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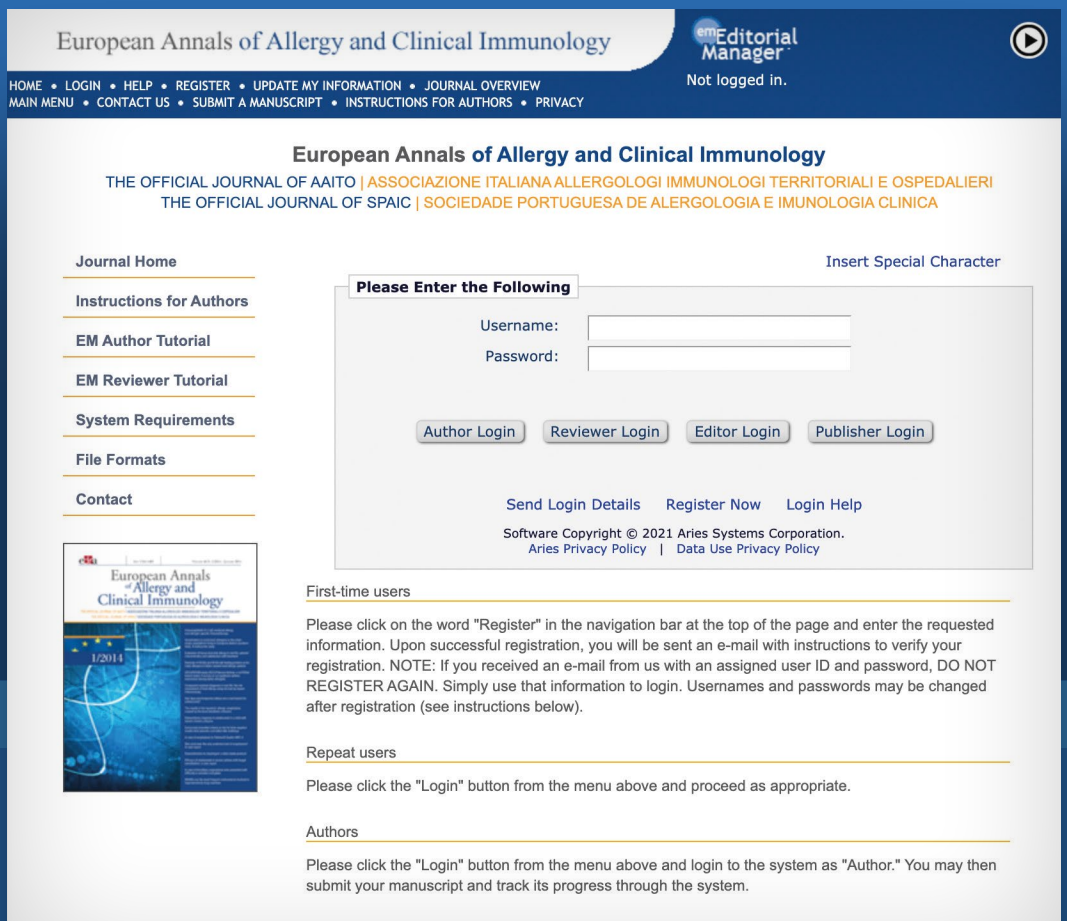

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EDITORIAL | Eur Ann Allergy Clin Immunol | Vol 57, N.5, 195-196, 2025

Adriano Vaghi[1], Maria Beatrice Bilò[2,3], Antonino Musarra[4]

Pollen-induced asthma: a unique model of mild to moderate asthma

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Asthma is a disease that affects approximately 300 million people worldwide (1), and patients with mild asthma represent approximately 50-75% of this population (2).

Although patients with mild asthma constitute the vast majority of asthmatics, it is a subgroup still understudied and mistakenly considered to be easy to manage clinically. In fact, the so-called mild asthma remains a poorly researched clinical area despite its significant impact on the life of some patients, particularly because of the possibility of experiencing severe exacerbations (3).

The definition of mild asthma itself is not consistently agreed upon by the main guidelines, and this lies in the assumption that the level of severity and frequency of symptoms is stable over time, and in the attempt to standardize the different characteristics of all patients under a single umbrella diagnosis (4).

Instead, it has been emphasized recently that the so-called mild asthma is in fact a heterogeneous condition characterized by different pathogenic and inflammatory mechanisms and clinical manifestations which may benefit from a differentiated and personalized management approach (5).

From this perspective, the works by Cecchi *et al.* on pollen-induced asthma (PIA) (6, 7), which has previously been classified as one of the clinical manifestations of mild-moderate asthma due to its long asymptomatic or paucisymptomatic periods, suggest that PIA might be considered as specific phenotype of asthma.

Early phenotyping, even in non-severe asthma, has recently been highlighted as a useful tool to improve a "precision" approach to asthma therapy (8). PIA meets the criteria for defining a phenotype as outlined by Han *et al.* (9). An ongoing issue in defining phenotypes is ensuring their long-term stability and addressing potential overlaps or transitional features with other phenotypes. However, several reviews and cohort studies indicate that this phenotype generally remains stable in the long-term (10, 11). The definition of PIA as a phenotype paves the way for a specific diagnostic algorithm, where T2 biomarkers, particularly FeNO, but also eosinophils, play a significant role as they can be considered endotypic diagnostic tests for PIA (6). The paper of Cecchi *et al.* also highlights how the diagnosis of PIA can be difficult outside the exposure period (6). In fact, the low expression of T2 markers and the low level of inflammation minimize the instability of the airway caliber and therefore the variability of FEV1, the positivity of the bronchodilation test and also the airways hyperreactivity, which are markers common to all asthma phenotypes (12).

The interpretation of PIA as a phenotype has important implications from a management point of view. The paper of Cecchi *et al.*, in fact, underlines how in these subjects the use of ACT as well as the use of cut-off values for the frequency of symptoms usually used to evaluate the "control domain" can be falsely reassuring when assessed outside the exposure periods and therefore lead to an underestimation of the patient's possible therapeutic needs during periods of maximum pollen exposure (6). The authors (6, 7) therefore suggest the adoption of a multidimensional "risk prediction" score that includes clinical history, symptoms, respiratory function, biomarkers, and comorbidities, in order to assess, even if indirectly, the patient's actual future risk and to personalize the therapeutic strategy.

Severe exacerbations and near-fatal asthma or fatal asthma episodes cannot be predicted in an individual subject because they are the result of a number of risk factors that add up and are potentiated in a variable way depending on the circumstances. However,

they can be preventable through the adoption of specific clinical tools such as an accurate risk stratification and the adoption of the maximum precautionary principle, which aims to adopt the more appropriate therapeutic approach (6, 7).

Therefore, adopting a proactive therapeutic strategy, when indicated, and a rapid step-up during the exposure season represents an advantageous strategy at a time when the patient is particularly vulnerable and when the transition from the onset of symptoms to a flare-up can be extremely rapid.

The aim of minimizing and preventing symptoms by adopting a proactive and non-reactive approach offers the additional advantage of avoiding phenomena of repeated instability of the caliber of the airways. Repeated bronchoconstriction episodes associated with allergic exposure favours crosstalk between the epithelium, inflammatory cells (eosinophils and mast cells) and smooth muscle cells which determines the activation and persistence of a T2 type inflammation and bronchial remodelling phenomena (12, 13).

From a clinical point of view, pharmacodynamic and pharmacokinetic characteristics of the inhaled corticosteroid should maximize bronchoprotection (14), thus effectively attenuating the airways hyperreactivity, which represents an important factor that influences the subjective threshold for allergen-induced bronchoconstriction (8).

In conclusion, the works by Cecchi *et al.* (6, 7) provide us with a new management strategy for PIA and offer us indications on how to look beyond the appearances and to recognize in time the "slumbering fire" and to adopt the most appropriate therapeutic strategy to control asthma during the pollen exposure season and avoid severe exacerbations.

This new interpretation of PIA allows us to overcome the simplistic indication of a "one-size-fits-all therapy" and to initiate a "precision" approach based on a careful and individual stratification of "future risk".

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edra REVIEW | Eur Ann Allergy Clin Immunol | Vol 57, N.5, 197-210, 2025

Lorenzo Cecchi[1], Matteo Martini[2,3], Kliljeda Jaubashi[4], Alessandro Maria Marra[5], Antonino Musarra[6], Francesco Papia[7], Adriano Vaghi[8], Giuseppe Valenti[7], Baoran Yang[9], Maria Beatrice Bilò[2,3]

Pollen-induced asthma: a specific pheno-endotype of disease?

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Key words

Asthma phenotype; pollen sensitization; pollen-induced airway inflammation; pollen concentration; allergic asthma.

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Impact statement

Pollen-induced asthma could be considered a specific phenotype. Pollen allergenicity depends not only on genetic and environmental factors, but also on the immunostimulatory components of the pollen matrix, that contribute to airway disease and may represent a defining feature of allergic asthma.

Summary

Asthma is a heterogeneous syndrome with a significant social and economic impact. While the knowledge of pheno-endotypes has advanced in severe asthma, little attention has been paid to the phenotypes of mild-moderate asthma. Along this line, a systematic review of the current literature on pollen-induced asthma was carried out, targeting the question whether it can be considered a specific phenotype of disease, with a focus on the role of pollen and its interplay with asthma.

This article reports the first part of the review, which covered background information on the multiple atmospheric and environmental factors affecting pollen concentration, the molecular bases of pollen-induced allergenicity and the pathogenic effector circuits that sustain and amplify inflammatory signals in response to allergens in sensitized subjects.

