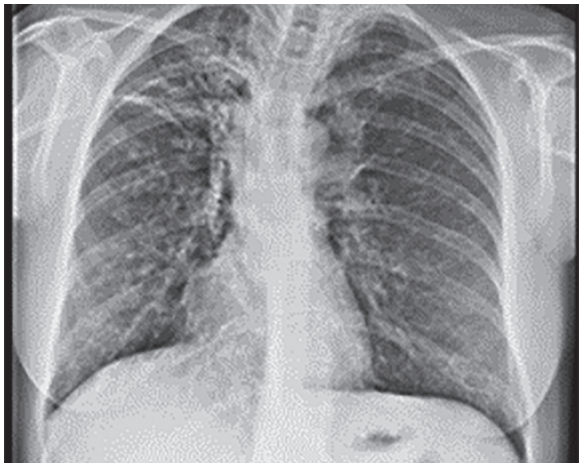

***Figure 5**. 28-year-old girl (F508del/F508del) with cystic fibrosis. Chest X ray after two months from therapeutic bronchoscopy.*

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.

***Table 1**. Summary of 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)

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

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

Data falsification and fabrication

All the data correspond to the real.

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