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Utility of biomarkers in the diagnosis and monitoring of asthmatic children

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ABSTRACT

Asthma imposes a heavy morbidity burden during childhood; it affects over 10% of children in Europe and North America and it is estimated to exceed 400 million people worldwide by the year 2025. In clinical practice, diagnosis of asthma in children is mostly based on clinical criteria; nevertheless, assessment of both physiological and pathological processes through biomarkers, support asthma diagnosis, aid monitoring, and further lead to better treatment outcomes and reduced morbidity. Recently, identification and validation of biomarkers in pediatric asthma has emerged as a top priority across leading experts, researchers, and clinicians. Moreover, the implementation of non-invasive biomarkers for the assessment and monitoring of paediatric patients with asthma, has been prioritized; however, only a proportion of them are currently included in the clinical practise. Although, the use of non-invasive biomarkers is highly supported in recent asthma guidelines for documenting diagnosis and supporting monitoring of asthmatic patients, data on the Pediatric population are limited.

In the present report, the Pediatric Asthma Committee of the World Allergy Organization (WAO), aims to summarize and discuss available data for the implementation of non-invasive biomarkers in the diagnosis and monitoring in children with asthma. Information on the most studied biomarkers, including spirometry, oscillometry, markers of allergic sensitization, fractional exhaled nitric oxide, and the most recent exhaled breath markers and "omic" approaches, will be reviewed. Practical limitations and considerations based on both experts' opinion and critical review of the literature, on the utility of all "well-known" and newly introduced non-invasive biomarkers will be presented. A critical commentary on biomarkers' use in diagnosing and monitoring asthma during the COVID-19 pandemic, cost and availability of biomarkers in different settings and in developing countries, the differences on the biomarkers use between Primary Practitioners, Pediatricians, and Specialists and their role on the longitudinal aspect of asthma is provided.

Keywords: Asthma, Children, Pediatric asthma, Non-invasive biomarkers

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INTRODUCTION

Asthma in children is a heterogenous disorder underpinned by chronic airway inflammation; however, considerable variability in both symptoms and natural history exists. It is well recognized that most asthma cases begin in childhood, while the disease causes a significant morbidity and health care expenditure in this age group.[1] The identification of different phenotypes in pediatric asthma, with distinct clinical presentation, pathophysiologic mechanisms and response to therapeutic interventions, has led to the recognition of underlying molecular pathways (endotypes) and related specific indicators (biomarkers). A biomarker is "a defined characteristic that is measured as an indicator of a normal biological process, pathological process or response to an exposure or intervention, including therapeutic interventions".[2] In clinical practice, diagnosis of asthma in children is mostly based on clinical criteria; nevertheless, assessment of both physiological and pathological processes through biomarkers, may support asthma diagnosis, aid monitoring, and further lead to better treatment outcomes and reduced morbidity.[3]

Recently, identification and validation of biomarkers in pediatric asthma has emerged as a top priority across leading experts, researchers, and clinicians from countries of different income.[4] The invasiveness of certain biomarkers, such as bronchoalveolar lavage and bronchial biopsy, limits considerably their use in most asthmatic children with controlled or partly controlled asthma.[5] Limited data exist for sputum induction, a semi-invasive and technically difficult method especially for younger children, and only for children with severe, therapy resistant asthma.[6] Thus, utilization of noninvasive biomarkers is currently becoming reinforced in guidelines such as the Global Initiative for Asthma (GINA) and National Institute for Health and Care Excellence (NICE) for supporting diagnosis, classification, and follow-up of asthma in the pediatric population.[7] Despite emerging evidence on new biomarkers, only a few are currently used in the clinical practice, while many of them are applied only in highly specialized centers.[8] The most studied biomarkers include spirometric and markers of allergic sensitization and fractional exhaled nitric oxide (FeNO);[9] although, the latter may have a role in identifying a type 2 inflammatory treatable trait, its role on monitoring asthma is still debated.[10] Data on oscillometry in the diagnostic algorithm of children with asthma are encouraging.[11] The more recent exhaled breath markers and "omic" approaches provide information on specific asthma phenotypes, however, their clinical applicability is still unresolved.[12]

Although recent reports have extensively dealt with the utility of biomarkers in adult asthma, respective information for Pediatrics is significantly less. It is well accepted that the use of non-invasive biomarkers has a potential clinical utility, contributing to tailored management of asthmatic children.[13] In the present report, the Pediatric Asthma Committee of the World Allergy Organization (WAO), aims to summarize and discuss available data in respect to the utility of implementing non-invasive biomarkers in the diagnosis and monitoring in pediatric asthma, discussing practical limitations and considerations, the potential utility of newly introduced biomarkers and practices in different parts of the world, and clinical settings.