Introduction

Currently, asthma is no longer considered a single disease but a complex and heterogeneous syndrome that includes variable clinical presentations (phenotypes) and specific pathophysiological mechanisms (endotypes) (1-6).
Asthma impacts over 300 million individuals of all ages worldwide, with a high count of disabilities, and premature deaths (7). According to the Global Burden of Disease (GBD), asthma is the second leading cause of death among chronic respiratory diseases, with 457.01 thousand deaths in 2017 (7, 8). Asthma is often associated with various comorbidities, such as allergic rhinitis, nasal polyps, gastroesophageal reflux disease, obstructive sleep apnea, and anxiety, leading to increased morbidity and seriously affecting patients' quality of life (7).
Prevalence data on asthma are important for the understanding of the clinical and economic burden of the disease. However, the estimation of the epidemiology of asthma at global level is challenging, because of the complex nature of the disease and the lack of universally accepted case definition and tests that are confirmatory for asthma (9-11). The results from a systematic analysis of the literature, including data extracted from the Global Burden of Diseases, Injuries and Risk Factors Study 2019, show considerable variation across countries in the estimation of asthma prevalence, ranging from 1.43 to 11.25% (10). Regarding incidence, an increase was observed globally over the 30-year period 1990-2019, which occurred especially in Africa countries, with the number rising from 6,487,957.18 (95 %UI: 4,578,735.08-8,736,387.55) to 7,604,488.39 (95% UI: 5,428,024.98-10,177,808.25) (7, 12). Mortality for asthma in adolescent and young adults has exhibited a consistent downward trend over a period of 30 years, which may be linked to improved asthma management. However, areas with lower socio-demographic index have higher age-standardized mortality rates for asthma and deserve attention and priority support for medical resources.
Allergic asthma, usually defined as asthma associated with sensitization to by otherwise harmless environmental substances, i.e. allergens (as pollen, fungal spores, animal hair, house dust mite), is the most common asthma phenotype (13, 14). It is estimated that up to 80% of childhood asthma and more than 50% of adult asthma cases may have an allergic component (15, 16). Molecular studies by Kaur *et al.* (2019) also found that T2 signature, with high sensitization to allergens, increased airway and blood eosinophils and good response to ICS, concerns a significant proportion of adult patients with asthma (6). The average age of onset of allergic asthma is younger than that of nonallergic asthma (13). Although the spectrum of allergic asthma may vary from mild to severe, studies have reported that allergic *versus* nonallergic asthma is less severe (13).
Allergens are triggers for asthma symptoms and can lead to increased morbidity. The majority of children with asthma in US are found to be sensitive to at least one indoor allergen (mite, molds, cat, dog) (17). Exposure to airborne pollen grains is known to be associated with asthma exacerbations and hospital admissions, especially in sensitized individuals and in children (18, 19*).* A prospective cohort study demonstrated that the sensitization to specific aeroallergens differentially impacts the risk of developing asthma and rhinitis. Specifically, sensitization to perennial allergens, to dog in particular, was associated with higher asthma risk as compared to seasonal allergens. Poly-sensitization at all ages was greatly associated with increased asthma risk (17, 20).
In the last decade, important knowledge milestones have been achieved in the description of the pheno-endotypes of severe asthma, while little attention has been so far paid to the phenotypes of mild-moderate asthma (21). Indeed, while current clinical guidelines underline the importance of phenotyping severe asthma, to target the appropriate therapy (*i.e.*, biologics), phenotyping mild-moderate asthma is not considered relevant, as the therapeutic approach recommended in these patients is considered to be independent of the phenotypes. In addition, the role of pollen, a major causal agent of respiratory allergy, in the complex interplay with asthma has not been completely elucidated. Along this line, the aim of our work is to investigate whether pollen-induced asthma (PIA) can be considered a specific phenotype in patients with mild-moderate asthma.

Materials and methods

A systematic review of the literature was conducted on Medline to identify English papers published up to March 31, 2024. Hand searching of references of interest was also performed within the selected studies. The search strategy included at least one keyword, in the title/abstract, for each of the following domains: pollen as a source of allergens (factors affecting pollen concentration, pollen size, immunologic mechanisms of response to airborne allergens), pollen-induced asthma (epidemiology, pollen-induced airway inflammation).
The research and selection of the studies were performed independently by five allergists, who collected and summarized the data from the studies. All the authors contributed to the definition of the research question and related keywords, and to the final selection of the studies to be included in the systematic review.

Results

Pollen as a source of allergens

Factors affecting pollen and allergen concentration
The concentration of pollens can be significantly affected by multiple atmospheric, environmental and botanical factors, thereby increasing the risk of respiratory symptoms and exacerbations in allergic pollen-driven asthma (18, 22-24).

Table I *- Main factors affecting the concentration of pollen and allergens.*

Atmospheric factors
Temperature
Humidity
UV radiation
Thunderstorms
Wind speed, distance, and direction (long-distance transport, air mass trajectories)
Pollution
Environmental and botanical factors
Soil contaminants
Microbiome
Tree biotic and abiotic stressors (*e.g.*, infections, other cultivated or native plants)
Urbanization and urban infrastructure topology
Tree urban planning (type and topology of trees)
Cultivar (plant variety that has been produced incultivation)
Land use

Most studies assessing the impact of pollen on respiratory health have used pollen count (number of airborne pollen grains) as a proxy for the concentration of airborne allergen. However, this may not reflect the true potential of allergens to exacerbate allergic respiratory symptoms, as subpollen particles (SPPs) carrying the allergens might come into play because of its size, small enough to reach the lower airways. The relationship between pollen count and pollen allergen levels (pollen potency, *i.e.*, amount of allergen per pollen) has been shown to be nonlinear, as the amount of allergen released from grains may vary significantly according to factors such as geographic location, time of the year, plant growth, weather conditions (25-27). Altogether, these observations explain why allergy symptoms are experienced even on days with low pollen counts and suggest that pollen count may not be a reliable proxy of allergen exposure (27, 28). Notably, Fuerte *et al*. (2024) provided the first evidence that levels of airborne Phl p 5, an important grass pollen allergen, are more consistently associated with the occurrence of allergic and respiratory symptoms than pollen counts, after accounting for meteorological and environmental factors (27).
The main factors affecting the concentration of pollen and allergens are reported in **table I**.

Atmospheric factors

Temperature has been shown to be linked to an increase in sensitization frequency and allergic diseases. The emission of anthropogenic carbon dioxide (CO_2) into the atmosphere and global warming can fertilize vegetation, enhancing the photosynthetic capacity and the growth of the plants, and are associated to an extended duration (mainly due to an earlier start) of pollen season and higher peak of pollen concentration (29, 30).
The effects of precipitation and humidity levels on pollen emission are complex (31, 32) and may depend on the specific pollen type (33). Heavy short-term precipitation significantly reduces atmospheric pollen concentrations, but, on the other hand, high humidity may induce hydration of pollen grains, sometimes followed by osmotic rupture, with generation of fragments of sub-micron diameter (0.5-2.5 μm) carrying allergens that can be dispersed by the wind into the atmosphere (31). However, the role of precipitation and humidity is rather complex to analyze, because there is not a standard definition of precipitation used across the studies and different scales of measuring precipitation are used (32). Under current climate change scenarios, heavy rainfall episodes, such as thunderstorms, cyclones and hurricanes, are expected to increase in intensity and frequency. Although mechanisms remain to be fully clarified, there is evidence in favor of a causal relationship between thunderstorms and epidemics of asthma attacks, including fatal and near-fatal (34). The most prominent hypotheses for "thunderstorm asthma" is that these events may concentrate aeroallergens at ground level to release respirable allergenic particles or other paucimicronic components after rupture of pollen grains by a combination of osmotic, mechanical, and electrical shock related to humidity, rainfall, wind gusts, and lightning strikes (34-37).
Wind speed and direction also play an important role in the process of lifting and transport of airborne pollen and allergens and in determining their load in the atmosphere (38, 39). The allergenic capacity of long-distance transport of pollen remains unclear. Pollen allergenicity could decrease or be lost altogether during flight in the higher layers of the atmosphere, where the action of factors such as air temperature, humidity and solar radiation on the pollen grains could impact on their ability to maintain allergenic potency (40). Air pollution may also aggravate the allergenicity of pollen (41-44) via different mechanisms: increase of pollen potency, damage of pollen surface with release of more allergens (45), change of its elemental composition, resulting in the release of more airborne SPPs. For instance, gaseous pollutants (nitrogen dioxide and ozone) have been shown to damage the pollen cell membranes in SPPs from plane tree pollen, leading to an increase in *Pla 3* allergen released into the atmosphere (46). When investigating these interactions between pollution and pollen, several variables should be considered, such as weather, urbanization, pollen species, type of pollutant, conditions of exposure, and individual susceptibility.

Environmental and botanical factors

Multiple atmospheric factors joint with environmental and botanical factors influence the concentration of allergens in pollen.

