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Priorities for future research into asthma diagnostic tools: A PAN-EU consensus exercise from the European asthma research innovation partnership (EARIP)

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SUMMARY

The diagnosis of asthma is currently based on clinical history, physical examination and lung function, and to date, there are no accurate objective tests either to confirm the diagnosis or to discriminate between different types of asthma. This consensus exercise reviews the state of the art in asthma diagnosis to identify opportunities for future investment based on the likelihood of their successful development, potential for widespread adoption and their perceived impact on asthma patients. Using a two-stage e-Delphi process and a summarizing workshop, a group of European asthma experts including health professionals, researchers, people with asthma and industry representatives ranked the potential impact of research investment in each technique or tool for asthma diagnosis and monitoring. After a systematic review of the literature, 21 statements were extracted and were subject of the two-stage Delphi process. Eleven statements were scored 3 or more and were further discussed and ranked in a face-to-face workshop. The three most important diagnostic/predictive tools ranked were as follows: "New biological markers of asthma (eg genomics, proteomics and metabolomics) as a tool for diagnosis and/or monitoring," "Prediction of future asthma in preschool children with reasonable accuracy" and "Tools to measure volatile organic compounds (VOCs) in exhaled breath."

1. INTRODUCTION

The European Commission launched the European Innovative Partnerships (EIPs) in the FP7 program as a way of shortening the process from the initiation of research to the marketing of the findings, whenever possible, in all kinds of fields. According to the EU Commission, "EIPs act across the whole research and innovation chain, bringing together all relevant actors at EU, national and regional levels to: (1) step up research and development efforts; (2) coordinate investments in demonstration and pilots; (3) anticipate and fast-track any necessary regulation and standards; and (4) mobilize 'demand' in particular through better coordinated public procurement to ensure that any breakthroughs are quickly brought to market." In accord with this philosophy, Asthma UK applied for an EIP for asthma and constituted the European Asthma Research Innovative Partnership (EARIP) with the aim of producing a European roadmap to quickly, effectively and drastically reduce asthma morbidity and mortality, and to look for the eventual prevention and cure of asthma.

Led by Asthma UK, EARIP gathered a considerable number of stakeholders from health professionals, patient associations and pharmaceutical companies. The project was divided into different work packages, including one on diagnostic tools. In the present consensus statement, we present the asthma diagnostic tools on which stakeholders agreed warranted the future investment.

The group, with external consultation, agreed on a list of relevant topics (Table 1), which constitute the different topics of the systematic review presented wherein. Working group members performed literature searches based on expertise and knowledge in this area (Table S1). The framework shown in Table 2 was applied to each topic in order to perform a short and focused review, providing up-to-date, in-depth information on each topic, to provide the base for consensus prioritization and ranking.

A first general literature search was carried out between January 2015 and March 2015. After a first report was written and the Delphi exercise was ended up, a new search to update the review was made between September 2016 and March 2017.

Table 1 *List of topics agreed to be covered by the structured review*

Diagnostic markers
Inflammation markers and cells
Blood
Eosinophils
Periostin
Exhaled breath
Exhaled nitric oxide
Volatile organic compounds
Markers in breath condensate
Markers in sputum and bronchoalveolar lavage
Systems biology
Lung function tests
Spirometry
Body plethysmography
Impulse oscillometry and forced oscillation technique
Interrupter technique
Infant lung function testing
Inert gas washout
Bronchodilation
Bronchial challenge tests
Asthma prediction

Table 2 *Framework applied for each of the topics in the structured review*

What is the current status of the specific diagnostic tool?
Please describe very briefly the literature search strategy
Is it a useful tool to diagnose/follow up asthma?
Should it be part—currently and in the near future—of the diagnosis/follow-up of asthma in every case and age?
Is it suitable for point of care detections?
Will it enable self-evaluation and follow-up of patients?
What the future would be with respect to the specific tool?
Do you expect further advances here? If so, which ones?
If you expect several advances, please state the one most important in your opinion

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2. STRUCTURED REVIEW

2.1. Introduction

With no single genetic or environmental cause, asthma is difficult to define. The diagnosis of asthma is a clinical one and should be based on the history of characteristic symptom patterns and evidence of variable airflow limitation; however, symptoms do not correlate very well with other hallmarks of asthma such as variable airway obstruction, airway hyperresponsiveness and chronic inflammation; it is unclear how all these features relate to each other. It is unknown if and how these features should be used in the diagnosis and monitoring of asthma.

Several diagnostic tests exist the sensitivity and specificity of which are not as high as would be desired, and thus, diagnosis can be considered a mosaic of many pieces including tests of lung function, tests for inflammatory markers and patterns of characteristic symptoms and signs in a clinical history. A further problem is the lack of standardization of existing diagnostic tests and in particular their applicability or even usability in young children.

As we become more knowledgeable about the different asthma phenotypes, there is a desperate need for new diagnostic tools to prevent over-, under-diagnosis and over-, under-treatment of patients to reduce asthma morbidity and mortality.

2.2. Inflammatory markers and cells

2.2.1. Blood

Eosinophilic inflammation is a hallmark in the vast majority of schoolchildren with asthma. Blood for measuring eosinophils and markers of eosinophilic inflammation is easy to obtain and may therefore be suitable for diagnosis and monitoring of asthma in children and adolescents. A differential cell count will give numbers for eosinophilic granulocytes. Eosinophil cationic protein (ECP) is a molecule that is released from activated eosinophils. Periostin is a protein secreted by airway epithelial cells and lung fibroblasts in response to IL-4/IL-13, which are key mediators of Th2-driven asthmatic inflammation.

Some further possible biomarkers of Th2-mediated asthma are currently being investigated: YKL-40, osteopontin, eosinophil-derived neurotoxin and some metabolites (choline, arginine, acetone, protectin D1); however, existing data need to be validated and their usefulness for clinical practice remains to be elucidated.[1]

Eosinophils and eosinophil cationic protein

Eosinophils can be easily measured in peripheral blood, but may be of use only when asthma is eosinophilic, which is not necessarily the case, especially in adult patients. Blood eosinophils >4% increase the probability that recurrent wheeze in combination with other risk factors in a child is asthma.[2] Although being significant, the correlation between blood and sputum eosinophils is far from perfect.[3] Setting the abnormal threshold of blood and sputum eosinophils at 400/μL and 3%, respectively, a discrepancy was found in 32% in a large asthma cohort, the group with high sputum eosinophils but normal blood eosinophil count representing 25% of the patients.[4]

Even though studies have confirmed the association of ECP with allergic asthma, ECP determination does not appear to be a valuable diagnostic marker of asthma, as changes in serum ECP concentrations can also be found in other atopic diseases such as allergic rhinitis, and even in conditions not related to allergic inflammation such as bacterial sinusitis.[5] Determination of ECP concentration has been discussed for assessing the severity of asthma, particularly in children, but it has not evolved as a helpful marker of inflammation in clinical practice.