AVAILABLE DATA FOR DIAGNOSING AND FOLLOWING ASTHMA IN CHILDREN BY COMMONLY USED NON-INVASIVE BIOMARKERS

Peak expiratory flow (PEF) measurement

Peak expiratory flow (PEF) is a simple measure of the maximal flow rate that can be achieved during forceful expiration following full inspiration.[14] Although less reliable than spirometry, PEF measurement aids in asthma diagnosis and monitoring, seen especially during the COVID-19 pandemic, due to restrictions in spirometry use in clinical settings.[15]

Measured over 1–2 weeks, PEF aids in demonstrating expiratory airflow reversibility, by either diurnal (>13%), day to day, or visit to visit (>15%) variability or by a>15% drop following exercise challenge compared to the baseline PEF values. PEF improvement can also confirm reversible airflow limitation within 10–15 min after inhalation of a rapid-acting bronchodilator.[16] In children, it is highly recommended to interpret PEF values relative to the personal best reading, as the highest PEF over

the preceding 2 weeks, if one is known.[17] It has been suggested that PEF assessment coupled with a written action plan, can be used for monitoring asthma control, evaluating triggers and worsening symptoms, and monitoring recovery following an exacerbation.[18] Long-term PEF monitoring has been used for early detection of exacerbations, in case of poor symptom perception, in patients with prior abrupt severe exacerbations and in difficult-to-control or severe asthma. However, studies evaluating the usefulness of PEF monitoring for improving asthma outcomes have yielded conflicting results, since it is well accepted that PEF long term measurements are associated with poor compliance. Recently, smart devices and applications, with relatively low or now cost for the patient, have been introduced into the market aiming at improving adherence to self-monitoring of PEF, however, the majority of them failed accuracy testing.[19]

Besides history and physical examination, PEF can serve as an objective measure of exacerbation severity in patients >5 years (mild to moderate exacerbation if PEF >50% and severe if PEF≤50% of predicted normal value or personal best). However, in a prospective single-center cohort study, only 48% of children with acute asthma, that were able to perform PEF measurements before and after the use of bronchodilators, provided valid information at both time points.[20] Despite several limitations on PEF utility, we recommend, and this agrees with several guidelines,[21-23] that PEF assessment can guide chronic management decisions and stratify risk in acute exacerbations, especially in areas where spirometry is not practical or available.

Spirometry

Spirometry is the most frequently used clinical laboratory test for assessing and monitoring asthma in children. Spirometry can contribute to the diagnosis of asthma, moreover in the presence of asthma-related symptoms. Nevertheless, spirometry can range within normal values in asthmatic children, while spirometric alterations in asthma are not unique or constantly repeated.

To allow comparisons over time and between locations, spirometry should be performed according to established guidelines and standards.[24,25] For asthma, the most useful spirometric variable for assessing airflow obstructive pattern, is Forced Expiratory Volume (FEV) in the first second (FEV_1), as it presents the best combination of low variability and good sensitivity. This combination results from moderate expiratory effort producing flow limitation over most of the expiratory flow-volume curve, so that further effort has little effect on the level of flow. Longitudinal measurements of FEV_1 are best expressed as percent predicted for height, and standards for this should be appropriate for the gender and ethnicity of the subject.[26] Other useful spirometric variables are: Forced Vital Capacity (FVC), where low levels are indicative of airway restriction as well as airway obstruction; FEV_1/FVC– low levels indicate airway obstruction and are consistent with asthma, chronic obstructive pulmonary disease (COPD), and bronchiectasis; and Forced Expiratory Flow $(FEF)_{25-75}$ – measured over the part of the flow-volume curve, is more reflective of small airway caliber and in asthma it has high sensitivity and variability. For all spirometric variables, the cut-off between normal and abnormal limits, should be taken from reference values that are appropriate for age, gender and ethnicity.[24] Bronchodilator response (BDR), which represents an increase in baseline values of at least 12%, 15–20 min following 2–4 puffs of b2 short acting agonist (SABA), is useful in establishing asthma diagnosis, while an increase in FEV_1 or FEV_1/FVC values of over two subject-specific Z scores have been suggested as indicators of airflow limitation and reversibility, although the latter is rarely used in the clinical practice.[27] More practically, an approximately 12% or 200 mL increase in FEV1 can be used as a measure of BDR, as suggested by the European Respiratory Society (ERS) recommendation.[25] A positive BDR is highly suggestive of asthma, but not diagnostic, as increases can also occur due to poor initial spirometric technique or airway clearance between measurements.

