INTRODUCTION

Allergic diseases are considered a major problem for healthcare systems in both developed and developing countries **(Da Silva et al., 2017).** Over the past years, longitudinal epidemiologic studies have shown an increased prevalence of sensitization to common allergens, and increased prevalence of asthma, atopic dermatitis, food allergy, and allergic rhinitis in children **(Salo et al., 2014).**

The increase in allergy is a phenomenon that is being observed in all fast-developing countries. For a long time, science has taken as a starting point that solely a genetic predisposition is a precondition for the development of an allergy. Today, knowledge of environmental factors that can alter genes or the transcription of genes in the cells, has improved **(Lake et al., 2016).**

Grass pollens are one of the most important airborne allergen sources worldwide. About 20 species from five subfamilies are considered to be the most frequent causes of grass pollen allergy, and the allergenic relationships among them closely follow their phylogenetic relationships **(D'amato et al., 2007).**

The allergic immune response to pollen of several grass species has been studied extensively over more than three decades. Eleven groups of allergens have been identified and

described, in most cases from more than one species. The allergens range from 6 to 60 kD in apparent molecular weight and display a variety of physicochemical properties and structures ***(Andersson et al., 2003).***

Grass pollens are amongst the most clinically important allergen sources worldwide and are responsible for triggering allergic rhinitis and exacerbating asthma ***(Davies, 2014).*** The most complete set of Timothy grass allergens has so far been isolated and cloned from Phleum pratense pollen ***(Hatzler et al., 2012).***

AIM OF THE WORK

We sought to investigate the rate of sensitization to Timothy grass pollen in a group of asthmatic Egyptian children in relation to other markers of disease expression. Our ultimate objective was to add information to the map of aeroallergen sensitization in our country.

ALLERGIC SENSITIZATION AND ASTHMA

The complex interplay between allergic sensitization and asthma, and a series of phenotyping studies that discriminated distinct sub-types of asthma **(Belgrave et al., 2013; Deliu et al., 2018)**, eczema **(Paternoster et al., 2017).** and atopy **(Kurukulaaratchy et al., 2005; Simpson et al., 2010; Lazik et al., 2013; Hose et al., 2017; Lee et al., 2017; Schoos et al., 2017; Stoltz et al., 2013; Garden et al., 2013)**, have provided support to the concept of heterogeneity of asthma and allergic sensitization.

Recognition of asthma heterogeneity has led researchers away from a focus on a single biological marker or clinical test that may be applicable to all patients with asthma, towards more personalized approaches that can address specific challenges in individual patients, or groups of patients. Over the last two decades, a substantial effort has been devoted to understanding asthma heterogeneity, and its variable clinical expression in individuals **(Pavord al., 2017; Anderson, 2008; Wenzel, 2004).**

One approach to understanding disease heterogeneity involves integrating a series of statistical models that account for the unobserved heterogeneity between individuals, with mechanistic information about underlying pathophysiology

(Belgrave et al., 2017). For example, the latent class analysis relies on the assumption that observable characteristics are imperfect indicators of an underlying (latent) construct **(Howard et al., 2015).** Over the last two decades, many attempts have been made to identify longitudinal trajectories of childhood wheeze **(Belgrave al., 2013; Spycher et al., 2008; Kurukulaaratchy et al., 2003)**, atopy **(Paternoster al., 2017; Simpson et al., 2010; Hose et al., 2017; Roduit et al., 2017; Herr et al., 2012)** and asthma **(Weinmayr et al., 2013; Depner et al., 2014; Boudier et al., 2013)** by means of latent class analysis. Such 'phenotyping' studies have traditionally been based on clinical characteristics that are mostly observed and recorded by physicians in the clinical/research settings.

However, features, which are collected to ascertain asthma presence or severity, may not be the most informative for the discovery of true disease endotypes. A recent study has shown that a careful synergy of data-driven methods and clinical interpretation may help better understand the heterogeneity of asthma, and enable the discovery of true asthma endotypes **(Deliu et al., 2018).** This study has identified allergic sensitization as one of the four potentially important features for disaggregating childhood asthma, the other three being the age of onset, asthma severity and recent exacerbations.