In summary, blood eosinophils may play a role in the diagnosis of eosinophilic asthma and have a value in risk-assessment and response to treatment. In addition, as the correlation between airway eosinophilia and blood eosinophilia is low, the differential role in subendotypes will have to be better defined. There does not seem to be an advantage for measuring ECP compared to the absolute eosinophil count.

Periostin

Periostin appeared to be a good systemic biomarker of Th2-driven or eosinophil airway inflammation in adults.[6] However, expectations about the usefulness of periostin to diagnose asthma have not been fulfilled as it is increased during growth and can be also be elevated in many inflammatory processes.[7]

2.2.2. Exhaled breath

Fractional exhaled nitric oxide (FeNO) in asthma diagnosis and monitoring

Asthma diagnosis Exhaled nitric oxide is a user-friendly biomarker, which has become increasingly popular among clinicians. While first measured online by chemoluminescence with the fixed machine, it can now be measured with a portable electrochemistry system. As FeNO value is flow sensitive, it is important to standardize the flow rate at which the measure is performed. It has become accepted that FeNO is best measured at a flow rate of 50 mL/s.[8]

Early studies found that patients with asthma displayed raised level of FeNO in their exhaled breath compared to healthy subjects. Treatment with ICS results in a dramatic reduction in the level of FeNO.[9] Current smoking also causes a sharp reduction in measured values.[10] Median values of FeNO in large asthma cohort studies were found to range between 25 and 35 ppb.[10,11] Several studies have investigated the use of FeNO to make an asthma diagnosis. A threshold value of 20 ppb combined to symptom was proposed as a tool for asthma diagnosis that was superior to the measurement of the fluctuations of peak expiratory flow rate in mild-to-moderate asthmatics. This threshold was probably too low and more recent studies in patients with symptoms of asthma and normal baseline FEV1 value, or no significant bronchodilation has shown that FeNO threshold of 34 ppb (at the flow rate of 50 mL/s) yielded the best compromise for an asthma diagnosis with a high positive predictive value but a low negative predictive value.[12]

While it was initially thought that elevated FeNO was a key marker of asthma in general, it later appeared that FeNO was mainly reflecting the presence of eosinophilic airway inflammation.[10] Therefore, elevated FeNO is assumed to reflect an eosinophilic asthma phenotype, which account for approximately 50% of all asthmatics seen in clinical practice although higher in children.[4,13,14] The threshold values that predict eosinophilic inflammation vary according to the dose of ICS, the smoking status and atopy.[10] Combining FeNO and peripheral eosinophil counts seems to be a better approach than just using FeNO values.[15] However, it should be always be kept in mind that asthma cannot be ruled out when symptoms are compatible with the condition despite low values of FeNO, as asthma is not necessarily eosinophilic.[16,17]

Asthma monitoring FeNO has been proposed as a biomarker that may help the clinician to manage asthma.[18,19] FeNO values above 33 ppb in one study[20] and above 47 ppb in another[21] were found to predict a good clinical response to ICS in patients with chronic respiratory symptoms. This is in line with the observation that only eosinophilic asthma convincingly responds to inhaled corticoids and add to the importance of asthma phenotyping in clinics.[22]

The utility of using FeNO to adjust the dose of ICS to reduce exacerbations in asthmatics already receiving treatment has been investigated in several studies with controversial results.[23,24] In this setting, FeNO seems to perform less well that sputum eosinophils. However, thresholds to adjust the dose of ICS may not have been chosen adequately in some of those trials.[25]

Accuracy of FeNO measurement is firmly established, and it is recognized that elevated value reflects an eosinophilic phenotype and predicts good response to ICS.[18] There is a need to study the cost-effectiveness of FeNO as a tool for asthma diagnosis and choice of treatment in a large-scale cohort study.

Volatile organic compounds

Assessing VOCS using electrosensor (eNose) and, even better, qualifying and measuring them using mass spectrometry has great potential but to date has only been done in a limited series in research settings.[26] This has been done to distinguish asthmatic lungs from those normal ones, but no study has been yet published which has assessed the measurements of VOCs to monitor asthma treatment, although one study used VOCs to predict exacerbations in children with reasonable accuracy.[27]

Further exploring the value of VOCS to assist phenotyping asthma and predicting major clinical outcomes such as exacerbations and response to treatment would be of great interest.

Markers in breath condensate

Exhaled breath condensate (EBC) enables the study of the pathological processes undergoing in the lung.[28] As EBC is a non-invasive technique, it can be easily applied even in children too young to be able to perform other tests. In EBC, several molecules have been studied that may have a role as biomarkers in asthma.

Many studies have investigated the role of eicosanoids in asthma. The presence of these mediators in EBC has been documented using both immunoassays and reference analytical techniques. Increased levels of LTB4, a potent inflammatory mediator, have been demonstrated in steroid naïve children[29] and adults[30] with asthma.

Likewise, increased levels of cysteinyl leukotrienes, which are powerful broncho-constrictors and pro-inflammatory mediators, have been reported in both children and adults, with the highest concentrations found in subjects with severe asthma, despite ongoing ICS therapy.[31,32]

Beside leukotrienes, which are 5-lipoxygenase metabolites, increased levels of other inflammatory metabolites of the 15-lipoxy-genase pathway such as eoxins, have been reported in asthmatic children.[33] Interestingly, the simultaneous assessment of a set of metabolites of arachidonic acid led to the identification of a profile of eicosanoids (including prostaglandins and leukotrienes) capable of discriminating asthmatic and healthy children with high accuracy.[34]

Several markers of oxidative stress have also been studied in EBC, pointing to an increased oxidative burst in asthmatic airways. 8-isoprostane and hydrogen peroxide (H_2O_2) are the oxidative stress biomarkers better studied in EBC. 8-isoprostane is a product of arachidonic acid peroxidation. High levels of this mediator have been found in asthma, and, in particular, in subjects with problematic asthma, suggesting that oxidative stress may play a role in the pathogenesis of this asthma phenotype.[31,35,36]

H_2O_2 belongs to reactive oxygen species (ROS) deriving from the dismutation of superoxide anions. A meta-analysis conducted on 8 studies analysing the role of EBC H_2O_2 in asthma demonstrated that this biomarker is increased in adults with asthma, shows a trend towards a correlation with the degree of asthma severity and control, and it seems sensitive to corticosteroid treatment.[37] Similar results were found in children.[38] These features suggest a possible role of this biomarker in the follow-up of asthmatic patients. Noteworthy, the measurement of oxidative stress biomarkers in EBC may help in the identification of asthmatic subjects with higher oxidative stress, who may likely benefit from the development of novel anti-oxidant treatments.