Spirometry in preschool children is possible with careful training of the child and adherence to guidelines. Due to young children having relatively larger airways compared to lung volume, and the fact that the expiration phase may last less than 1 sec, the $FEV_{0.5}$ has been proposed as a valid spirometric variable in asthmatic preschoolers. Although we believe that the specific measurement can be used in this age group, more data are warranted to support its widespread clinical utility.[28]

Impulse oscillometry

Impulse Oscillometry (IOS) measures the mechanical resistance of the respiratory system during a spontaneous breathing at tidal volume.[29] The technique is based on the use of a loudspeaker to send small-amplitude oscillatory pressure waves at the entrance of the airway and, from the recording of the pressure and flow variations that these impulses impose to the respiratory system, to be able to calculate the impedance (Zrs) a concept that encompasses both the "resistance" (Rrs) and the Reactance (Xrs) of the respiratory system. Though less data are available due to the newness of the technique, forced oscillometry, or airway oscillometry has the added advantage of portability and ease of use, and is of particular use in young children, where it is difficult to maneuver reproducible lung function measures.[30]

IOS has been used for the diagnosis and monitoring of respiratory tract diseases such as asthma, virus-induced wheezing,[11] bronchopulmonary dysplasia,[31] cystic fibrosis,[32] eosinophilic bronchitis,[33] bronchiectasis,[34] vocal cord dysfunction,[35] and tracheal stenosis,[36] etc., and to assess bronchial hyperresponsiveness [37] and the response to asthma treatment.[38,39]

In short, in an obstructive problem that affects the central airways, the main affectation will be detected in the Resistance curve, which will be significantly increased in parallel to the reference values, with similar increases in R5 (Total Resistance) and of the R20 (Central Resistance); that is, the resistances are increased independently of the oscillation frequency. Resistances at 5 Hz are increased when the value obtained is greater than 150% of the theoretical value, which, compared to spirometry, would be equivalent to a 20% drop in FEV_1. On the contrary, no relevant changes will be detected in the Reactance curve (which will be superimposed on the reference values). In the case of peripheral airway obstruction, R5 is increased, but as this occurs at the expense of peripheral airway involvement, central resistance (R20) is normal. And, because of the peripheral involvement, the capacitive reactance of the lung is decreased so that the reactance curve is displaced downwards with respect to the predicted values, the X5 is also decreased, the Frequency of Resonance (Fres) displaced to the right, and the area under the reactance curve (AUC-AX) increased (Table 1). It is necessary to highlight that those central and peripheral obstructive patterns are prototypical patterns, which are not very frequently seen in daily clinical practice, where what is usually observed are mixed patterns.

Additionally, the area under the AX curve (AUC) reflects the changes of the obstruction in the peripheral airway and allows a more sensitive assessment of the bronchodilator response and changes induced by long-term treatments. It is perceived as a more sensitive marker of peripheral airway obstruction compared to the isolated use of R5 or X5 and can better differentiate between exacerbation and stable patient in asthma, and between asthmatic and healthy patient.[40]

There are no unanimously accepted criteria in relation to the bronchodilator test in children but, a bronchodilator response is considered positive if,

	Total Resistance (RS)	Central Resistance (R20)	Peripheral Reactance (X5) (*)	AUC Reactance (AX)
Central obstruction	↑↑	↑↑	=	=
Peripheral obstruction	↑↑	=	↑↑	↑↑
Mixed obstruction	↑↑↑	↑↑	↑↑	↑↑
Restriction	=	=	↑↑	↑↑

Table 1. Interpretation of impulse oscillometry using prototypical changes in the values of the Total Resistance (R5), Central Resistance (R20), peripheral Reactance (X5) and AUC of Reactance (AX) that can usually be observed depending on the underlying involvement of the airway. *(*) Being a negative value, although X5 mathematically decreases its absolute values increase compared to the theoretical ones*

after administration of the bronchodilator agent, there is a decrease in the R5 value greater than 20% and greater than 15% in the R10.[41]

In conclusion, IOS is a highly sensitive technique in respect to asthma diagnosis and monitoring, providing complementary information to that obtained through conventional respiratory function techniques such as spirometry in all age groups during childhood; while its use is increasing mostly in specialized centers in Europe, United States, Asia, and Australia[42]

Fractional exhaled nitric oxide (FeNO)