To date, the identification of asthma subgroups described in different studies, and how the current approach to 'asthma

phenotyping' may lead to more effective and targeted asthma treatment strategies, has not been translated to clinical setting **(Pavord al., 2017; Belgrave et al., 2017; Deliu et al., 2017).**

Heterogeneity of allergic sensitization and its relevance to asthma

Atopy can be defined as the genetic tendency to become sensitized to common allergens that most people do not react to, as a result of ordinary exposure. Atopic diseases cover a heterogeneous group of symptoms and disorders, ranging from wheezing, coughing, breathlessness, hay fever and rhinitis, to skin conditions such as atopic dermatitis (eczema, or atopic eczema), each of which might be triggered by different factors. Different manifestations of atopic disease may co-exist in the same patient, or develop at different times **(Belgrave et al., 2014).** However, a history of atopy and confirmation of IgE-mediated sensitization (e.g. using skin prick tests and/or measurement of specific serum IgE) does not necessarily indicate the presence of an immunologically mediated allergic reaction and/or allergic disease, as sensitization may be asymptomatic **(Custovic et al., 2013).**

Recent evidence has shown that similar to asthma, 'atopic sensitization' is heterogeneous, and that there are several distinct sub-groups of sensitization, differing in their risk factors, associations with asthma progression and response to treatment **(Simpson et al., 2010; Lazik et al., 2013; Stoltz**

et al., 2013; Garden et al., 2013; Custovic et al., 2015; Havstad et al., 2014; Custovic et al., 2015; Wickman et al., 2017).

Four different classes of sensitization were uncovered, including predominantly dust mite non-dust mite, multiple early and multiple late sensitization, and have shown distinct associations of these classes with asthma. These findings have been subsequently replicated in another birth cohort **(Lazic et al., 2013)**, and have demonstrated that the association with asthma and diminished lung function is strong for multiple early, but not other sensitization classes **(Belgrave et al., 2014).**

In another study, **Garden et al. (2013)** described three latent atopic phenotypes using longitudinal data on skin prick sensitivity (late mixed inhalant sensitization, mixed food and inhalant sensitization, and dust mite monosensitization).

In another study **Hose et al. (2017),** atopic sensitization was classified into three phenotypes (benign atopy, symptomatic atopy and severe atopy). More recently, **Dharma et al. (2018)** revealed four distinct patterns of allergic sensitization (atopic dermatitis; inhalant sensitization; transient sensitization and persistent sensitization).

The finding that the timing of onset and types of sensitization are the key discriminative factor for atopy subtypes **(Simpson et al., 2010; Lazik et al., 2013; Custovic**

et al., 2015) has been independently confirmed by a recent study **(Lee et al., 2017)**, which reported the existence of four distinct atopy phenotypes, differing in the time of onset and types of sensitizing allergens (later sensitization to indoor allergens, multiple early sensitization, early sensitization to outdoor allergens followed by indoor allergens, and early sensitization to indoor allergens followed by outdoor allergens).

Similarly, **Schoos et al. (2017)** explored the longitudinal patterns of atopic sensitization in early childhood based on sIgE responses and revealed a total of seven latent sensitization patterns: cat/dog/horse; timothy grass/birch; molds; house dust mites; peanut/wheat flour/mugwort; peanut/soybean and egg/milk/ wheat flour. Although direct comparison between studies is difficult because of differences in subject ascertainment, population structure and diagnostic criteria, there is increasing evidence that the risk of asthma is significantly higher among children who are highly sensitized **(Hose et al., 2017; Havstad et al., 2014)**, sensitized early in life **(Simpson et al., 2010; Lazik et al., 2013; Custovic et al., 2015)**, or sensitized to multiple allergens **(Lazic et al., 2013; Garden et al., 2013).**

Additionally, sensitization to allergens from specific sources as dogs **(Stoltz et al., 2013),** cats or horses **(Schoos et al., 2017)** was reported to be strongly associated with asthma development during childhood. Summary of cross-sectional and longitudinal studies in children, which employed investigator-

led and data-driven approaches to identify distinct patterns of allergic sensitization, and their specific relationship with asthma, is shown in **Table 1**.