Exhaled breath condensate pH is a robust marker, with a good reproducibility.[39,40] Reduced EBC pH has been reported in both adults and children with asthma.[41] In particular, a significant reduction in PH of the airways has been reported during acute asthma exacerbations.[36,42,43] Nonetheless, in the epidemiological setting, EBC pH could not discriminate between asthmatic and healthy subjects.[44] In a large cohort of subjects with severe and non-severe asthma, EBC pH turned out to be on the whole normal, but there was a subgroup of asthmatic subjects with very low EBC pH (<6.5)[45] EBC pH may be a useful biomarker in the characterization of a specific asthma subphenotype.

Increased levels of adenosine have been found in the EBC of asthmatic children and adults.[46] Using multiplex immunoassay technology, increased levels of cytokines, chemokines and soluble adhesion molecules have been reported in children with asthma and also in preschool children found to have persistent wheezing at 5 years of age.[47-49] Eventually, increased levels of the inflammatory and oxidative stress mediator ADMA (asymmetric dimethylarginine) have been showed in EBC from asthmatic children.[50]

The EBC technology has a significant potential for asthma diagnosis and monitoring, but the technique needs to be better standardized if we want to move it from research to clinical practice. Investments are urgently needed to achieve a full standardization of the methodologies used for sample collection and analysis.

2.2.3. Sputum and bronchoalveolar lavage

Ideally, any material obtained directly from the lower airways of asthmatic patients could be very useful diagnostic biomarker. A *large number of studies* have been conducted over the last decades to evaluate the diagnostic utility of sputum and *bronchoalveolar lavage* (BAL). Sputum samples collection is not always a simple procedure, especially in children and BAL is an invasive technique.[51,52]

Sputum

Studies on sputum look mainly at the potential informative role of sputum as a material with airway inflammatory biomarkers. Most studies have focused on asthma severity assessment and provision of more efficient *biomarker-guided treatment.*[53-55]

The technique of induced sputum that allows to collect airway secretion after inhalation of saline is valuable in approximately 80% of the patients and has been key in the emergence of the concept of inflammatory phenotype.[56] Sputum eosinophil count, neutrophils and several soluble mediators—most recently periostin—have been examined.[54] They are considered as the only non-invasive measure of airway inflammation that has a clearly proven utility in clinical practice in adults.[56] Recent studies have further highlighted the role of sputum eosinophils in poor asthma control. A retrospective study on a large asthma cohort has shown that patients combining sputum eosinophil >3% and blood eosinophil counts >400/μL have poor asthma control and are prone to exacerbate.[4]

In a prospective study conducted in severe asthmatics uncontrolled despite high dose of ICS and LABA, the

repeated presence of eosinophils in the sputum was associated with increased of excacerbations.[57] Furthermore, in a large retrospective cohort of asthmatics, it has been shown that both fluctuation in FEV1 and fluctuation in sputum in sputum eosinophils independently correlated with change n ACQ6.[58] Sputum induction can be effectively performed in adults, but there might be difficulty in collecting sufficient sputum in children under 8 years.[51,56] Furthermore, one study in children using sputum eosinophils for monitoring asthma was negative.[53]

More studies are needed to clarify the biomarker (or combination of biomarkers) that could be most valuable in practice as a tool in precision medicine.

Bronchoalveolar lavage

BAL is a major tool in the diagnostic procedure for a number of pulmonary diseases, including asthma. It can provide useful information about the pattern of airway inflammation in terms of total cellularity, differential cell profile and several inflammatory mediators.[59,60] However, the information available is fragmentary, as most of the studies look at either refractory asthma or certain biomarkers from patients in whom bronchoscopy could be justified and suffer from lack of specificity.[59,61] Subsequently, these studies are affected by methodological difficulties and selection bias, with patients having the sufficiently severe disease to justify an *invasive* sampling procedure. Data interpretation arising from such studies needs special caution. BAL has also been used for specific asthma phenotypes identification.[61]

Because of the invasive nature of fiberoptic bronchoscopy, BAL could be used in certain patients only. It is still useful for difficult asthma to detect other implicated causes of symptoms such as silent aspiration or persistent bacterial infection or congenital abnormalities (children).

2.2.4. Systems biology

In the study of chronic complex diseases, such as asthma, beside the assessment of individual biomarkers, it is of utmost importance to study several mediators simultaneously, through a systems biology approach. An overall profile can better mirror the complexity of asthma, in the pathogenesis of which a large number of cell types and molecular pathways contribute, interacting in complex networks.[62,63]

The "-omic" technologies (genomics, proteomics and metabolo-mics) are systems biology platforms. Being guided by no a priori assumptions, they look into which components are associated with a given pathological condition, shedding light on pathogenic pathways and phenotypic characteristics with a hypothesis-generating approach.[63]

The "-omic" technologies, proteomics and metabolomics in particular, have been applied in the study of asthma. The proteomic analysis of EBC proved that it is possible to identify profiles of differentially expressed proteins, capable of discriminating asthmatic children from healthy controls.[64]

The metabolomics approach has been applied to several biofluids. In adults, the metabolomics analysis of serum[65] and exhaled breath condensate[66] samples demonstrated a clear separation between asthmatic and healthy subjects. Moreover, the metabolomics analysis of urine samples showed that during an acute exacerbation, a profound alteration of the metabolic profile occurs, with a significant role of the metabolites indicative of oxidative stress.[67]

In children, metabolomic analysis of both urine and EBC samples proved capable of clearly discriminating between healthy and asthmatic children.[68,69] Metabolomics has been also applied in the characterization of different asthma phenotypes, and a separate metabolic profile has been demonstrated in children with severe asthma by applying the metabolomics approach either to plasma[70] or to EBC[50] samples.

An "-omic" approach has been applied also to the study of volatile organic compounds (VOCs) in exhaled breath. The VOCs profile could discriminate asthmatic from healthy children.[48] In addition, the study of VOCs profile seems to have promising application for the prediction of asthma exacerbation[27] and for the early identification of asthmatic children among preschool children with recurrent wheezing.[71]

The "-omic" technologies may have a key role in the development of personalized medicine, potentially contributing to shift the focus of medicine from the traditional symptom-oriented diagnosis and treatment of diseases (reactive medicine) towards the so-called P4 medicine, which concentrates on preserving health through the prevention and early diagnosis of disease.[72]

The "-omic" technologies, in fact, enabling the simultaneous assessment of several mediators can lead to the discovery of early diagnostic profiles and can shed light on new, sometimes unexpected, biomarkers that may be applied to monitor asthma and to guide therapy.