Increasing evidence indicates that the microbial composition of pollen (pollen microbiome) may affect its allergenicity (47, 48), as suggested by the observation that significantly higher amounts of major endotoxins synthesized by bacteria occur in high allergenic pollen in contrast to low allergenic pollen (48).
Pollen release and allergenicity may be also affected by soil pollutants and contaminants, such as cadmium (49) and indirectly by factors that influence plant growth and development, such as biotic stressors (living organisms like virus, bacteria, fungi and insects) and abiotic stressors (pollution, heat, cold, drought, salinity, high UV light, wounding, hypoxia) (50, 51).
Other environmental factors to be considered are land use (agriculture, pasture, plant varieties produced in cultivation by selective breeding), urbanization and urban infrastructure topology. Urban areas, where vegetation coverage is limited, may become "islands" of higher temperatures relative to outlying suburban or rural area ("urban heat island effect"), with possible impact on plant growth and pollen emission (52, 53). This may have implications in epidemiological studies, as large temperature differences between the pollen monitoring station and the study area could result in differences in pollen count and allergen content.
In summary, pollen exposure and allergenicity are influenced by multiple specific and nonspecific environmental stressors (pollen exposome) and their consequences at organ and cell level are considered to play a role in the development, progression and exacerbation of pollen-induced asthma (28).
An important question concerns pollen threshold used in warning systems, that are intended to inform people of the risk of developing allergy symptoms. There is no consensus about which pollen concentrations provoke allergy symptoms (54). First of all, pollen traps are usually installed on roofs at a height of 15–20 m, but the pollen concentrations may differ from ground level, where exposure mainly occurs (55), and where it is highly variable both locally and spatially (56). Secondly, the clinical threshold of pollen is very variable as well. In fact, the relation between pollen/allergen exposure and symptom development is complex, and the dose threshold above which symptoms are experienced is influenced by factors such as individual sensitivity, sensitization, allergen content of pollen, age, geographical areas (54, 57).

Pollen and the airways: a matter of size

Experimental models aimed at predicting the relationship between aerosol particle size and lung penetration show that large particles, with aerodynamic diameters > 6 mm, mainly deposit at the oropharyngeal, whereas smaller particles penetrate the bronchiolar tree (58, 59).

Factors influencing pollen deposition in the airways

The deposition of pollens in the airways can be significantly affected by multiple factors (28, 31, 32). Besides the factors affecting pollen and allergen concentration reported in **table I**, pollen-specific characteristics such as size and morphology may also play a role.
Intact pollen grains are typically between 22 mm (birch) and 100 mm (corn) in size, thus too large to reach the lower airways where asthmatic reaction occur. For instance, grass pollen is present in the atmosphere both as whole grains (approx. 20 to 55 μm in diameter) and as smaller size fractions (< 2.5 μm) (60); ragweed pollen has a geometric diameter ranging between 16 and 27 μm (61), Parietaria pollen between 16-18 μm (62, 63). The question how the pollen grains may affect the respiratory system (the "size paradox") and the processes by which pollen allergens become airborne particles of respirable size have been investigated. As previously reported, during heavy precipitation or periods of high humidity pollen grains are hydrated and may undergo osmotic rupturing into SPPs that can penetrate deeper into the lung (28, 31). These data are supported by recent studies based on the measurement of chemical and biological markers demonstrating a significant increase in the SPPs with diameters 0.25-2.5 μm during thunderstorms and rain events in the pollen season, with peak concentrations occurring during convective thunderstorms with strong downdrafts, high rates of rainfall, electrical ions, and lightning (64, 65). Importantly, SPPs derived from pollen after osmotic shock have been shown to retain allergenicity (37). The main allergens of *Parietaria Judaica* (Par j 2), olive tree (Ole e 1) and grass pollen (Phl p 2 and Phl p 5) are detectable in SPPs and all of them are consistently associated with the epidemic of thunderstorm asthma (37).
The impact of pollen morphology on its deposition in the airways has also been investigated. High-resolution imaging techniques have revealed pollen grain is commonly found in round, ellipsoidal, triangular, disc or bean-shape, with a smooth to spiky texture. Wind-pollinated plants produce lots of lightweight, smooth pollen, whereas the pollen of insect-pollinated plants is heavy and sticky. Experimental studies by Hassan (2011) have investigated the effect of size and surface morphology of pollen-shape carriers on drug delivery performance. The results might be extrapolated to the actual pollen morphology and showed that, at low flow rates, sparse surface asperity was associated to a significant improvement in the delivery of the drug fine particle fraction (the dispersed drug powder with diameter ≤ 5 μm) as compared to pollen-shape carriers with dense surface asperity (66).
In the study by Inthavong *et al.* (2021), pollen particles exhibited higher drag coefficients (*i.e.*, resistance in a fluid environment, such as air or water) and lower particle density compared to aerodynamic equivalent spheres, suggesting that pollen has greater mobility in its aerodynamic flight and greater potential to penetrate the nasal cavity (67).

Site of inhaled pollen airway deposition

As the SPPs are several times smaller than intact pollen grains, they can evade filtration by the nasopharynx and penetrate deeper into the airways, provoking respiratory symptoms.
The association between grass pollen exposure and early markers of asthma exacerbations, such as lung function changes and

increase in airway inflammation, is limited, yet results from available studies suggest the evidence of a correlation (68). In a community-based cohort of 936 adult participants, increasing grass pollen concentrations were significantly associated to changes in FEF25%-75% and FEV_1/FVC ratio, measured 2-3 days after exposure, but not in FEV1, suggesting that the greatest impact might be on medium-sized to small airways (69). Modifications in lung function parameters (FEV1 and FVC) following pollen exposure have been reported also in children and in pollen sensitized adolescents (68).

The study by Nassikas *et al*. (2024) on a large cohort of 490 adolescents exposed to high concentrations of pollen reported a significant increase in airway inflammation (assessed by the measurement of FeNO levels), even in the absence of allergic sensitization and asthma (70).

The results from study on 85 asthmatic patients suggest that there are differences between house dust mite (HDM) mono-sensitized subjects and weed pollen mono-sensitized subjects, not only in airway wall thickness, but also the indices of small airway obstruction, reflecting airway remodeling (71). The results need to be confirmed on a larger population of patients.

Altogether, increasing evidence suggests that a large proportion of allergens is associated with particles of respirable size, either fragments of pollen, soluble allergen adsorbed to air pollutants of various origin or part of the dehiscing anther releases at the time of pollen shedding. These particles are small enough to deposit in the peripheral airways and induce inflammation and respiratory symptoms in predisposed subjects. Limited evidence shows effects on lung function parameters, reflecting a deposition on medium to small airways.

Mechanisms of innate and adaptive immune response to aeroallergen

The concept of the pollen matrix in allergic sensitization

Allergic asthma may involve various types of hypersensitivity reactions to allergens (antibody-mediated, immune cell-mediated, tissue-driven or linked to metabolic mechanisms), resulting in the development of symptoms (72). Classically, the mechanisms of allergies are associated with the type 1, IgE-dependent immune response, characterized by involvement of T helper 2 (T2) cells and production of cytokines including IL-4, IL-5, and IL-13. However, recent evidence shows endotypes of allergic diseases related to T1 or T3-driven activation pathways (72).

Up to now, 987 different allergens have been officially described, of which 195 are registered as plant-derived airborne allergens (https://www.allergen.org).

The key question is why only some environmental proteins cause allergic sensitization and others do not. The molecular bases of allergenicity, *i.e.*, the capacity of certain molecules to induce type 2 inflammation and specific IgE antibodies, are not fully understood. Results from epidemiological and experimental studies support the notion that allergic sensitization is not only dependent on the genetics of the host and environmental factors, but also on intrinsic features of the allergenic source itself, specifically the composition of the pollen matrix (73-80).

The intrinsic and extrinsic compartment of the pollen matrix

Pollen allergens are embedded in a complex and heterogeneous matrix composed of a various bioactive molecule that are co-delivered during the allergic sensitization. The pollen matrix can be divided into two compartments, an intrinsic part consisting of compounds inherent to the pollen (proteins, metabolites, lipids, carbohydrates) and an extrinsic fraction, that includes viruses, aerosols and particles from air pollutants and a pollen-linked microbiome (73, 81-87). Together these components of the matrix provide a specific context for the allergen and are determinant of T2 sensitization (**figure 1**).

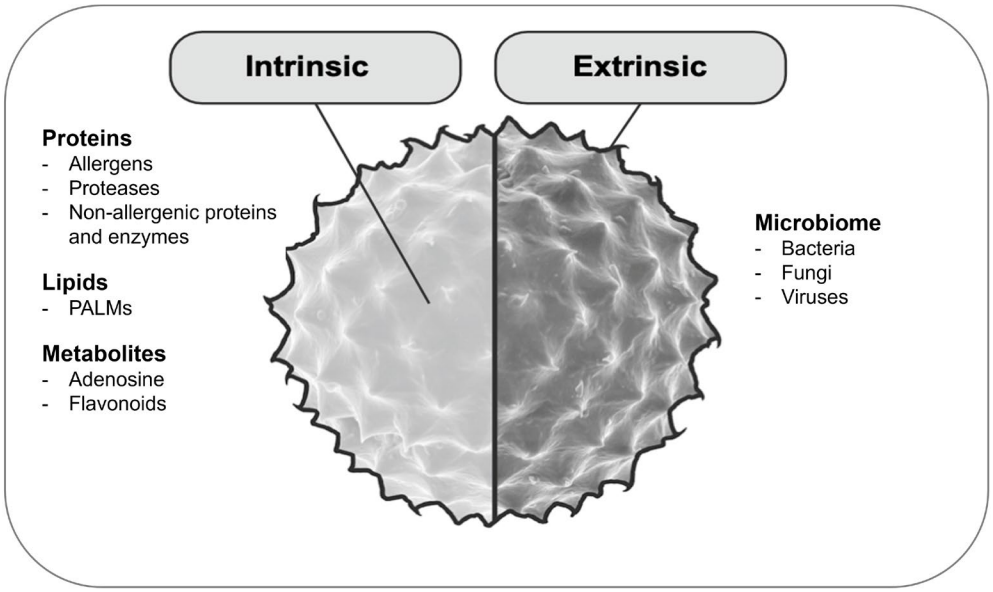
Specifically, the initiation of allergic sensitization to pollen is likely to occur via distinct molecular mechanisms, involving pollen species-specific immune adjuvants that may contribute to the generation of a pro-inflammatory microenvironment to favor T2 polarization. Indeed, experimental studies have shown that several purified allergens were lacking inherent sensitizing potential, supporting the role of pollen-derived components as key players in the initiation of the inflammatory allergic response in predisposed subjects (73, 74, 82, 88).