Table (1): Atopy phenotypes and their association with asthma

Reference	Cohort	Age group (years)	Biomarker	Atopic sensitizations paterns	Strongest association with asthma
Kurukulaaratchy et al., 2005	IOW	4–10	SPT	Early childhood atopic; chronic childhood atopic; delayed childhood atopic	Chronic childhood atopic
Simpson et al., 2010	MAAS	1–8	SPT and sIgE	Nondust mite, dust mite, multiple early, multiple late	Multiple early sensitization
Lazic et al., 2013	MAAS	1–11	SPT and sIgE	Early sensitivity to grass, eggs but not mite; dust mite; early sensitivity to mite, grass, pollens and late sensitivity to pets; multiple sensitivity	Multiple sensitivity
	IOW	4–18	SPT	Sensitivity to grass, late sensitivity to peanut; dust-mite; sensitivity to mite, grass and late sensitivity to pets; multiple sensitivity	Multiple sensitivity
Stoltz et al., 2013	COAST	1–9	sIgE	Any sensitization; mono sensitization; polysensitization; cat; dog; dust-mite; Alternaria alternata; cockroach; ragweed; silver birch; grass	Dog sensitization
Garden et al., 2013	CAPS	1.5–8	SPT	Late mixed inhalant; mixed food and inhalant; dust-mite	Mixed food and inhalant
Havstad et al., 2014	WHEALS	2	sIgE	Highly sensitised; milk and egg-dominated; peanut and inhalants	Highly sensitised
Schoos et al., 2017	COPSAC	1–6	sIgE	Dog/cat/horse; grass/birch; molds; dust-mite, peanut/wheat flour/mugwort; peanut/ soybean, and egg/milk/wheat flour.	Dog/cat/horse sensitization
Hose et al., 2017	MAS	1–6	sIgE	Food sensitization; seasonal sensitization; mite sensitization; severe atopy	Severe atopy
	PASTURE			Cow's milk sensitization; food sensitization; inhalant sensitization; severe atopy	

Reference	Cohort	Age group (years)	Biomarker	Atopic sensitizations paterns	Strongest association with asthma
Lee et al., 2017	CHEER	7–11	SPT	Later sensitivity to indoor allergens; multiple early, early sensitivity to outdoor allergens followed by indoor allergens; early sensitivity to indoor allergens followed by outdoor allergens	Early sensitivity to outdoor allergens and late sensitivity to indoor allergens
Dharma et al., 2018	CHILD	1–3	SPT	Atopic dermatitis; inhalant sensitization; transient sensitization; persistent sensitization	persistent sensitization

CAPS, childhood asthma prevention study; CHEER, children's health and environmental research; CHILD, Canadian healthy infant longitudinal development; COAST, childhood origins of asthma; COPSAC, Copenhagen prospective studies on asthma in childhood; IOW, Isle of wight; MAAS, Manchester asthma and allergy study; MAS, multizentrische allergiestudie; PASTURE, protection against allergy: study in rural environments; sIgE, specific IgE; SPT, skin prick test; WHEALS, Wayne County health, environment, allergy and asthma longitudinal study.