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2.3. Lung function tests

2.3.1. Cooperative patients

Spirometry

Asthma diagnosis should be based on both the presence of symptoms and objective demonstration of variable airflow obstruction. However, there may be important barriers to performing lung function tests not only in primary care settings but also in secondary care settings.[73,74] Some studies showed that spirometry has been used in diagnosis in only 21%-25% of paediatric asthma patients in a primary care setting.[75,76] The correct use of spirometric measurement is crucial for an accurate diagnosis.

This may result in both over-diagnosis and under-diagnosis of asthma.[74] One-third of adults individuals with physician-diagnosed asthma may not have asthma when objectively assessed.[77] Spirometry is an objective tool that may help to prevent misclassification of asthma severity and inappropriate underuse or overuse of asthma medication among paediatric asthma patients: nearly one-third of patients had their treatment plans changed after clinicians viewed their spirometry results.[78]

Spirometry is normal in many patients with asthma at the time of clinical presentation, and objective confirmation of variable airflow obstruction may be challenging. Most adults in primary care have mild asthma and well-preserved lung function. Airflow obstruction defined as a ratio of $FEV_1/FVC < 70\%$ was found only in 21% adult patients diagnosed with asthma in a primary care setting[79], but this proportion raises to 60% in severe adult asthmatics despite treatment with high-dose ICS/LABA.[80] Most asthmatic children also have a normal spirometry, with 94.2% of 3626 children having a $FEV_1 > 80\%$ predicted[81] and only 10.5% of 3612 asthmatic children having a $FEV_1/FVC < 80\%$.[82] In this study, FEV1/FVC < 80% had a high sensitivity for asthma diagnosis (>90%) but low specificity (<20%), being associated with asthma diagnosis in a patient with concomitant allergic rhinitis but not in children without allergic rhinitis.

A fixed limit FEV_1/FVC ratio (<70% in adults) is often used to identify airflow obstruction instead of the lower limit of normal (<5th percentile).[83] This fixed cut-off points have been shown to cause much misidentification of airflow obstruction specially in young adults, increasing the likelihood of under diagnosis of obstruction.[84] The use of Z-scores in children is probably more appropriate.

A decreased $FEF_{25\%-75\%}$ is indicative of small airway obstruction. The utility of an isolated decrease in $FEF_{25\%-75\%}$ in the setting of otherwise normal spirometry is unclear, because values are more variables than FEV_1. Evidence suggests than a reduced $FEF_{25\%-75\%}$ correlates with bronchial hyperresponsiveness on bronchoprovocation testing.[85,86]

The utility and limitations of spirometry in asthma diagnosis are well established. A wider use of this technique in primary care with the implementation of new algorithms will allow a better diagnostic classification of both paediatrics and adult patients. However, there is no single randomized control trial showing that guiding treatment on FEV1 improves asthma outcomes.

Plethysmography and asthma diagnosis

Plethysmograhic measurement of lung volumes does not provide much additional information for clinical decision-making in most patients with asthma, and it is not recommended in current guidelines.

Lung hyperinflation is frequent in uncontrolled asthma, and a significant proportion of both children and adult asthmatic patients have elevated residual volume and abnormal RV/TLC ratio in the presence of normal FEV_1/FVC ratio and the absence of significant bronchodilator response.[87,88] The clinical significance of these findings in asthma needs further prospective studies. In adults, the role of airway resistance (Raw) and specific airway conductance (sGAW) as an aid to asthma diagnosis has been explored in a real-life study and found to predict disease with a positive predictive value around 75% but a poor sensitivity.[89]

Measurement of specific airway resistance (sRaw) using plethysmography could be useful. In epidemiological studies as early as age 3 years, sRaw differs between children with a history of wheezing and those without,[90] and higher sRaw at age 3 years is associated with subsequent persistence of wheezing.[91] sRaw seems to be also adequate to assess bronchodilator response in children.[92] However, in some studies, the use of sRaw in schoolchildren has not been adequate to diagnose asthma, because of high variability and huge overlap between healthy children and those with asthma.[82]

In summary, the value of plethysmography in asthma diagnosis is very limited, with sRaw having some role.

2.3.2. Non-cooperative patients

Diagnosing asthma in preschool children is often challenging.[93] The demonstration of bronchial reversibility after administration of a bronchodilator helps clinicians to establish the appropriate treatment. Unfortunately, in this age group, spirometry is often not applicable because results depend on effort and effective co-operation by patients. Therefore, non-invasive lung function measurements requiring only passive co-operation, while the patient is breathing at normal tidal volume, such as impulse oscillometry, the forced oscillation technique, and interrupter technique, have been proposed.[94]

Impulse oscillometry and forced oscillation technique

Impulse oscillometry (IOS) measures the resistance and resonance capacitance of the lungs, both at small and large airway level, performing measurements in a non-invasive, effort-independent manner during spontaneous breathing. The most relevant outcomes of IOS are R5, the resistance in small airways, R15 or higher, the resistance in larger airways and the low frequency integrated impedance reactance at R5.[94,95] All these measurements can be compared to baseline following bronchodilator or longitudinally in patients with chronic asthma that require regular treatment.[95]

Impulse oscillometry could be a more sensitive method to evaluate small airway than spirometry parameters such as FEF 25-75, because, in contrast with spirometry that requires a deep inspiration, *forced* oscillation technique (FOT) does not modify the airway smooth muscle tone.[95]

It has been shown that IOS provides effective measures of lung dysfunction in 4-year-old children at high risk for persistent asthma.[96] This was confirmed and extended in another study evaluating at this age range the effects of short- and long-acting bron-chodilators.[97] More recent studies demonstrated the efficacy of IOS as an alternative to FEV1 in older asthmatic children.[98,99] Interestingly, it has been shown that R5 but not FEV1 showed improvement in patients with persistent asthma after inhaled steroid treatment.[100]

Forced oscillation technique holds the perspective of improving the diagnosis of airway obstruction, quantifying the amount of airway reversibility and hyperactivity even in non-collaborative patients.

Impulse oscillometry practice requires well-trained technicians and physicians both for performing and evaluating the tests. Pitfalls of IOS are airway leak and poor holding of the cheeks, as well as tongue effect, cough, swallowing, shallow breaths and vocalization, with a significant influence of the upper airway shunt in preschool children.

Normal values of respiratory impedance (Zrs) for preschool children have been obtained, as well as of respiratory resistance (Rrs).[101-103] However, there is a lack of standardization in measuring procedures and equipments.

The interrupter technique

The interrupter technique is able to detect changes in airway calibre.[104] The principles of the interrupter technique are that, during a sudden and rapid airflow interruption at the mouth, the alveolar and mouth pressure will equilibrate. The interrupter resistance (Rint) is defined as this pressure divided by the airflow measured immediately before interruption.[105]

There are reference values for preschool children, which are difficult to compare being often obtained using different methods. The technique is able to measure the magnitude of changes in airway calibre after inhalation of a bronchodilator, but the cut-off value for a decrease in Rint beyond which a response may be considered clinically effective remains to be established.[94,104,105]

To make the technique more reproducible and uniform between centres, a series of recommendations have been presented.[94] However, many issues remain to be clarified, particularly to establish the cut-off values beyond which a clinical response to bronchodilator could be considered clinically relevant.