Pollen grains are rich in lipids displaying immunomodulatory effects (74). For instance, in sensitized individuals, but not in healthy controls, cypress pollen-derived phospholipids were shown to be presented to T cells by major histocompatibility complex (MHC)-related molecules on dendritic cells, an interaction causing T cell proliferation and secretion of IL-4 (playing a key role in the initiation of sensitization) and IFNy (73, 83, 84, 89). Further evidence comes from human studies with olive pollen and *in vitro* murine models with birch pollen, showing that pollen lipids activate invariant natural killer T cells by upregulating CD1d expression on dendritic cells (90, 91).

Regarding the extrinsic compartment of the matrix, the pollen microbiota, whose composition is variable and specific for each pollen species (47, 92), seems to play a role in allergenic inflammation. In fact, besides intrinsic pollen-derived lipids, microbial lipids constitute a source of immunomodulators and act as strong adjuvant of the sensitization process (83, 87).

The influence of plant viral infection on the sensitizing potential of pollen is still largely unknown. A pilot study on a small sample (n = 15) of subjects with a history of seasonal allergic rhino-conjunctivitis enrolled outside the pollen season observed that virus-induced modifications in components of grass pollen have the potential to alter its allergenic potency, as assessed by skin testing (85). The results suggest that virus infection of grasses deserves consideration as a factor in pollen-induced allergic disease.

Figure 1 - *The composition of the pollen matrix influencing the sensitizing potential of allergenic pollen source (adapted from ref. 73).*



Additionally, air pollutants, such as diesel exhaust particles, ozone, carbon dioxide and nitrogen oxides, may influence the composition of the pollen matrix as well as of the pollen microbiota, displaying an assistive role in the development of the allergic inflammation (41-43, 93, 94). In this regard, a correlation between exposure to atmospheric pollutants and the content of allergens and immunostimulatory compounds in pollen was reported (95, 96).

The role of epithelium in the initiation of the sensitization process

Increasing evidence suggests that an epithelial dysfunction, coupled with inherent properties of environmental allergens, can be responsible for the inflammatory response (97, 98).

Epithelial cells are endowed with a series of specialized pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) and protease activated receptors (PARs), which are required to provide first defense mechanisms towards pathogens. In atopic individuals, upon encounter with the epithelium, the pollen releases allergens and various matrix bioactive molecules that cause the disruption of the epithelial tight junctions, enabling the transportation of allergens across the membrane (81, 99-101), the activation of PRRs, the release of epithelial cytokines, like thymic stromal lymphopoietin (TSLP), IL-25, IL-33, and various pro-inflammatory cytokines (IL-8, IL-1, IL-6, TNFα). In turn, all these molecules activate the dendritic cell network and other innate immune cells, such as basophils and type 2 innate lymphoid cells, that drive pollen-induced T2 inflammation (88, 102-105). In this context, evidence is emerging on the epithelial cytokine TSLP as a critical player in the development and progression of allergy and asthma (106). TSLP is positioned at the early phase of the inflammatory cascade, therefore, its inhibition could simultaneously suppress multiple pathways of inflammation. In allergic asthma, TSLP promotes the differentiation of T2 lymphocytes secreting T2 cytokines targeting B cells, eosinophils, mast cells and airway smooth muscle cells (106). The pollen-induced secretion of TSLP and the associated type 2 inflammation were shown to be dependent on TLR4 and myeloid differentiation primary response 88 (MyD88), and probably linked to oxidative stress (107-109). In this respect, stimulation of epithelial cells with pollen extracts from short ragweed, birch, timothy grass and mountain cedar caused elevation in the levels of reactive oxygen species (ROS) (110-113). In addition to TSPL, a TLR4/MyD88-dependency was also observed for pollen-induced IL-33-mediated T2 responses for IL-25, which has the potential to initiate and activate type 2 innate lymphoid cells and T2 cells (73).

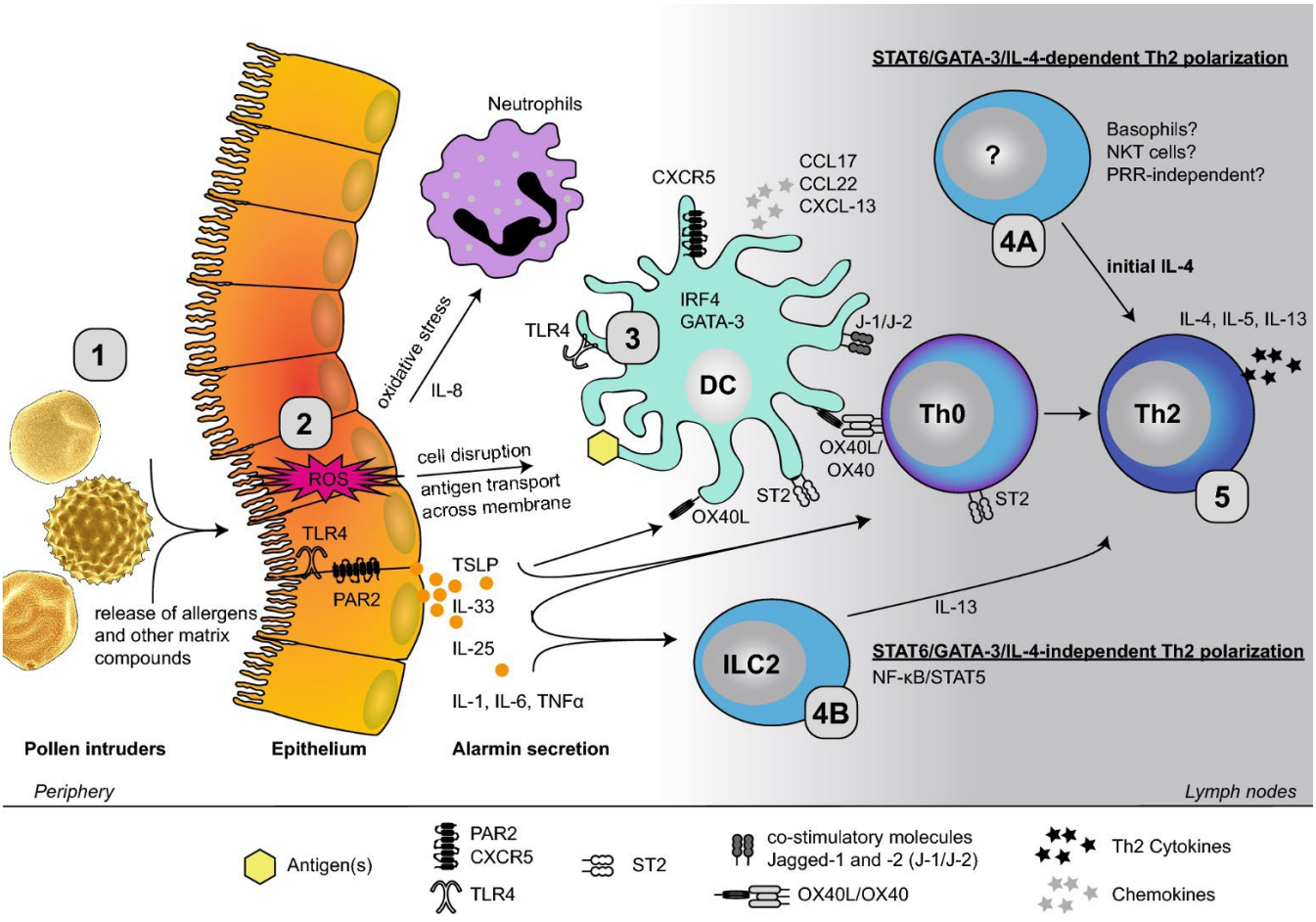
Once activated by pro-inflammatory cytokines, dendritic cells instruct T2 polarization through three types of signals to naïve T cells: 1) antigen-derived peptides presented via MHC-II, 2) expression of co-stimulatory molecules and 3) secretion of pro-inflammatory cytokines and chemokines (114). In addition, activated dendritic cells secrete chemokines (CCL17, CCL22 and CXCL13) and chemokine receptors enable them to migrate to the lymph nodes, where they prime naïve T cells to become antigen-specific T2 cells (115-119).

For efficient T2 priming IL-4 seems to be important. Basophils, mast cells and NKT cells were shown to produce IL-4 (120) and

once generated, T2 cells themselves represent the most important source of IL-4.
An overview of the initiation process of allergic sensitization is shown in **figure 2**.