To date, the Fractional concentration of Nitric Oxide in the exhaled air (FeNO) has been suggested as a biomarker that aids in the diagnosis and monitoring the level of control in childhood asthma.[3,43] Studies have established the positive correlation between FeNO levels and eosinophilic airway inflammation as assessed by blood, sputum and bronchoalveolar lavage eosinophils, supporting the diagnosis of T2 asthma, both in children and adults.[44,45] FeNO reflects IL-13 mediated inflammation, as shown by reduction in the FeNO levels with biologics specifically inhibiting that cytokine.[46] Moreover, in preschool children FeNO may be helpful in objectively defining different wheezing phenotypes, while increased FeNO values have strongly associated with increased risk of later asthma persistence/development. [47,48] In school aged children the diagnostic value of FeNO is high particularly in atopic children with asthma-associated symptoms, while children in the highest FeNO quartile have more than a 2-fold increased risk of new-onset asthma later in life.[49,50] International guidelines and technical standards are available for FeNO measurement[51,52] and its clinical interpretation.[53] As exhaled NO values are highly flow dependent, all measurements should be performed at a standardized exhalation flow rate of 50 mL/s. Patients exhale against a positive counter pressure of 10 cm H_2O to avoid cross-contamination with nasal NO. To guide the subjects in performing a valid exhalation maneuver, the flow parameters are controlled by visible and/or audible feedback. The procedure is noninvasive and can be easily and effectively performed starting from 5 to 6 years of age.

According to the American Thoracic Society (ATS) guidelines,[53] FeNO levels, expressed in part per billion (ppb), less than 25 ppb (<20 ppb in children) indicate that eosinophilic inflammation is less likely, while values > 50 ppb (>35 ppb in children) indicate that eosinophilic inflammation and corticosteroid-dependent inflammation are likely. Intermediate FeNO values (25–50 ppb and 20–35 ppb in children) should be interpreted cautiously and with reference to the clinical context. Moreover, a reduction of at least 20% in FeNO for values over 50 ppb or more than 10 ppb for values lower than 50 ppb as the cut point, indicate a significant response to anti-inflammatory therapy, such as ICS.

Epidemiological and mechanistic studies suggest that FeNO is a valid biomarker for eosinophilic airway inflammation in asthma, and its use is increasing in clinical practice due to the growing availability of affordable and portable devices. However, clinical trials in children have not consistently confirm the beneficial effect of adding FeNO to a symptom-based approach, thus its routine use in monitoring pediatric asthma is still debated. There are several reasons why FeNO has apparently failed to translate from promising biomarker to clinically useful tool, and one reason may be a lack of understanding of confounding endogenous and exogenous factors that influence FeNO levels.[54] Furthermore, FeNO levels in children may be considered as a moderately reliable biomarker for type-2 inflammation, rather than a marker for asthma itself. Future studies need to obtain appropriate reference values of different ethnic group or geographical different areas, as well as to rethink FeNO cut-off values in children, considering the effect of increasing age and height on developing airways.

Bronchial hyperresponsiveness

Bronchial hyperresponsiveness (BHR) has long been established as a cardinal characteristic in all asthma phenotypes. The utility of non-specific BHR for diagnosing asthma has been well established, while respective data on its use for monitoring asthma activity are scarce.[55] Assessment of non-specific BHR by stimuli directly affecting the smooth muscle, such as methacholine is highly sensitive in ruling out asthma when BHR is negative, while stimuli acting indirectly on inflammatory cells and nerves, such as exercise, adenosine, mannitol, cold air etc., have been long used in Pediatrics for assessing subjects with symptoms

highly suggestive of asthma, but with normal spirometry. Moreover, determination of indirect bronchial challenges has been used for diagnosing exercise induced asthma, adjusting anti-inflammatory treatment and to discriminate other asthma – mimicker conditions such as laryngeal dysfunction.[56] BHR has also been assessed as a marker of asthma persistence in school aged children with asthma.[57,58] Determination of non-specific BHR represents an important biomarker in diagnosing asthma, especially due to its negative predictive value and although we support its role in monitoring morbidity in asthmatic children, caveats regarding the technical requirements of the method such as high cost equipment, Moreover, difficulties in cooperation, the time consuming methodology, the need for acute interpretation of the results, and the effect of concurrent medications including inhalers/biologics and environmental factors, have underutilized the test in the clinical practice.[42]

Allergy assessment: SPTs, sIgE, eosinophils, ISAAC, alex test

Aero-allergen sensitization, assessed either by skin prick test (SPTs) or by total and specific IgE determination, is an established marker for atopy and a significant predictor for the differential response to inhaled corticosteroids as a maintenance therapy, even in preschoolers with asthma and to anti-IgE monoclonal antibody therapy in severe asthmatics.[59] IgE values can be easily determined in most of the clinical settings, although interpretation of the results should be cautiously performed, since sensitization, ie, positive IgE values, do not necessarily suggest clinical disease. In addition, SPTs are easily and routinely performed by allergy experts, causing minimal discomfort in children with respiratory allergy. Nevertheless, interpretation needs to be cautious and related to asthma morbidity, since sensitization per se might not be clinically relevant.[60]