Lung function tests in infants and asthma

Cohort studies have shown that those infants who develop asthma could have, prior to any respiratory illness, impaired lung function. This premorbid condition has been described in different asthma phenotypes.[106-109] Considering infant lung function before the first episode of wheeze and the subsequent development of atopy during the first 6 years of life, 3 phenotypes have been described: transient wheezers, who have low neonatal lung function, do not develop atopy and maintain lung function during childhood, suggesting a small or malacic airway[106]; persistent wheezers, who have premorbid normal or decreased lung function (according to the Tucson cohort[106] or the Copenhagen cohort[107] studies, respectively), do develop atopy, and lung function decreases during the first years of life, suggesting airway remodelling very early in life; finally, late-onset wheezers have normal neonatal lung function, although they develop atopy their lung function is maintained.[106-108]

However, there are some limitations in those studies: most based their observations on very limited number of cases[106,108,109]; and although one[107] assessed 311 cases, all mothers had a history of doctor's diagnosis of asthma after age 7. Moreover, the techniques to assess lung function are different between studies (VmaxFRC, FEV0.4 and FEV0.5). Furthermore, there is a significant overlap of lung function values in infants between those with and without future asthma and between different asthma phenotypes. On the other hand, regression equations based on data from 429 healthy infants aged 4-118 weeks have been published very recently.[110]

It is essential to have adequate normal population-based reference values of infant lung function. Normal lung function values of raised-volume-rapid-thoracic-compression technique, for instance, are based on only 155 tests,[111] and on top of that, it is mandatory to correct the regression equations of normality if the most extended equipment in Europe is used,[112] not to speak of different ethnicities.

If decreased lung function very early in life, or even in utero, plays any role in the development of asthma, we need to understand how and why this decrease is produced in order to design strategies to prevent it. Thus, it is important to study prenatal risk and protective factors (other than the known ones, such as prematurity and tobacco smoke exposure) related to lower lung function very early in life. And although we have some clues in infants born from high-risk mothers,[113] research should be extended to the whole population.

Inert gas washout for measurement of ventilation inhomogeneity

The majority of lung function tests measure flow; airway resistance; or lung volumes. While such measures are undeniably helpful, one of the greatest contributions to impaired respiratory function in asthma is the effectiveness of gas mixing or ventilation distribution. We know that ventilation distribution has some degree of heterogeneity in healthy individuals is more pronounced those with stable airways disease; and that this situation can lead to catastrophic shifts—in turn leading to hypoxaemia—during bronchoconstriction.[114] There is therefore increasing interest in the objective monitoring of ventilation heterogeneity in both stable and unstable asthma, and the most promising tools for this are inert gas washout tests.

These investigations rely upon measurement of exhaled inert gas (either nitrogen or a previously inhaled gas such as Sulphur hexafluoride) during multiple breath or single breath washout (MBW or SBW). The former test usually employs tidal breathing and produces indices of overall ventilation heterogeneity such as the lung clearance index (LCI), or mechanism dependent indices such as S_{cond} and S_{acin}, which broadly measure conducting airway generated heterogeneity and more peripherally generated heterogeneity, respectively.[115] SBW usually employs a raised volume inspiration and expiration, and the phase III slope of the expiration is analysed. Performing SBW using multiple inert gases of differing molecular weight and therefore diffusivity can provide additional information as to where in the airway tree changes in airway calibre are likely to be occurring.[116,117]

Calculation of the S_{cond} and S_{acin} indices from MBW has demonstrated that proximal conducting airways and more peripheral airways generate heterogeneity in subjects with asthma, and that both mechanisms are partly but not wholly reversed by bronchodila-tor.[115,118,119] Intriguingly, the degree of baseline heterogeneity appears to predict the severity of airway hyperresponsiveness in adults and children with asthma,[120-122] in a way that differs from subjects with COPD.[123] One possible explanation is that baseline heterogeneity is predictive of airway closure during bronchoconstric-tion.[114,124] Further stud ies have identified relationships between heterogeneity and symptom control[125]; clinical stability[118]; and response to inhaled corticosteroids.[126] The link between heterogeneity and inflammation, as measured by exhaled nitric oxide, is not clear, like with most lung function tests.[121,122,127] There are limited data relating heterogeneity to asthma phenotype, although one study in children with preschool wheeze has identified S_{cond} as an excellent discriminator between the episodic viral wheeze and multi-trigger wheeze phenotypes,[128] although those phenotypes have not been shown to be very stable over time.[129] It is noted that the majority of studies utilizing MBW have employed the S_{cond} and S_{acin} indices. Global indices such as LCI are widely used in the study of CF lung disease and in bronchopulmonary dysplasia, but how LCI relates to S_{cond} and S_{acin} in asthma is not well understood.[128,130,131]

The SBW test is quicker and more straightforward to perform and interpret than MBW, and some of the earliest studies investigating ventilation heterogeneity in asthma and COPD used this method.[132-134] These studies identified links between asthma diagnosis and severity and the Phase III slope from SBW. More recent studies have confirmed these findings[135] and have utilized multiple gases to localize presumed bronchoconstriction.[116,117,136]

The field is new, and not yet well understood. However, the use of MBW is in the process of transforming the monitoring and care of early/mild CF lung disease, and the promising early data in subjects with asthma should not be ignored. It is possible that inert gas washout tests could prove to have advantages over more traditional lung function tests in asthma monitoring, particularly given that ventilation heterogeneity is known to be present at baseline, and to lead to dramatic changes in ventilation distribution during bronchoconstriction.

An additional, crucial advantage is that most inert gas washout measurements can be collected during tidal breathing and can therefore be performed in infants; preschool children; and older subjects who are unable to co-operate with more traditional lung function tests.