In summary, the mechanisms involved in pollen-induced activation of the innate immune system and T2 polarization are complex and not fully understood. It seems that different allergenic pollen sources interact with distinct innate receptors and signal-

Figure 2 *- Pollen-induced activation of the innate immune system and T2 polarization (reproduced from ref. 73). Upon encounter with the epithelium the pollen hydrates and releases its content including allergens and various other bioactive molecules* ***(1)****. At the epithelium* ***(2)****, this immunogenic cocktail causes the disruption of the epithelial membrane, activates PRRs such as TLR4 and PAR2, triggers the release of alarmins (TSLP, IL-25 and IL-33), and induces oxidative stress and secretion of IL-8 and other pro-inflammatory cytokines (IL-1, IL-6 and TNFα). In turn, DCs are activated (upregulation of surface markers including OX40L and notch ligands), migrate to the lymph nodes (expression of CXCR5), where they present processed antigens via MHC-II to naïve T cells* ***(3)****. Th2 polarization occurs either STAT6/GATA-3/IL-4-dependent* ***(4A)*** *or -independent via the NF-κB/STAT5 pathway and the contribution of ILC2s* ***(4B)****. The origin of initial IL-4 for Th2 polarization is still a matter of discussion; proposed candidate are basophils and NKT cells. Once a Th2 immune response has been initiated, a class-switch of B cells to antigen-specific IgE-producing plasma cells occurs resulting in the sensitization of susceptible individuals to pollen allergens* ***(5)****.*



CCL17; CCL22 chemokine (C-C motif): ligand 17 and 22; CCR7: C-C chemokine receptor type 7; CD80; CD86 and CD40: cluster of differentiation 80; 86 and 40; CXCL-13: C-X-C motif chemokine 13; CXCR5: C-X-C chemokine receptor type 5; DCs: dendritic cells; GATA-3: GATA binding protein 3; IL-: Interleukin; ILC2: type 2 innate lymphoid cells; IRF4: interferon regulatory factor 4; NF-κB: nuclear factor "kappa-light-chain-enhancer" of activated B cells; NKT: natural killer T; OX40L: OX40 ligand; PARs: protease activated receptors; PRRs: pattern recognition receptors; ROS: reactive oxygen species; ST2: IL-33 receptor; STAT5, STAT6: signal transducer and activator of transcription 5 and 6; Th: T helper cells; TLR4: toll-like receptor 4; TNFα: Tumor necrosis factor alpha; TSLP: thymic stromal lymphopoietin.

ing pathways, that are also influenced by genetic polymorphisms affecting epithelial pattern recognition, barrier function, and cytokine production. Altogether, the data suggest that allergic sensitization to pollen most likely results from particular combinations of pollen-specific signals rather than from a common determinant of allergenicity.

Pollen-induced airway inflammation: specific features on allergic asthma

Experimental evidence suggests that allergen-specific T2 cells and their cytokines orchestrate allergic airway inflammation, induce mucus production from airway epithelium and promote airway hyper-responsiveness (121-123).

Along this line, studies on a human model of allergen-induced asthma exacerbation have been conducted aimed at exploring differences between allergic asthmatics and allergic non-asthmatic controls in the airway response to allergen, that could provide fundamental insights into asthma pathogenesis and possibly identify novel therapeutic targets (124, 125).

Cho *et al*. (2016) showed that both groups developed prominent allergic airway type 2 inflammation in response to allergen. However, allergic asthmatic subjects compared to allergic non asthmatic controls had markedly higher levels of innate type 2 receptors on allergen-specific CD4+ T cells recruited into the airways and increased levels of type 2 cytokines, total mucin as well as airway baseline smooth muscle mass (124).

Further research by Alladina *et al*. (2023) showed that transcriptional profile of airway epithelial cells upon allergen challenge with allergens was markedly altered in allergic asthmatics subjects as compared to allergic non-asthmatic controls (125). Specifically, in asthmatic subjects a subset of epithelial cells – goblet and suprabasal quiescent goblet cells as well as basal cells – displayed the greatest response to allergen, with upregulation of genes involved in type 2 inflammatory cell recruitment and signaling, mucus metaplasia, and genes that promote extracellular matrix degradation and connective tissue regeneration. In contrast, in allergic non-asthmatic subjects the basal and suprabasal cells were able to promote an injury-repair response to allergen challenge, with increased expression of alarmins (IL33 and HMGB1) and neutrophil chemoattractants. Collectively, these results identify airway basal and secretory cells as highly dynamic cells during allergic inflammation and reveal mechanisms by which they may drive asthma pathogenesis.

IL9-expressing pathogenic T2 cells, that amplify type 2 inflammation and promote the expression of profibrotic mediators and pathologic airway remodeling, have also been shown to be highly specific to asthmatic airways and were only observed after allergen challenge (125).

Additionally, airways of allergic asthmatics, after allergen challenge, were uniquely enriched for conventional type 2 dendritic cells (that express *CD1C*) and *CCR2*-expressing monocyte-derived cells, with up-regulation of genes that sustain type 2 inflammation and promote airway remodeling. In contrast, airways of allergic non-asthmatic subjects were enriched for macrophage-like monocyte cells (MCs), characterized by production of factors modulating endocytic clearance, cell differentiation and survival, and expression of trophic factors promoting angiogenesis and tissue repair, as shown in animal models (126, 127). This finding suggests that these populations play an important role in the resolution of inflammation and protection against airway remodeling, as opposed to IL-4/IL-13 signaling via STAT6 in the airways of asthmatics, that may prevent or arrest macrophage differentiation and direct a pathogenic monocyte cell phenotype characterized by up-regulation of genes involved in inflammatory signaling, antigen presentation, and pathologic airway remodeling. Cellular crosstalk between airway epithelial and immune cells is also critical to the initiation and resolution phases of allergic inflammation (128-130). Cellular communication pathways in allergic controls were characterized by growth factor signaling and injury-repair response to allergen, whereas asthmatics were dominated by basal cell-Th2-mononuclear phagocyte interactions that may sustain and amplify type 2 signals, leading to failure to engage antioxidant response, loss of growth factor signaling, increase in mediators of airway remodeling.

In summary, allergen challenge leads to increased eosinophilia and type 2 cytokine levels in the airways of both allergic asthmatic and allergic non-asthmatic subjects, but the effector pathways elicited by T2 inflammation are distinct. The airway epithelium of asthmatic subjects is highly dynamic, with basal and secretory epithelial cells up-regulating the genes involved in matrix degradation, mucus metaplasia, and remodeling, while failing to induce the epithelial injury-repair and antioxidant processes observed in non-asthmatic controls, that are possibly protective against pathologic remodeling.

How pollen interacts with the respiratory mucosa remains largely unknown due to a lack of representative model systems. In this respect, Van Cleemput *et al*. (2019) demonstrated that pollen proteases of three plants, Kentucky bluegrass, white birch and hazel, selectively destroy the integrity and anchorage of columnar respiratory epithelial cells, but not of basal cells, in both *ex vivo* respiratory mucosal explants and *in vitro* primary equine respiratory epithelial cells (131). Interestingly, Blume *et al*. (2013) analysed the effect of grass pollen exposure on differentiated human primary bronchial epithelial cells derived from severe asthmatic donors and non-asthmatic controls. The results show a differential response in terms of inflammation mediator release, without any difference in physical barrier properties (132).

Discussion and conclusions

Asthma is a global problem and a significant social and economic burden. Although specific epidemiological data on pollen-induced asthma are scarce, overall allergic asthma, which is the most com-

mon phenotype, is costly for the healthcare systems, with large additional societal costs due to lost work productivity.
Clinical manifestations are intimately linked with the release of plant pollen into the environment. The factors that influence pollen concentration and potency are multiple, region- and species-specific, difficult to identify, quantify and predict in terms of type of effect, as it is increasingly clear that they all have independent and joint effects on respiratory health. The variability of pollen and allergen concentration is often overlooked in clinical studies, even in randomized controlled trials, suggesting that allergic-type asthma is not always properly investigated and introducing a possible bias in studies on allergic populations.
In the future, temperature and precipitation are projected to increase, all factors that will potentially augment pollen emission and allergenicity, with negative impact on respiratory health. Also, urbanization will further increase in the next decades, with negative consequences on the health and survival of urban trees, leading to loss of biodiversity. In this context, tree urban planning and the integration of green infrastructure may mitigate the impact of urban development.
The molecular bases of allergenicity are not fully understood. There is evidence that allergic sensitization dependents not only on the genetics of the individuals and the environmental factors, but also on species-specific immunostimulatory components of the pollen matrix that may contribute to the generation of a pro-inflammatory microenvironment to favor T2 polarization. Future investigation will contribute to elucidate the pathogenic effects of pollen in the airway.
Importantly, in allergic asthmatics, as compared to allergic non-asthmatics, the pathogenic effector circuits sustain and amplify T2 signals in response to allergens, while the circuits facilitating the resolution of inflammation and tissue repair are inhibited: therefore, tissue reprogramming in response to T2 inflammation could drive structural airway disease and may represent a defining feature of allergic asthma.
The observation that many allergic individuals develop asthma over time (133), suggests that the pathogenic mechanisms leading to asthma may be incremental. Thus, a key question is whether a pharmacologic intervention may slow down or at least partially revert the cellular pathways driving airway remodeling.
Inhaled glucocorticoids reduce airway inflammation and some aspects of remodeling, as proliferation of lung fibroblasts, metaplasia of goblet cells and thickening of subepithelial basal membrane (134, 135), but currently there are no drugs or other interventions available that can definitely reverse this process (134).
In vivo animal models of allergen-induced airway inflammation, using sensitized rats exposed to repeated allergen challenge, showed established structural alterations of the airways could not be reversed by the treatment with inhaled corticosteroid administered post challenge, but concomitant treatment could partly prevent these changes (136). In addition, glucocorticoid could inhibit *in vitro* the differentiation of human lung fibroblasts to contractile myofibroblasts, that are involved in the development of the inflammatory cascade. The effect of reversion to the normal phenotype occurs both at the very early and also at a mild stage of the differentiation process (137).
The clinical relevance of these findings is not known, since no animal model of allergic airways disease encompasses all features of the human disease, and results cannot be easily translated to the clinic; however, the data support the hypothesis that early intervention with inhaled glucocorticoids could at least in part prevent or slow down airway remodeling in asthma.
Advances in the understanding of the molecular circuits underlying airway structural changes and remodeling in response to allergens as well as repair mechanisms may facilitate the development of novel and more effective therapeutic approaches.