Blood eosinophils is an easy point of care test, even in primary care and is currently considered as a proxy for airway eosinophilias, and response to asthma treatment, more so steroids and anti-eosinophilic monoclonal antibodies.[61] Recently, the utility of blood eosinophils on asthma diagnosis in preschoolers with asthma, has been suggested by treatment testing (steroid responsiveness),[62] with increased blood eosinophils ($\geq$250/mm^3) being strongly associated with frequent asthma attacks/nocturnal symptoms and persistent wheezing later in life.[63] It should be noted however, that blood eosinophils do not accurately correlate with airway eosinophilia, more so in preschoolers with asthma.[64] Although studies in adults have shown that peripheral and airway eosinophilia are significantly correlated, data in children are equivocal, since elevated eosinophil counts can be detected even in the healthy state, thus altering the cut-off normal limit. In addition, atopy per se, and asthma triggers/activity impact the peripheral eosinophil values.[65]

More recently, component-resolved diagnostic assays have been introduced for simultaneous measurement of several allergen components, due to their innovative microarray technology (biochip).[66,67] The Thermo Fisher ImmunoCAP ISAC (Immuno-solid-phase Allergen Chip) contains 112 allergens from 51 (or more recently from 48) allergen sources, while the more recently developed MADx Allergen Explorer (ALEX), contains 282 allergens: 156 extracts and 126 components. The Euroline microstrips is based on the immunoblot technique. The major advantage of such comprehensive analysis is the identification of clinically relevant sensitizations, which supports asthma diagnosis.[68] As with other allergy–related assays, positive results should be interpreted in the presence of asthma-related symptoms, although sensitization to major allergen proteins, is of highly decisive clinical importance. Multiplex tests are still high costly, and clinicians should be adequately trained and familiarized with allergen protein families. Moreover, patterns of sensitization may vary considerably between different geographical areas, thus clinician experts should be aware of the local molecular epidemiology for interpreting the results properly.[69]

EMERGING BIOMARKERS OF ASTHMA IN PEDIATRICS

The demand for using noninvasive biomarkers for pediatric patients with asthma is steadily increasing, due to inefficient diagnostic indices in this age group and the increased need to identify specific asthma endotype for better disease monitoring. During the last years emerging biomarkers for the diagnosis and monitoring of

asthma have been explored for clinical practice, with limited however data in children. We report data for emerging biomarkers which are mainly derived from cells infiltrating the airway tissues and the exhaled air, potentially reflecting different aspects of airway inflammation.

Sputum

Sputum cell counts and/or soluble mediator assays could serve as potential biomarkers of allergic inflammation. Eosinophil perioxidase in sputum samples has been shown to represent a valid and repeatable surrogate marker of eosinophils and/or eosinophil degranulation in adults with asthma and COPD.[70] However, relevant studies in children are scant and most current speculations are extrapolated from adult's studies, while the currently available biomarkers for Type 2 inflammation are insufficiently sensitive and specific, even in adults.[71] Moreover, sputum sampling is technically challenging in children and procedures to process and assess cells need specific technical requirements, thus limiting the usefulness of the specific biomarker mostly for research purposes.

Cellular biomarkers

A multivariate analysis showed that asthma control was independently associated with individual fluctuations in sputum eosinophil count,[72] although a poor correlation between blood and sputum eosinophil counts was revealed in children.[71] Elevated sputum eosinophil count was linked to a significantly shorter time to first exacerbation, greater risk of exacerbation in one year of follow-up[73] and accelerated FEV_1 decline.[74] Spontaneous sputum eosinophilia (>3%) was recently reported in severe asthma, however the concomitant existence of food allergy in children is also associated with higher FeNO and sputum eosinophilia, suggesting enhanced eosinophilic inflammation.[75] Sputum basophil estimation may represent an additional indicator of T2-high asthma since it correlated positively with sputum eosinophils and inversely with sputum neutrophils in one study.[76] There is no reliable marker of non-TH2 asthma endotype although sputum neutrophil counting may have some value; exposure to certain environmental conditions such as cold was associated with increased airway obstruction and upregulation of sputum neutrophils.[77]

Potential soluble biomarkers

Vascular endothelial growth factor (VEGF), a key inducer of angiogenesis, was found elevated in induced sputum during acute pediatric asthma exacerbations.[78] Inverse correlations were demonstrated between sputum high mobility group box-1 (HMGB1) levels and pulmonary function indices in severe asthma.[79] Moreover, eosinophilic asthma in children was associated with increased expression of sputum clusterin, a sensitive cellular biosensor of oxidative stress[80] and thymus and activation-regulated chemokine (TARC). The latter correlated positively with sputum eosinophils, serum total IgE, FeNO, and bronchodilator response and inversely with FEV_1/FVC.[81] In non-T2 pediatric asthma, sputum overexpression of IL-10 and IFN-γ, TNF-α and thymic stromal lymphopoietin (TSLP) and interleukin (IL)-26 protein reflected disease control and could be potential biomarkers of severity.[82]