At present, the MBW technique is too complex and time-consuming to be employed in routine asthma monitoring but will continue to be researched to better understand asthma phenotyping and physiology. In the longer term, the development of tidal breathing SBW, possibly utilizing multiple inert gases, could become a valuable clinical tool. There is more than one approach to develop such a test, but it is noted that a multiple gas method has already been used to identify peripherally generated heterogeneity in children with mild asthma symptoms and normal spirometry.[136]

2.3.3. Bronchodilation

The diagnosis of asthma should be based on the history of characteristic symptom patterns and evidence of variable airflow limitation.[83] As the GINA guidelines point out, evidence of variable airflow limitation should be documented from bronchodilator reversibility testing or other tests. Hence, tests of airflow obstruction and airway responsiveness (including reversibility testing) may provide support for the diagnosis of asthma in children and in adults. In patients with normal or near-normal pre-treatment lung function, reversibility testing with a bronchodilator is of limited value, as there may be little room for measurable improvement.[83] However, should still be performed, in particular in children who may have supranor-mal values. In contrast, in cases of established airflow obstruction upon initial assessment, measuring the bronchodilator response to β2-agonists appears helpful to demonstrate variability of airflow limitation. A significant increase in airflow (as determined by FEV1 or FVC or PEF, depending on the protocol employed) after administration of a bronchodilator indicates reversibility of airflow obstruction and supports the diagnosis of asthma.[83,137]

There is no consensus about the drug, dose or mode of administering the bronchodilator in the lung function laboratory. Current guidelines recommend inhalation of 100-400 µg (children) and 200-400 µg (adults) salbutamol or equivalent.[83] As an alternative to measuring the immediate response to a bronchodilator in the lung function laboratory, it is also recommended by some guidelines to test the response to 2-8 weeks of a therapeutic trial with inhaled corticosteroids (ICS). Also, there is no clear consensus about which degree of lung function improvement constitutes significant reversibility in subjects with airflow obstruction.[137] There is as yet no consensus on how a bronchodilator response should be expressed (percent of initial spirometric value, or percent of predicted value, or absolute change), and which variables should be used (FEV1, FVC, PEF). These differences are due to the heterogeneity of study designs and results, and also due to different interpretations of the outcomes of these studies that have been conducted in the general population and in patient populations (studies referenced by[137]). Also, the bronchodilator response tends to increase with decreasing baseline FEV1.[138] So, upon establishment of a generally applicable guideline, decisions will always be well-founded, but nonetheless, the definition of a universal cut-off level for a "positive" bronchodilator response will finally be arbitrary. Increments of lung function parameters of <8% are likely to lie within the range of measurement variability.[137,139,140] The recent 2017 GINA guidelines indicate the following criteria for making the diagnosis of asthma: for adults, an increase in FEV1 of >12% and >200 mL from baseline, and for children, an increase in FEV1 > 12% predicted. The ATS/ERS task force on standardization of lung function testing considers post-bronchodilator FEV1 or FVC > 12% and 200 mL compared with baseline as "significant" bronchodilation.[137]

Even though international recommendations regarding reversibility testing do differ in various aspects, knowledge of the ATS/ERS task force suggestions appears useful as this proposal has been set up to help minimize differences within and between laboratories. Beyond numerical criteria, it also appears useful in clinical practice to judge and compare the shapes of the flow-volume curves before and after bronchodilator inhalation.

A large worldwide study on the bronchodilator response in adult healthy general populations recently reaffirmed the 12% criterion[141] which approximates the 95th percentile for the percentage change in FEV1 after bronchodilator inhalation in general population studies mainly consisting of adults.[142] Still, the recommendations of the ATS/ERS task force have very recently been a subject of animated scientific debate.[143-146] In any case, sensitivity and specificity of the bronchodilator response for the diagnosis of asthma are limited. One major problem in judging reversibility tests in the diagnosis of adult asthma is the fact that there is also a significant bronchodilator response in COPD.[147] Patients with asthma may tend to show a larger increase in flow and volume after inhalation of a bronchodilator than COPD patients,[137] and a > 400 mL improvement in FEV1 in response to a bronchodilator is considered to strongly suggest underlying asthma.[83] However, the response to a bronchodilator has never been shown to add to the differential diagnosis, and the Global Initiative for Chronic Obstructive Lung Disease recommends that the degree to which airflow is reversible should not be used as a criterion in making the differential diagnosis between asthma and COPD.[148]

In children, diseases other than asthma may be associated with significant bronchodilator responses, such as allergic rhinitis and bronchopulmonary dysplasia.[149] On the other hand, also in children, an absent response to bronchodilators does not exclude asthma.[150] If the bronchodilator test is positive, it has been shown in children that this is predictive of a good response to ICS.[151] However, as most children with asthma have baseline FEV1 within the normal range,[152] the diagnostic value of bronchodilator responses during stable disease may often be limited in this age group. Different cutoff values have been proposed for the paediatric age group to increase sensitivity and specificity of bronchodilator response tests. The most recent of these studies on the validity of current criteria of a significant bronchodilator response included a large cohort of 1041 children with mild-to-moderate asthma from the US Childhood Asthma Management Program (CAMP) that were compared to 250 control subjects. Here, the conventional "adult" cut-off of 12% improvement in absolute FEV1 was associated with a good specificity for asthma diagnosis of 89.5%, but with a poor sensitivity of 35.6%.[153] This poor sensitivity may be due to the fact that only a minority of CAMP children had a baseline FEV1 of <80% predicted. Even though a cut-off of 8% resulted in a better sensitivity (54.4%), the authors do not recommend to choose a lower specific general cut-off criterion, given the variability of this test in children.

To summarize, the bronchodilator response test is one of the pieces in the mosaic of diagnosing asthma, along with a characteristic pattern of symptoms and signs in clinical history, and maybe signs of inflammation, such as increased FeNO. With baseline, lung function showing an obstructive pattern, serial monitoring, such as serial peak flow readings, may be useful for demonstrating variation and variability of airflow limitation. With normal baseline lung function, the bronchodilator response test is less valuable, and other tests like bronchoprovocation tests appear more expedient.

In children participating in the CAMP study, it has been shown that a consistent bronchodilator response of >10% over 4 years predicted night-time awakenings, oral steroid bursts, hospital visits and missed days of school.[154] Accordingly, in long-term asthma management, a positive bronchodilator response may indicate the need for an intensification in asthma treatment, for example by adding a long-acting bronchodilating agent to an ICS or increasing the ICS dose.[155,156]

2.3.4. Bronchial challenge tests

Fluctuation in airway calibre, a critical feature of asthma can be demonstrated in several ways including significant bronchodilation to β2-agonists and hyperresponsiveness towards direct stimulating agents like methacholine and histamine. When significant reversibility to inhaled salbutamol is not demonstrated, bronchial challenge with a direct agonist of smooth muscle is essential. The common way to express the bronchial hyperresponsiveness is determine the provocative concentration of the inhaled agent that causes a fall of 20% in FEV1 (PC20 FEV1).[157] Compared to measuring fluctuation of peak flow, measuring blood eosinophil count or reversibility to inhaled β2 agonist measuring PC20 methacholine was shown to have the higher accuracy to make a correct diagnosis in mild-to-moderate asthma.[158] Methacholine or histamine bronchial responsiveness is mainly seen as a marker of airway wall remodelling or intrinsic smooth muscle abnormality.[159] The role of eosinophilic inflammation in bronchial hyperresponsiveness, though not absent, is limited.[160]