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Contributions

All authors equally contributed to the manuscript draft and critical revision for important intellectual content, to data collection, analysis and interpretation. All authors read and approved the final version of the manuscript.

Conflict of interests

The authors declare that they have no conflict of interests.

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POSITION PAPER

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Pollen-induced asthma: diagnostic and therapeutic implications

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Summary

Evidence supports the hypothesis of pollen-induced asthma as a specific asthma phenotype, with defined clinical features and tailored pathways for its clinical management.

The probability of diagnosis varies significantly in the pollen season, in which allergic patients are symptomatic, as compared to asymptomatic periods outside the pollen season. In this context, a novel diagnostic scheme for pollen-induced asthma has been developed.

Pollen exposure is the key risk factor for symptoms and exacerbations. Therefore, we proposed a therapeutic algorithm for pollen-induced asthma based on a risk stratification model that considers the medical history of the patients and the measurement of objective markers, allowing a tailored therapeutic approach.

Impact statement

Pollen-induced asthma can be considered a specific asthma phenotype, with defined clinical features and tailored diagnostic and therapeutic pathways for its clinical management.

Introduction

Pollen-induced asthma (PIA) could be considered a specific phenotype. As reported by Cecchi *et al.* (1), pollen allergenicity depends not only on genetic and environmental factors, but also on immunostimulatory components of the pollen matrix, that contribute to airway disease and may represent a defining feature of allergic asthma.

A phenotype is commonly defined as "*the visible characteristics of an organism resulting from the interactions between its genetic*

patrimony and the environment". In this article, we will adopt an operational description, useful from a clinical point of view. Therefore, by asthma phenotype we mean *"the characteristics of the disease, single or in combination, which describe the difference between individuals affected by the same disease, and which are correlated with clinical outcomes: clinical history and symptoms (onset, duration, control of symptoms, exacerbations), impaired respiratory function, disease progression, biomarkers, comorbidities and response to the treatment*". Thus, the identification of specific phenotypes should have a predictive value in terms of clinical outcomes and response to therapy (2-4).

The Global Initiative for Asthma (GINA) document highlights the importance of phenotyping in severe asthma for the purpose of indicating biological drugs, while, although the definition recognizes that asthma is a heterogeneous disease, the identification of the phenotypes of mild-moderate asthma is not considered relevant because the therapeutic approach recommended in these patients is in any case independent of the phenotypes (5).

Evidence supporting PIA as a specific phenotype can be derived using both a down-type investigation methodology (expert clinical judgement) and an unsupervised one (button up, cluster analysis):

- Pollen-induced asthma as a clinical phenotype: respiratory symptoms, exacerbations, impaired respiratory function, and increase in T2 biomarkers are all elements that are quantitatively linked to the seasonal exposure to pollen to which the patient is sensitized, while in the remaining period of year the patient remains asymptomatic (1). The strategy for evaluating asthma control, in particular the risk of exacerbations and clinical worsening, is strongly influenced by exposure to allergens. Similar to the severe asthma phenotypes, for PIA a targeted therapy is available, represented by specific immunotherapy, as well as a mainly seasonal symptomatic and anti-inflammatory pharmacological therapy.
- Pollen-induced asthma phenotype identified with cluster analysis: three large cohort studies using different clustering techniques to describe possible asthma phenotypes (SARP, U-BIOPRED, UK cohort), identified a cluster characterized by mild allergic asthma (cluster 1 in the SARP cohort and cluster 3 in the U-BIOPRED cohort), with characteristics compatible with those above described as PIA (6-8). Despite the difference between the studies, Kaur *et al*. (3) identified 4 phenotypes: 1) early onset mild allergic asthma; 2) early onset moderate-severe allergic asthma; 3) late onset non-allergic eosinophilic asthma; 4) late onset non-allergic non-eosinophilic asthma. The main factors discriminating the heterogeneity of asthma common to the different phenotypes are the age of onset, respiratory function, atopy and eosinophils. Other patient characteristics, such as sex, obesity and smoking, although commonly detected, play a less important role when comparing studies.

Altogether, the identification of PIA as a clinical phenotype has a predictive value in terms of clinical outcomes and response to therapy (4). According to Han *et al.* (4), it is possible to identify a clinical phenotype when subjects are characterized by similar clinical presentations (respiratory symptoms occurring during the period of exposure to pollen), pathogenic mechanisms, diagnostic pathways, biomarkers, and availability of an endotype-specific therapy (disease modifying such as immunotherapy).

Materials and methods

A narrative systematic review of the literature was conducted on Medline to identify English papers published up to March 31, 2024. Hand searching of references of interest was also performed within the selected studies. The search strategy included papers with the terms "asthma" and "pollen/allergic" asthma in title/abstract, associated with at least one keyword, in the title/abstract, for each of the following domains: adherence to medications, risk of exacerbations, diagnosis, and treatment.

The research and selection of the studies were performed independently by five allergists, who collected and summarized the data from the studies. All the authors contributed to the definition of the research questions and related keywords, and to the final selection of the studies to be included in the systematic review. Considering the paucity of data about PIA and the low-quality evidence of the obtained studies, a formal process to assess the certainty in the body of evidence or the strength of the recommendations was not performed. Consensus was sought from a panel of asthma experts from the Asthma Interest Group of AAIITO (Association of Italian Hospital Allergists and Immunologists), with a formal voting process implemented in case of disagreement during the discussion. The final consensus paper was reviewed and approved by all the authors.

Pollen-induced asthma: diagnostic flow chart

Allergic asthma is the most common asthma phenotype, characterized by early onset, immunoglobulin type E (IgE) sensitization to allergens, IgE-related Th2-mediated background (9).

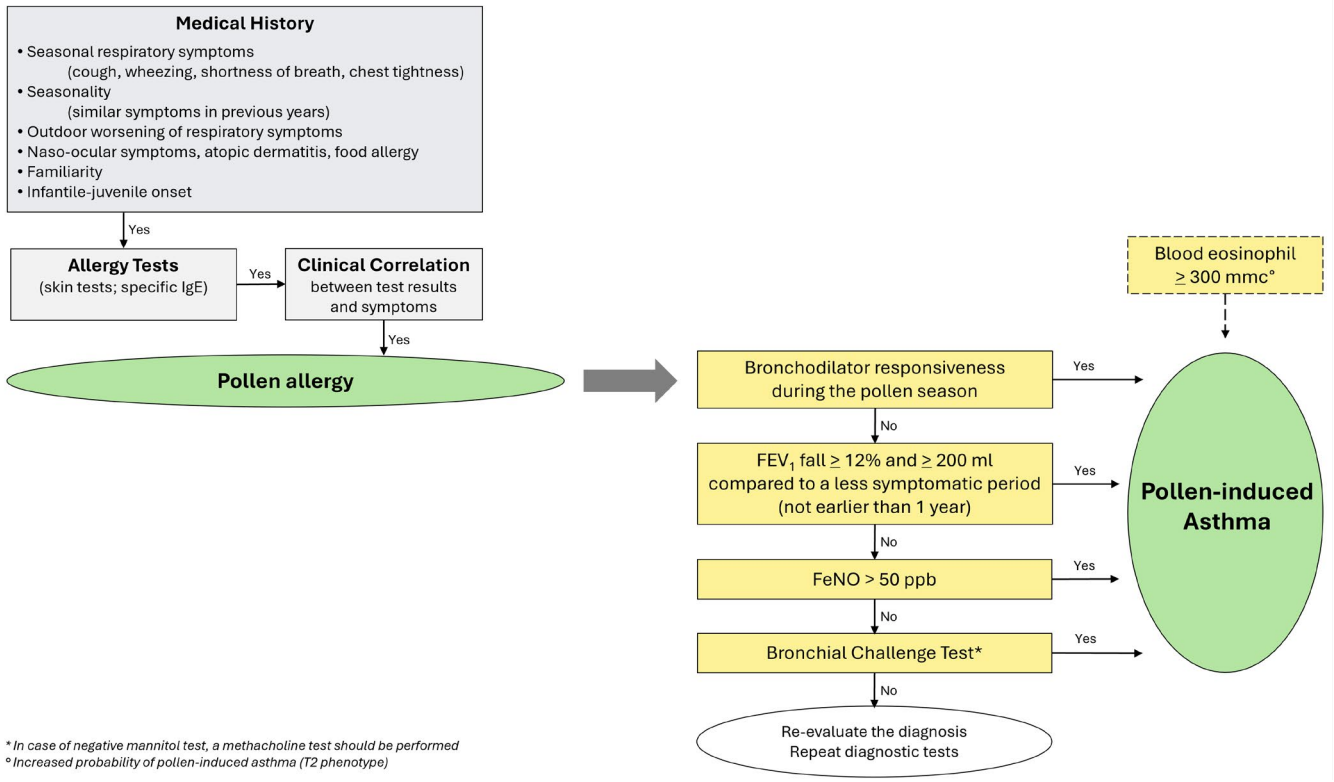
Allergic rhinitis is a common comorbidity of asthma and, in the case of PIA, is observed in the vast majority of patients, over 80% (10).