Exhaled breath condensate

Exhaled Breath Condensate (EBC), which was introduced more than 20 years ago, is a non-invasive technique to assess airway inflammation. EBC mainly consists of water vapor containing both volatile and non-volatile compounds. Despite the promising early results regarding its utility in the diagnosis and monitoring of childhood asthma, high variability in the sampling and analytical methods of EBC has precluded its widespread clinical application. To address this, the European Respiratory Society (ERS) recently provided guidance on the standardization of sampling, analyzing, and reporting of relevant data.[52] A variety of biomarkers in EBC have been assessed, with generally mixed results.[83] Hydrogen ions (as pH), cytokines, markers of oxidative stress (e.g., hydrogen peroxide), oxides of nitrogen and leukotrienes are the most frequently examined biomarkers in the available literature. However, most of the published studies are cross-sectional, include a small sample size and are characterized by significant clinical and methodological heterogeneity.[83,84] In addition, other factors which could affect EBC composition such as concomitant upper airway diseases or medications, should be also considered.[85] Therefore, future studies using uniform definitions and standardized methodology in the collection and analysis of EBC will provide valuable insight in the potential of EBC as a diagnostic and monitoring tool in children.

New – upcoming (omics, breath biopsy)

Omics approaches, including genomics, transcriptomics, epigenomics, proteomics, metabolomics, and microbiomics, have emerged as promising tools for precisely endotyping asthma in the last two decades. Studies have confirmed their utility in differentiating patients with asthma from healthy subjects, expanded our view regarding the internal heterogeneity of asthma, enhanced our understanding of asthma pathophysiology at the cellular level and provided insights into the biological mechanisms, which may affect treatment response.[86] Nevertheless, small, and heterogeneous samples, lack of standardization and variations in analytical methods and lack of longitudinal follow-up in the individual studies have impeded clinical interpretation of the obtained results.[87] Of note, although single omics approaches are thought to capture only a dimension of the underlying disease process and may be of limited clinical utility, only a limited number of studies have conducted integrated omics analyses. In specific, McGeachie et al integrated metabolomic data with genome-wide genotype, gene expression, and methylation data of 20 asthmatic children and identified two implicated pathways – arachidonic acid and linoleic acid metabolism – in asthma control as well as altered sphingolipid metabolism as an underlying feature of uncontrolled asthma and cellular response to albuterol.[88] As asthma research networks and cohorts are expanding and methods to integrate omics data are growing, multi-omics approaches in combination with clinical features and laboratory parameters may allow for accurate disease classification and implementation of individualized targeted treatment options.[89]

COMMENTARY

Biomarkers use in diagnosing and monitoring asthma during the COVID-19 pandemic

In December 2019 a new infectious disease started in Wuhan, China, and the pathogen Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was identified as the cause.[90] This pandemic challenged the health care systems organization, and the management of chronic diseases to minimize risks of infection for both the medical professionals and the patients. Elective healthcare visits and procedures were postponed by considering risk and benefits individually.[91]

In fact, with COVID-19-related lockdown, asthma diagnosis evaluation and monitoring changed significantly. Many consultations were cancelled or turned into teleconsultations and access to disease biomarkers changed. In allergy diagnosis, skin prick tests were not recommended, and, in asthma diagnosis and monitoring, pulmonary function tests (PFTs) were cancelled or postponed, as these procedures could potentially disseminate infectious particles by various mechanisms, including by generated aerosols.[92] Clear recommendations regarding the safe practice in pulmonary function laboratories were made and individual protective equipment and other safety recommendations for performing PFTs must still be followed.[93]

The real-life situation experienced by physicians managing asthma patients during the first lockdown of the COVID-19 pandemic, was assessed in a survey promoted by the EAACI asthma section, that was completed by 339 healthcare professionals from 52 countries. 79% of follow-up consultations were replaced by phone calls, whereas only 49% of newly referred patients attended the clinic. 62%, 76%, 66%, 76%, and 87% of responders did not conduct spirometry, impulse oscillometry, bronchodilator test, FeNO, or methacholine provocation, respectively, for asthma diagnosis in adults, with similar rates in children. In the paediatric patients, 56% of the PFT were cancelled. About $^{3}/_{4}$ of responders based the initial asthma diagnosis and the prescription of inhaled therapy on clinical parameters only and PFT were used in only 29% of cases to monitor asthma worsening. The authors considered that all necessary resources should be allocated to ensure the performance of PFT in asthma management.[94]

In another online survey, including 91 physicians, members of the Pediatric Asthma in Real Life (PeARL) think tank and the World Allergy Organization (WHO) Pediatric Asthma Committee, caring for an estimated population of more than 133 000 children with asthma, it was found that COVID-19 significantly impacted paediatric asthma services:

39% ceased physical appointments, 47% stopped accepting new patients, and 75% limited patients' visits. Virtual clinics and helplines were launched in most centers. Better than expected disease control was reported in 20% of patients, whereas control was negatively affected in only 10%. Children with asthma do not appear to be disproportionately affected by COVID-19, and clinical departments have rapidly responded to the pandemic by limiting and replacing physical with virtual appointments.[95] Finally, in the multinational PeARL childhood asthma cohort, it was evaluated the impact of the COVID-19 pandemic on asthma activity. It was confirmed that during the pandemic, children with asthma experienced improved disease control. Pulmonary function during the pandemic compared with the year before was evaluated in a subgroup of asthmatic children; paired analyses suggested that pre-bronchodilation FEV1 and PEF were significantly improved during the pandemic, while there was also a non-significant trend for improved post-bronchodilatation FEV_1.[96]

Management of asthma patients during the current pandemic peaks was mostly supported by clinical data and with exceptional use of *in vivo* and *in vitro* biomarkers to achieve the best control of this chronic disease. In the future, most of the allergy community will benefit from e-health, including not only e-consultations but also other health digital tools allowing biomarkers acquisition, always with strict quality control and regulations.

Cost and availability in different settings/ developing countries

While diagnosis of asthma in children has been largely considered by recurrence of indicative signs and symptoms, objective measurements and procedures emerge as a real unmet need.

Classical biomarkers are available for adult population not only for diagnosis but also for phenotyping patients, to provide the most appropriate management, since "one size fits all" is no longer supported for asthma treatment, not even in children.

However, the chance for objective diagnosis and phenotyping in pediatric population is restricted by limited access to biomarkers particularly in developing sites, while availability is not global either.

We describe biomarkers available in different countries (reported by representative specialists from cited places), with equivalent cost in euros, as shown in Table 2. We observe that total IgE is the only biomarker available everywhere, with a wide range of cost, from a very accessible 3€ in Brazil to 45€ in the United States. The cost of Prick Test emerges as widely available at an affordable cost, from 6€ in Egypt going up to 100€ in México. The cost of specific IgE is also offered at most places but at higher cost, from 25€ in Egypt to tenfold higher value in Argentina and Mexico. Molecular diagnosis is not generally available, with a range cost of 75€ to almost 700€, depending on platform and country.

Pulmonary function tests (spirometry) are also available everywhere at a range cost of less than 8€ in Japan to 50€ in Mexico. However, a much cheaper test as Peak Flow is free of charge in Egypt to 5€ in Greece but is not used in some countries. Also, Impulse Oscilometry is available in same places at 5€ in Egypt to 15€ in Greece.

The most restricted access corresponds to a) Exhaled Breathe Condensate that is not offered to children anywhere, b) Sputum Eos is offered only in Japan and Greece, and c) Bronchial Provocation Test is performed in several specialized centers across Asia, Europe, and North and South parts of the world. Respective costs through bronchial hyperresponsiveness testing with methacholine are: in Japan at 15€, 480 Spain at 20€, 35€ in Greece, while in the United States approximately $880 when billed to insurance companies, and costs to the patient will vary.

Lastly, FeNO is possible to be measured in both research and clinical settings (mostly in Europe). A rough estimation of the cost is 11€ in Japan up to 50€ in Mexico. Currently sensors are available even for home monitoring.

In conclusion, the most accessible tests are related to allergy markers, with the exemption of molecular components, while tests related to T2 inflammation is scarcely reachable for pediatric asthma evaluation, no matter its cost.

Differences in the use of biomarkers for monitoring pediatric asthmatic patients between Primary Practitioners, Pediatricians, and Specialists

Asthma patients often have a poorly controlled disease, attributed to poor adherence to treatment and wrong inhalation technique (50–90% and 70–80% of the patients respectively), coupled with other factors such as high tolerance to asthma symptoms or the inability to perceive the chronic nature of the disease.[97,98] Primary care doctors should address these treatable traits, as asthma control visits are highly irregular in different countries, seeking care only when there is an exacerbation.[99,100] The primary care doctor, pediatrician and specialist should be able to make the diagnosis of asthma in any child with asthma symptoms by applying a multidimensional approach. The implication of tools such as the validated questionnaires to assess asthma control and quality of life and lung function determination, should be available and known to all doctors dealing with children with asthma-associated symptoms.[101] Adherence, inhalation technique and correct dose of the drug must be verified. Of note, the primary care physician or pediatrician has the ability to recognize, avoid or eliminate triggering factors (eg, comorbidities), and indicate non-pharmacological interventions.[21] Nevertheless, symptoms in children are often nonspecific, while even following diagnosis, monitoring can be challenging. In case of inadequate asthma control, referral to an asthma specialist optimizes the diagnostic approach and treatment. The use of noninvasive biomarkers potentially aids at phenotyping the asthmatic child and guide a more personalized treatment according to the pathophysiological mechanism, especially in patients with poor asthma control (difficult-to-treat asthma or severe asthma).[102]