Indirect challenges such as mannitol or exercise challenge are complementary to direct agent challenges and may reflect more accurately the underlying airway inflammation.[161] Direct challenge tests are sensitive and better to exclude asthma, while indirect challenge tests are seen as more specific and better to confirm the presence of the condition.[162]

The level of bronchial hyperresponsiveness to indirect agents is highly and rapidly sensitive to ICS treatment.[161] The effect of ICS on responsiveness to histamine and methacholine is much less impressive but sustained decrease over time was observed with continuous treatment.[159] Some study suggests that looking at FVC rather than FEV1 during a methacholine challenge may more informative on disease severity.[163] The slope dose-response curve to methacholine was shown to correlate to ACQ in a population of unselected asthmatic patients, the stronger the responsiveness the poorer the asthma control.[164]

The utility to include bronchial hyperresponsiveness (BHR) as a parameter to adjust asthma treatment has been less studied than with FeNO. There is one study that showed that monitoring methacholine responsiveness using PC20M to adjust the dose of ICS improved asthma control.[165] Although another study could not find any difference in terms of asthma-free days, however, adjustment by BHR produced a better outcome with respect to pre-bronchodilator FEV1 in allergic asthmatic children.[166]

Most of asthmatics are seen in the primary care setting. As asthma diagnosis is difficult in primary care and often leads to overdiagnosis[167,168] and wrong treatment allocation, it is an urgent need to find a convenient test for assessing bronchial hyperresponsiveness that may be applicable for general practitioners, that is easy to learn and administer and not too time-consuming. Mannitol challenge might do it, but it has to be demonstrated in large-scale study in general practice.[169]

Table 3 *Summary of prediction tools from birth cohorts*

	API[2]		**IoWight**[172]	**ECA**[a,173]	**PIAMA**[174]	**Leicester**[175]
General characteristics						
Children surveyed	1246		1456	449	1921	1226
Age at assessment (y)	0-3		0-4	0-2	0-4	1-3
Age at prediction (y)	6-13		10	10	8	6-8
Outcome prevalence (%)	13.7		37.2	N.A.	11.7	28.1
Parameters						
Frequency of wheezing episodes	✓			✓	✓	✓
Wheezing without colds	✓				✓	✓
Recurrent nasal symptoms	✓		✓			
Diagnosis of eczema	✓				✓	✓
Parental history of asthma	✓		✓			✓
Blood eosinophilia	✓					
Sensitization to aeroallergens			✓			
Recurrent respiratory infections			✓			
Duration wheezing of episodes				✓		
Hospitalizations due to wheezing				✓		
Parental use of inhaled medication					✓	
Male gender					✓	✓
Medium/low parental education					✓	
Post-term delivery					✓	
Age >1 y						✓
Activity disturbance					✓	✓
Shortness of breath					✓	✓
Exercise-related wheeze/cough					✓	✓
Aeroallergen related wheeze/cough					✓	✓
Diagnostic performance						
Cut-off point	L[b]	S[b]	≥ 3	> 5	≥ 20	≥ 5
Sensitivity	40	15	53	52	60	72
Specificity	80	96	85	88	76	71
PPV (%)	27	42	68	54	23	49
NPV(%)	88	86	74	87	94	86
Youden's index	0.20	0.11	0.38	0.40	0.36	0.43

[a]Nested case-control study.
[b]Loose and Stringent indexes at 11 y.

Table 4 *List of the 21 statements extracted from the review, ranked according to the mean score obtained in the two Delphi rounds*

Statement	Score
1. Prediction of future asthma in preschool children with reasonable accuracy	3.67
2. New biological markers of asthma (eg genomics, proteomics and metabolomics) as a tool for diagnosis and/or monitoring	3.54
3. New/improved tools to monitor lung function in the clinical setting	3.38
4. Assessing variability over time as a tool for diagnosis	3.33
5. FeNO as a tool in the diagnosis of asthma in patients older than 5 y of age	3.29
6. Refinement of symptom scores, for example Asthma Control Test	3.21
7. Tools to measure volatile organic compounds (VOCs) in exhaled breath condensate	3.17
8. Exhaled nitric oxide (FeNO) as a tool to guide the adjustment of inhaled corticosteroid dose in primary, secondary and tertiary care	3.13
9. Definition of standardized, normal values and cut-offs of lung functions tests at any age in EU populations for diagnosis and/or monitoring	3.13
10. Bronchodilation test as a tool for diagnosis and/or monitoring	3.04
11. Functional indexes other than FEV_1 (eg FVC, FRC, RV or RV/TLC) as a tool for diagnosis and/or monitoring	3.00
12. Serum periostin as a biomarker of allergic asthma as a tool for diagnosis and/or monitoring	2.92
13. Assessment of blood eosinophils as a tool for diagnosis and/or monitoring	2.88
14. Measurement of ventilation inhomogeneity (multiple breath washout) as a tool for diagnosis and/or monitoring	2.79
15. Bronchial challenge/hyperresponsiveness as a tool for diagnosis and/or monitoring	2.79
16. Tools to measure oxidative stress markers in exhaled breath condensate	2.75
17. Tools to measure non-volatile compounds, such as cytokines or chemokines, in exhaled breath condensate	2.67
18. The interrupter and forced oscillometry techniques as tools for diagnosis and/or monitoring in non-cooperative children	2.63
19. Plethysmography as a tool for diagnosis and/or monitoring	2.33
20. Measurement of blood eosinophil cationic protein as a tool for diagnosis and/or monitoring	2.25
21. Peak flow variability testing in routine practice as a tool for diagnosis and/or monitoring	2.08

2.4. Asthma prediction

Although some previous papers[170] looked for early markers for future asthma, the first index built on a prospective cohort of newborn children was the Asthma Predictive Index (API).[2] Several indicators were used to use a loose and a stringent index which included major and minor criteria. These indices were used to predict asthma at the age 6, 8, 11 and 13 years. API was modified to be used as an inclusion criteria tool in the Prevention of Early Asthma in Kids (PEAK) study as an expert opinion: one minor criterion (recurrent nasal symptoms) was substituted for two (sensitization to aeroallergens and food allergy).[171]

The Isle of Wight score[172] predicted persistence of wheeze at age 10 of children who wheezed at 1, 2 and 4 years of life (see Table 3 for specific items of the score, range 0-4). A cut-off point of >3 was found to be the highest discriminative.

Another predictive score was developed from the data of a nested case-control study of 449 children included in the Environmental and Childhood Asthma birth cohort (ECA).[4,173] Children with recurrent [≥2 episodes of or ≥4 weeks (persistent)] doctor confirmed bronchial obstruction by their second birthday were cases. The authors built a severity score (0-12 points, see Table 3 for items in the score) and used a cut-off of >5 to predict asthma at age 10.