Usually, the diagnosis of PIA is suspected during the symptomatic period of exposure to the pollen to which patients are sensitized.

The proposed diagnostic path for PIA is summarized in the flow chart (**figure 1**).

The process starts from the medical history, that may suggest the presence of a pollen-induced respiratory disease, followed by allergy tests and assessment of the compatibility between the seasonality of symptoms and the positivity towards the identified allergens. In fact, the presence of a positive skin test or positive sIgE does not necessarily mean that the allergen is causing symptoms and

Figure 1 *- Pollen-induced asthma diagnosis.*



there is still no evidence regarding sIgE thresholds necessary to confirm or exclude clinical disease (5, 11). The clinical relevance of sensitization needs to be confirmed by patient's history (5, 11). A recent diagnostic technique, known as "component resolved diagnostics" (CRD) is used to determine the specific molecules (or components) against which the IgE have been produced, to distinguish between genuine sensitization and clinically irrelevant IgE cross-reactivity due to panallergens or carbohydrate determinants (12-14), and to guide the choice of allergen specific immunotherapy (AIT).

In the case of symptoms suggestive of asthma (cough, wheezing, chest tightness, shortness of breath, nocturnal awakenings for asthma) along with seasonal onset (*i.e.*, temporal association between symptoms and pollen exposure), a pollen-induced variability in expiratory lung function must be also documented to confirm the diagnosis of PIA. The first line recommended test is spirometry showing a decrease of ≥ 12% and ≥ 200 ml compared to a previous test carried out in a less symptomatic period but not earlier than one year (5, 15). This diagnostic process can be carried out in any clinic where a spirometer is available, even a portable one; the only limiting factor is the correct technical execution of the test. A bronchodilation test with SABA during pollen exposure is recommended, as a ≥ 12% and ≥ 200 ml increase in FEV1 confirms the diagnosis of PIA. It was not considered appropriate to establish the finding of obstructive spirometry, with FEV1/FVC < the lower limit of normal (LLN) or < 75% (5, 15-17), as a pre-condition for carrying out the bronchodilation test, as the patients with PIA frequently show non-obstructive spirometry, especially when the prevalent symptom is cough. On the other hand, the fact that in these patients the respiratory parameters are frequently normal reduces the probability of a positive bronchodilation test, thereby limiting the sensitivity of the test, even if the specificity is good.

A negative bronchodilation test does not exclude a diagnosis of PIA: in this case it is suggested to perform a direct (methacholine) or indirect (mannitol) bronchial challenge during the pollen exposure, if the FEV1 change from extra-pollen period to pollen period is inconclusive.

A positive result with mannitol (PD15 < 635 mg) is indicative of a high degree of bronchial inflammation, but this test is less sensitive, although more specific, than the test with methacholine using a cut-off value of PC20 < 8 mg/ ml (18-20). It will be the

doctor's choice to carry out the test with mannitol first, being more informative regarding the activity of the inflammatory processes and easier in the execution. In the event of a negative result with the mannitol test, a test with methacholine should be performed (18). If even in this case the result is negative, the diagnosis of asthma can be excluded or, if the suspicion of asthma remains, the test can be repeated in a more symptomatic period (18). It is important to underline that in PIA, airway hyperresponsiveness (AHR) increases and can have clinically diagnostic value only during the pollen exposure (21, 22).

GINA report suggests lung function testing with the handled device peak expiratory flow (PEF) meter, when spirometry is not available, to assess excessive variability in expiratory lung function (5). Although PEF is less reliable than spirometry parameters, it is better than relying on symptoms alone.

The assessment of T2 inflammation should always be included in the diagnostic work-up for PIA, using appropriate biomarkers. Therefore, FeNO testing should be also performed, being a surrogate measure of eosinophilic lung inflammation, which could persist even in the absence of overt respiratory symptoms (23, 24). This test is recommended if spirometry is not available: the guidelines from the British Thoracic Society, the National Institute for Health and Care Excellence, the Scottish Intercollegiate Guidelines Network (BTS/NICE/SIGN), and from the European Respiratory Society (ERS) suggest FeNO measurement as a part of the diagnostic work-up in adult patients with suspected asthma, in whom the diagnosis is not established based by initial spirometry combined with bronchodilator responsiveness testing (15, 16). Values > 50 ppb are considered diagnostic for asthma (16, 25). This cut off is higher than the one previously recommended in the previous edition of NICE guidelines (40 ppb) and is considered more useful because it is characterized by greater specificity, although less sensitivity (17); this is particularly important if considering that atopic patients may show an increase in FeNO during the pollen season, especially in polysensitized individuals where a dramatic increase was observed (26).

Importantly, FeNO testing is part of the diagnostic work-up in the GARD (Global Alliance Against Chronic Respiratory Diseases) recommendations for the management of severe asthma (27) and is included in the essential levels of assistance (LEA) in Italy, *i.e.*, the services and benefits that the National Health Service (SSN) is required to provide to all citizens.

The higher the FeNO value measured, the greater the probability of asthma (17). However, a negative test does not exclude asthma, especially if the patient has taken oral glucocorticoids or used ICS regularly or as needed (28). On the other hand, high FeNO levels may also be observed in non-asthmatic respiratory conditions, as eosinophilic bronchitis and allergic rhinitis (5, 29).

In the proposed diagnostic work-up, FeNO measurement is suggested before bronchial challenge, as its execution is simpler, although its use is not widespread due to lack of the adequate equipment. The eosinophil count was not included as a diagnostic test, even if data are available in this regard, because of the variability of cut-off values between studies (3.4% and 360, 150, 500, 300 eosinophils/mmc) (25, 30-32); nevertheless, it is an important factor that may enhance the pre-test probability of confirming a diagnosis of PIA. The bronchial allergen challenge is not mentioned in the algorithm as, due to both safety and cost-efficiency concerns, its use is currently restricted to specialized centers with experienced staff, with protocols tailored to mild asthmatics for research purposes.

In conclusion, the probability of diagnosis of PIA phenotype can vary significantly in the pollination period, in which sensitized patients are symptomatic, as compared to asymptomatic periods outside the pollen season. Therefore, negative diagnostic tests should be contextualized with the presence of symptoms and the pollen calendar, to reduce the possibility of false negative diagnoses.

Risk stratification and control assessment in the pollen-induced asthma phenotype

Asthma control includes two domains: symptoms (impairment) and future risk (5, 33, 34). The assessment can be carried out with validated questionnaires, such as the ACT, which investigates a previous period of 4 weeks. In the PIA phenotype, the results on symptoms (impairment) can be highly discordant if carried out in a period of exposure to pollen compared to a period outside and far from the pollen season. Similarly, the interpretation of the "future risk" reflects the same peculiarity because, unlike other forms of asthma, in this phenotype the major trigger factor for exacerbations, *i.e.*, pollen exposure, is clearly identifiable and directly correlated, in a quantitative measure, to the risk of exacerbations (**figure 2**). Therefore, the information obtained from assessment tools should be contextualized to the period of the year investigated and the pollen calendar.

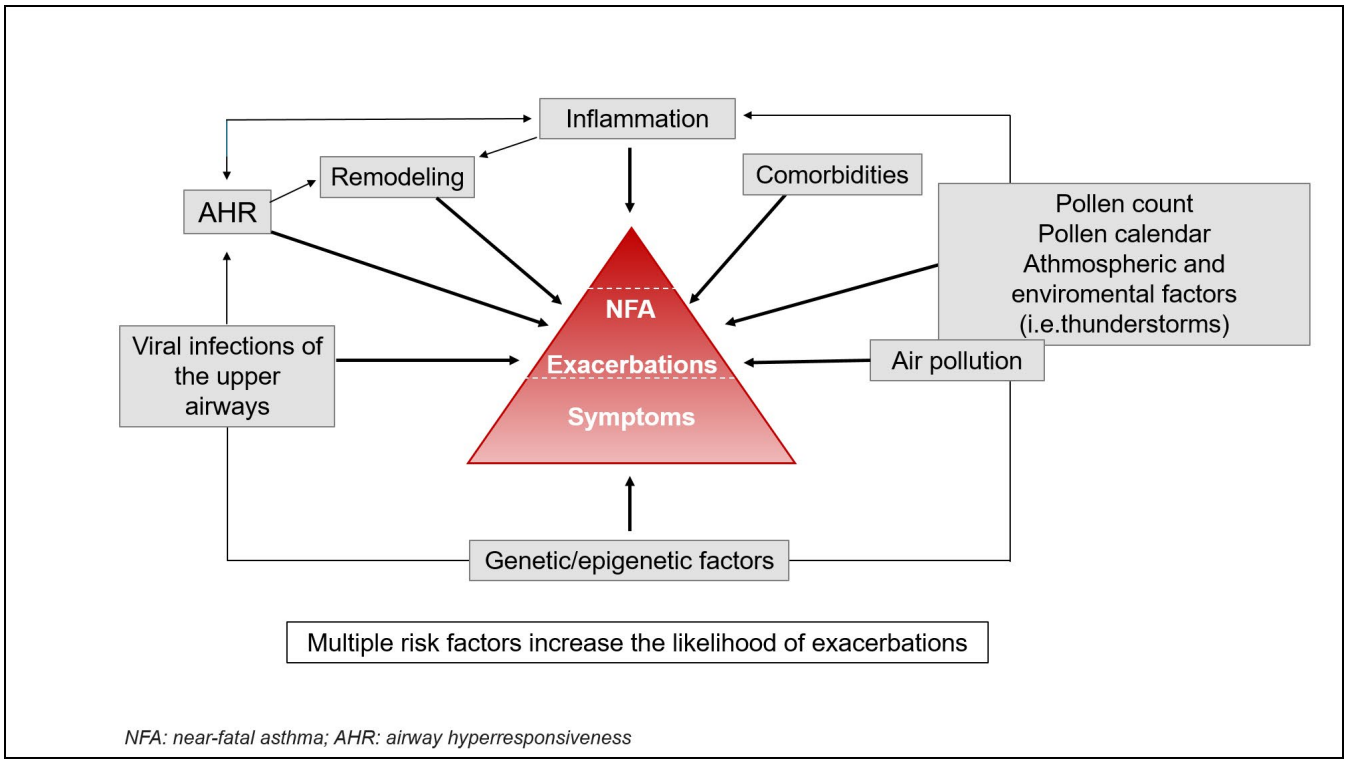
The predictability of the main future risk plays a central role in the clinical management of PIA. Even patients with mild asthma may experience episodes of severe exacerbations (5). Indeed, a significant proportion of subjects who have experienced episodes of "near-fatal asthma" or death from asthma were atopic and were classified as mild asthmatics, frequently not taking any controller ICS-based therapy (5, 35), suggesting that the impairment domain and the future risk domain are not closely related (36-38). These observations suggest that in PIA the risk stratification should be carried out in the pre-seasonal period, to identify the most suitable pharmacological strategy.