Diagnostic tests to ascertain allergic sensitization and its relation to asthma

Skin tests and blood tests are commonly used to diagnose clinical allergies and sensitivities. However, in the context of respiratory allergy, the interpretation of these commonly used allergy tests remains arbitrary, as it traditionally relies on predefined cut-offs that have relatively poor ability to distinguish between asymptomatic sensitizations and clinically relevant allergies, and does not take into account either age, sex or ethnicity of the patient. A recent study has demonstrated that both age and sex should be taken into account whenever interpreting the results of skin tests and sIgE measurement, and that age-specific and sex-specific normative data are urgently needed **(Mohammad et al., 2016).**

To increase the diagnostic accuracy of current allergy tests in relation to the presence and persistence of asthma, the results should be reported in a quantitative manner (e.g. the titre of IgE, or the size of skin test wheal diameter). There is a clear need to develop better ways of interpreting these tests to identify patients with clinically relevant allergies more precisely, for example, by taking into consideration sex, age and environmental exposure. **(Prosperi et al., 2014).**

In a recent publication, IgE reactivity to a limited number of allergen molecules early in life identified children with a high risk of both asthma and rhinitis in adolescence in two birth cohorts from the UK and Sweden **(Wickman et al., 2017).** In Sweden, early-life IgE reactivity to four risk molecules (peanut Ara h 1, birch Bet v 1, cat Fel d 1, and grass Phl p 1) predicted both incident and persistent asthma at 16 years, whilst in the UK, similar association was observed for five allergenic molecules (dust mite components: Der p 1 and Der f 2; Phl p 1 and Phl p 5; Fel d 1) **(Wickman et al., 2017).**

A relevant study has indicated that longitudinal trajectories of sensitization patterns to a limited number of grass and dust mite allergens from age 5 to age 11 years had different associations with clinical outcomes, indicating that the time of onset of specific patterns of IgE response may be critically important **(Custovic et al., 2015).** Similarly, **Posa et al. (2017)** have recently shown that IgE polysensitization to

several dust mite molecules predicts current rhinitis, and both current and future asthma.

Diagnostic and prognostic test for asthma

Difficulties related to confirming the diagnosis of allergy is only a small part of a bigger diagnostic problem in asthma. The ability to predict individuals who may become asthmatic in the long-term is equally important to prevent asthma, or minimize its impacts on many aspects of the lives of individuals. There has been a significant amount of work carried out on developing predictive models that could be incorporated into the decision-making process **(Savenije et al., 2012; Caudri et al., 2013; Kurukulaaratchy et al., 2003; Castro-Rodrı´guez et al., 2000; Pescatore et al., 2014),** which has been systematically reviewed and critically evaluated recently **(Smit et al., 2015; Luo et al., 2015). Figure 1** shows diagnostic aids for asthma in children and specific difficulties associated with each step.