Table 5 *Three most important fields ranked for research investment in tools for diagnosing and/or monitoring asthma (note that lower score means more importance)*

Statement	Score
1. Prediction of future asthma in preschool children with reasonable accuracy	1.63
2. New biological markers of asthma (eg genomics, proteomics and metabolomics) as a tool for diagnosis and/or monitoring	1.69
3. Tools to measure volatile organic compounds (VOCs) in exhaled breath condensate	2.69

The PIAMA (Prevention and Incidence of asthma and Mite Allergy) score[174] was calculated from the findings of this birth cohort by the age of 8 years. Several markers found in children who had wheeze and/or cough at night without colds during the previous 12 months at ages 1 through 4 years were used to build a score (0-55 points) in order to predict asthma at age 8 years. A cut-off value of ≥ 20 was found to be the best in diagnostic performance.

The latest predictive model published is the one from the Leicestershire Respiratory Cohort Studies,[175] which included children followed from birth who had at least a healthcare visit between ages 1 and 3 years for respiratory problems plus wheeze and/or cough without a cold and/or cough at night. The presence of wheeze plus asthma medication during the previous 12 months was used as asthma case definition at 6-8 years of age.

Although those prediction tools are easy to apply their diagnostic power needs to be improved: the best Youden's index does not reach even 0.5, and although the specificity value is quite acceptable, the sensitivity one is quite low. Performance of API and PIAMA scores in subsequent (validation) studies did not improve, producing relatively low rates of false positives (8%-67%) but quite high rates of false negatives (29%-80%).[174,176-178]

An additional limitation, which has not been fully addressed, is the exact definition of the dependent (outcome) variable used to diagnose asthma at the age when the condition is intended to be predicted. Different definitions may have important effects on the predictive probabilities as provided by the predictive model.[179] However, and based on the data in Table 3, there seem to be 2 different areas which might determine future asthma when present in infants or preschool children: allergy and severity of wheezing episodes.

Other attempts to build prediction indexes/scores to predict asthma in adulthood from data in childhood have reached to similar conclusions.[180] Some authors have suggested that biological markers might predict asthma. Among those biomarkers which might have some usefulness are as follows: eosinophils, ECP, specific IgE, filaggrin mutations, Th2 interleukins, FeNO, EBC characteristics and composition and, of course, genetics in the form of polygenic risk and genetic risk scores.[181-184]

To use prediction indices as a generalized tool, their predictive power needs to be improved. The current evidence suggests that a tool based only on clinical data is limited in its predictive capacity; and that adding one or several biomarkers could be helpful. Which biomarker(s) is(are) more useful remains to be elucidated; and might be a research priority.

There are probably 4 fields in which the new predicting tools need to be improved: (1) Clarification of the dependent variable (outcome definition); (2) Combination of clinical and biological markers; (3) Application of more sophisticated and available statistical methods; and (4) Uniformity of the population for which the score is developed.

The clarification of the outcome variable is closely related to a better profiling of asthma phenotypes and their stability at certain ages (adolescence and young adulthood). This clarification should not be solely based on the clinical and biological marker classification, but also on the response to the different available treatments.

3. CONSENSUS EXERCISE

The exercise included two Delphi rounds previous to a summarizing workshop in which the information obtained in the Delphi rounds was shared and the 3 most important topics among those obtaining a mean score of 3 or more in the Delphi rounds were chosen and ranked.

3.1. Delphi exercises (1st and 2nd rounds)

To reach an agreement on the most important diagnostic tools for future research investment, a list of statements extracted from the review, 21 statements (Table 4) describing the likelihood that further investment in each technique would result in the development of simple, accurate, inexpensive, non-invasive diagnostic tool/s were extracted from the review.

The statements were circulated among 37 European asthma experts including health professionals, researchers, people with asthma and industry representatives who were asked to rank the potential impact of research investment in each technique or tool (score 1-5; 1 = very low, 5 = very high). The e-Delphi exercise was conducted over 2 rounds: round one (August 2015) 25 responses were collected. Round 2 (September 2015) respondents from round 1 were asked to review their responses, along with the average score for each statement, and asked whether they would change their ranking and given an opportunity to provide any comments; in total, 16 responses were obtained from the 25 requests.

Statements with a mean score 3 or more were considered to have acceptable consensus on their likely impact on the investment on asthma diagnosis and/or monitoring. From the list of 21 statements extracted from the review, 11 obtained a mean score 3 or more (Table 4).

3.2. Summarizing workshop

To better refine and contextualize the priorities produced from the Delphi exercise, and to reach an agreement on those likely to have the biggest impact on people with asthma, a workshop was organized during the 2015 European Respiratory Society meeting on September 26th, 2015. Representatives from the working group, healthcare professionals, researchers, people with asthma, patient organization representatives and industry representatives, discussed the results from the exercise. Attendees were asked to rank the top 3 diagnostic tools which should be considered as priorities in the future. The 3 most impactful diagnostic and or monitoring tools were ranked from highest to lowest, as shown in Table 5.

4. CONCLUSIONS

Research on the prediction of asthma in preschool age with reasonable accuracy and how to integrate the new biological markers in the diagnosis and monitoring of asthma should be the two main research areas towards which the economic effort should be addressed. A third area of importance is the measurement of exhaled volatile organic (VOCs) compounds. If Volatilome is contemplated as a new biological marker, the importance of its measurement would be probably enhanced.

Conflict of interests

The authors declare no conflict of interest.

Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article.

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APPENDIX 1

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Paul Aurora; University College London; UK. Eugenio Baraldi; University of Padova; Italy. John Blakey; Liverpool School of Tropical Medicine; UK. Silvia Carraro; University of Padova; Italy. Chris Compton; GSK; UK. Louise Fleming; Imperial College; London; UK. Steve Fowler; University of Manchester; UK. Erol Gaillard; University of Leicester; UK. Monika Gappa; Marienhospital Wesel; Wesel; Germany. Luis Garcia-Marcos; University of Murcia; Spain. Frankie Gibson; EARIP Patient Advisory Group; UK. Glenn Glenn Crater; Aerocrine; UK. Renaud Louis; University of Liege; Belgium. Antonio Moreno-Galdo; University Hospital Vall d'Hebron; Barcelona; Spain. Rob Niven; University of Manchester; UK. Diego Peroni; University of Pisa; Italy. Kostas Priftis; University of Athens; Greece. Amanda Roberts; EARIP Patient Advisory Group. Dermot Ryan; General Practitioner, UK. Manuel Sanchez-Solis; University of Murcia; Spain. Antje Schuster; Heinrich-Heine-Universität Düsseldorf; Germany. Ulla Seppala; Aerocrine; UK. Omar Usmani; Imperial College, London; UK. Marc van der Schee; Academic Medical Centre, Amsterdam; The Netherlands. Jacob van Sont; Leiden University Medical School; The Netherlands.

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