Natural history/patterns: assessing the longitudinal aspect

Time is often underestimated regarding the clinical use of biomarkers. Cost and other logistic considerations underpin the most common scenario of biomarker use: that of a one-time, cross-sectional measurement. Clearly however, biomarker levels fluctuate in time,[103] in some cases rapidly and extensively. Consequently, clinical interpretation of any biomarker should be aware of the timing and possible trajectories of the measure. Both FeNO and lung function measures fluctuate, to some extent in parallel or preceding clinical symptoms and signs.[104] It is therefore important to appreciate change rather than absolute values in these measures; this is well accepted when it comes to e.g. peak flow variability in time – a surrogate for asthma activity, but also in regard to FeNO values for which variability might be within the overall "normal" range, however with significant fluctuations in between individual measurements.[11] Another frequent case where a fluctuating biomarker may influence management, is the level of total IgE when considering initiation of anti-IgE. Considering that the dosage schedule of *anti*-IgE depends on baseline total IgE levels, the variation of available values may generate a challenge in the clinical setting and requires medical judgement beyond the formal algorithm. Blood eosinophils may also fluctuate, therefore "cut-off" values of 150 or 300 cells/mm3, should be seen with some flexibility, while patient values need to be considered.[105] Of note, both blood differentials as well as IgE levels may increase in the context of an infection – and possible exacerbation – in allergic children;[106] therefore, to the extent possible, values obtained away from such events should be considered. Another longitudinal aspect is normal growth, particularly for the preschool age. While FeNO increases with age,[9] cut-offs are not age specific. In all, the use of biomarkers in children with asthma should be incorporated in a clear, pre-designed, monitoring plan.

CONCLUSION

Although asthma represents one of the most common chronic diseases in childhood, confirming diagnosis and follow up, based on solely clinical grounds can be challenging. The implementation of non-invasive biomarkers for assessment and monitoring of paediatric patients with asthma, has been widely studied, however, only a proportion of them are currently included in the clinical practise.

In the final and in the post-pandemic phases, it is time to revalue the use of biomarkers in the initial assessment and monitoring of asthmatic children,

	USA	Greece	Japan	Brazil	Spain	Argentina	Egypt	Mexico
Fractional exhaled Nitric Oxide	35	20	10.8	N/A (research only)	22	N/A (research only)	N/A (research only)	50
Peak Flow Measurement	N/A	5	N/A	N/A	N/A	3	No charge	N/A
Spirometry	200	10	7.6	25	10.6	20	5	40
Impulse Oscilometry	12 000 (the whole equipment)	15	11.5	N/A	10	N/A	5	N/A
Bronchial Provocation Test	$880 when billed to insurance companies; cost to the patient varies	35	15.4	N/A	20	N/A	N/A (research only)	N/A (research only)
Total IgE	45	12	7.7	3	7.5	10	6	12
Specific IgE (10 allergens)	400	120	84.6	30	80	250	25	250
Skin Prick Test (10 allergens)	50	20	12.3	N/A	10	30	6	100
ImmunoCAP ISAC Test	400	350	N/A	N/A	120	N/A	N/A	695
ALEX Test	N/A	150	N/A		75	N/A	N/A	391
Sputum eosinophils	N/A	5	1.2	N/A	N/A	N/A	N/A (research only)	N/A
Exhaled Breathe Condensate	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Table 2. Cost (in euros) of biomarkers and diagnostic procedures for pediatric asthma diagnosis. N/A: no data available from this country for this specific evaluation

respecting best practices, and ensuring safety measures for both health professionals and patients.

Abbreviations

IOS; impulse oscillometry, FeNO; fractional exhaled nitric oxide, WAO; World Allergy Organization, PEF; Peak expiratory flow, FEV1; Forced Expiratory Volume in the 1st second, FVC; Forced Vital Capacity, FEF; Forced Expiratory Flow, ERS; European Respiratory Society, Zrs; impedance, Rrs; resistance, Xrs; Reactance, Fres; Frequency of Resonance, AUC; area under the AX curve, BHR; Bronchial hyperresponsiveness, SPTs; skin prick test, EBC; Exhaled Breath Condensate, LFT; lung function test, PeARL; Pediatric Asthma in Real Life

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Authors' contributions

All authors have contributed to the writing and revision of the manuscript. PX and NGP are involved in the Concep tualization, writing, review and editing of the paper. YA, CFP, ZES, RMG, EH, IF, PLS, MMA, MM, AN, WP, PP, WJY and GW are involved in writing and original draft preparation and final editing.

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Declaration of competing interest

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