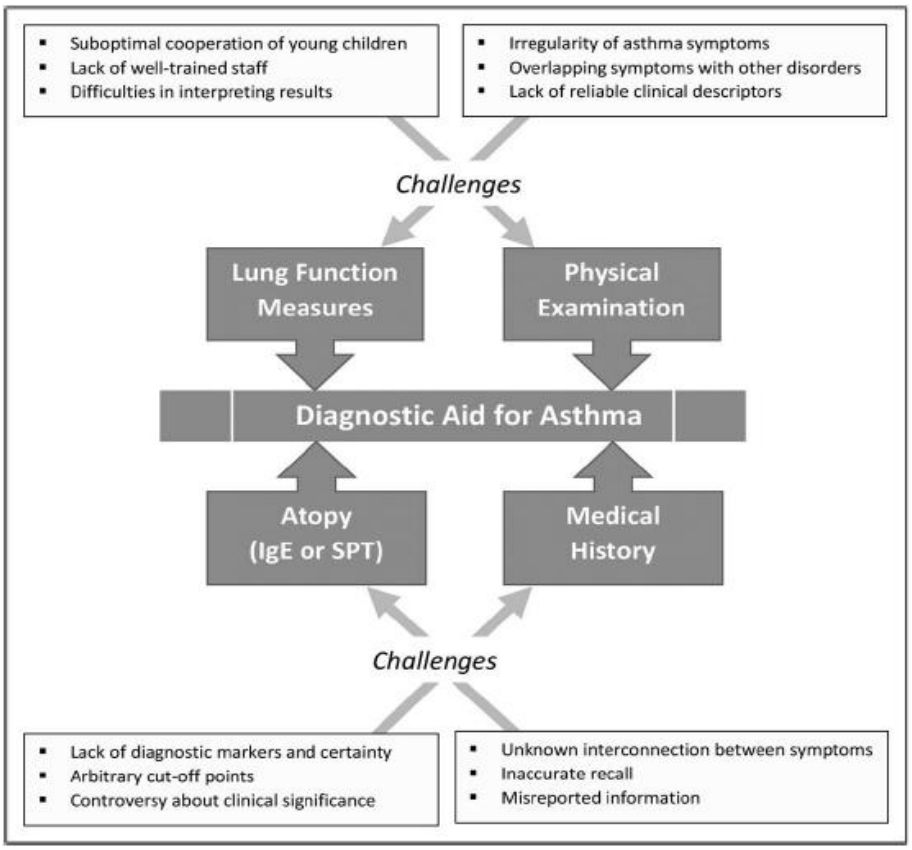

Figure (1): Diagnostic problems in asthma.

Although the majority of existing asthma prediction models were developed based on either clinical indices or regression equations **(Luo et al., 2015),** there is an increasing trend towards using machine-learning approaches to develop predictive models for asthma **(Chatzimichail et al., 2013; Finkelstein, 2017).** A proposed algorithm have been recently tested amongst children aged 13–16 years, and found poor agreement between the algorithm and asthma defined by physician diagnosis, presence of current symptoms and regular use of inhaled corticosteroids **(Murray et al., 2017).**

However, in the absence of standardized asthma outcome definitions, diagnostic criteria for asthma and independent validation studies in different cohorts, the clinical value of the developed predictive models, and their applicability to other populations and different healthcare settings, remains unclear. Moreover, the predictive accuracy of asthma predictive indices and models decreases with the increasing prevalence of asthma in the population studied **(Brand, 2011),** suggesting that, although these tools may be useful in low-risk populations, their prediction performance is still far from being satisfactory in clinical settings, especially for highrisk patients.

One of the key questions going forward is how best to incorporate tests for the assessment of allergic sensitization into diagnostic algorithms for asthma, both in terms of confirming asthma diagnosis, and the assessment of future risk (e.g. of asthma exacerbations, or disease persistence). It is possible that such diagnostic algorithms may include not only the measurement of allergen specific IgE but also allergen-specific IgG responses. In three birth cohort studies, it has been shown that dissociation between allergic airway symptoms and sIgE sensitization in children is associated with a high-level coproduction of sIgG1 **(Holt et al., 2016).**

GRASS POLLEN ALLERGY

Allergic diseases affect up to 20-30% of the world population, including children as young as 3, and are associated with disrupted sleep, impaired work performance and lower life quality **(Andersson and Lidholm, 2003; Bousquet et al., 2008; Dykewicz and Hamilos, 2010; Suphioglu et al., 1992; Wheatley and Togias, 2015).** Among the allergic diseases, hay fever is a widespread allergic upper respiratory condition triggered by airborne pollen allergens. Pollen allergen exposure can also exacerbate asthma in susceptible individuals **(Aalberse, 2000; Bousquet et al., 2008; Davies, 2014; Greiner et al., 2011; Scala et al., 2010).**

Genetic and environmental variations between individual plants, as well as differences between allergen proteins in various plants species, particularly grasses (Poaceae family), impact allergen exposure, sensitivity to allergens and symptoms of patients **(Aiubi et al., 2015; Amardip, 2014; Buters et al., 2015; Chen et al., 2016; Davies, 2014).**

Allergic sensitization to aeroallergens frequently leads to the production of allergen-specific IgE antibodies in susceptible people **(Moneret-Vautrin, 1997; Moneret-Vautrin and Kanny, 2007).** IgE typically recognises motifs or conformational epitope structures on the surface of allergen proteins, which contain diverse and complex motifs, making it difficult to attribute IgE binding to any single linear peptide