Figure 2 summarizes the factors associated with an increased risk of exacerbations in patients with PIA.

An accurate medical history can be sufficient to identify subjects who are more likely to develop symptoms and are at risk of exacerbations during periods of maximum exposure to pollen.

Notably, symptoms that are proxies of exacerbations and are possible markers of AHR, that affects the extent of the broncho-

Figure 2 *- Risk factors for exacerbations in pollen-induced asthma.*



spasm response to inhaled allergens, should be carefully identified (22, 39). They include wheezing, chest tightness, shortness of breath, and nocturnal awakenings.

From a clinical perspective, the main risk factor is a history of exacerbations in the previous year, in particular during the pollen season. Both severe exacerbations, easier to detect and remember because they are characterized by the use of oral steroid therapy, and moderate exacerbations, mostly characterized by an increased frequency in the use of reliever drugs (34), should be assessed. Exacerbations are the result of the concomitance of multiple risk factors: exposure to pollen acts both as a predisposing factor, increasing T2 inflammation and AHR, and as a trigger for symptoms (40).

The onset of symptoms and, to a greater extent, an exacerbation, varies from subject to subject and in the same subject over time due to the co-presence or absence of different predisposing factors (genetic and epigenetic) and triggers, mostly pollen-related factors in PIA, in addition to the others (**figure 2**). This multifactorial contribution explains the high possible variability of seasonal symptoms (41-43).

In addition to previous exacerbations, for risk stratification it is useful to investigate the symptoms that occurred during the previous pollen season and their frequency. The most specific symptom is wheezing, an indicator of the presence of a significant obstruction (44, 45), although there is no clear correlation between obstruction and the onset of wheezing. Therefore, wheezing is a cardinal symptom to be assessed both in the previous pollen season and in the months preceding the control examination, reflecting a significant degree of bronchoconstriction.

The presence of wheezing, coughing and chest tightness are associated with AHR especially if they appear occasionally after episodes of hyperventilation, as during running in children and young adults, or when the patient sings or speaks loudly for a long time (46-48).

A further element to assess is the persistence of respiratory symptoms (as cough, chest tightness) after viral infection of the upper airways, which the patient often does not pay attention to, believing it to be a normal evolution of the infection (41).

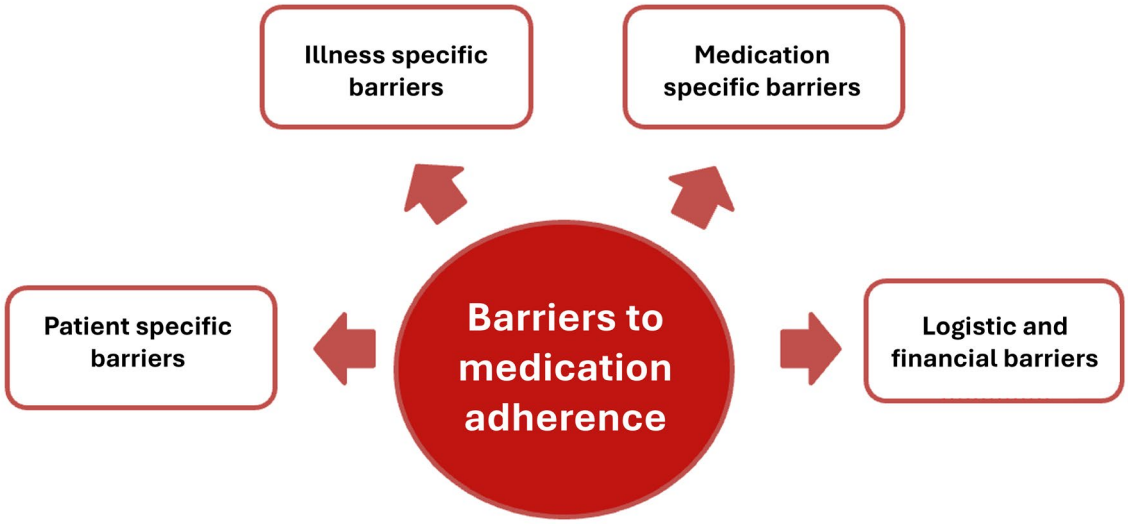
Correct perception of the obstruction by the patient is an important factor in evaluating the reliability of the reported symptoms. In clinical practice, hypoperception can be identified in the presence of a discrepancy between the level of obstruction verified by spirometry and the symptoms reported, or more generally by an overestimation of the patient's control of symptoms com-

pared to the evaluation of control obtained through questionnaires such as ACT, all factors that may increase the risk of exacerbations (49, 50).
The presence of comorbidities, in particular allergic rhinitis, gastro-esophageal reflux and obesity, also influence the risk of exacerbations (41).
Risk stratification can be further improved by using biomarkers related to bronchial inflammation: the greater the degree of inflammation in the pre-seasonal period, the greater the probability that the further release of T2 cytokines induced by allergic reactions can trigger seasonal symptoms.
High levels of FeNO reflect the presence of T2 inflammation and are indicators of positive response to ICS therapy. In previous versions of ERS/ATS guidelines, FeNO levels are considered low below 25 ppb, intermediate between 25-50 ppb and high > 50 ppb (28). Therefore, in patients with PIA, the finding of levels above 25 ppb in a period of non-exposure to pollen may be considered an indicator of future risk, and values above 40-50 ppb high risk; asymptomatic sensitized subjects in the period of non-exposure to pollen generally do not have significantly increased FeNO levels (26).
Different FeNO thresholds have been used, in mild allergic asthmatic subjects with FeNO values lower than the cut-off value and with positive clinical outcomes, to predict the possibility of reducing/suspending ICS (51, 52).
Regarding circulating eosinophils, large studies (Copenhagen General Population Study) including to a greater extent patients with mild asthma, indicate that high levels (400 eosinophils/mm^3) predict an increased risk of serious exacerbations and poor asthma control (53, 54). In addition, the *post-hoc* analysis of the Atlantis study showed that 16% and 26% of patients with mild asthma, respectively in the GINA 1-2 steps, have a post-bronchodilator FEV1/FVC < LLN and this functional impairment is related to eosinophilic inflammation and an increased risk of exacerbations (55).
The concomitant presence of high levels of FeNO and circulating eosinophils is also useful to identify subjects with greater risk of exacerbations. However, it should be noted that also smokers may show higher levels of circulating eosinophils and low levels of FeNO (56, 57).
The presence of an AHR together with allergic sensitization is known to be a prerequisite for the development of an early allergic response in terms of airway obstruction (58-60). High levels of AHR, especially if detected prior to the pollination season, also may constitute an important risk factor for the development of symptoms and exacerbations during maximum exposure to pollen (39, 61-68). Importantly, the finding of a concomitant fall in FEV1 and FVC during the bronchial challenge with methacholine allows to identify patients, even those suffering from mild asthma, who are at risk of episodes of near-fatal asthma, as there is a concomitant obstruction of the proximal and distal airways which can lead to respiratory arrest (69, 70).

Adherence to asthma medication during the pollen season

Although in clinical studies asthma can be well controlled in most patients with an appropriate therapeutic strategy (71), in clinical practice non-adherence with prescribed medications is very common and represents a significant barrier to optimal disease management.
To date, scientific literature does not report data on the adherence to medication in patients specifically affected by PIA. The available evidence comes from studies conducted on patients with allergic (sensitive to pollen or other allergens) or non-allergic asthma. In any case, the problem of therapeutic adherence appears to be independent of the trigger factors. Therefore, the findings emerging from these studies may be transferable to PIA.
Approximately 50% of adults and children on long-term therapy for asthma fail to take medication at least part of the time, resulting in poor quality of life, reduced work performance, and increased risk of exacerbation, associated with increased direct and indirect costs of disease management (5, 72, 73). Adherence may also decrease over time: a real-world study showed that adherence significantly declined with subsequent prescriptions (74). Furthermore, undetected suboptimal adherence, including the correct use of the inhalers, may be interpreted as poor therapeutic response, perpetuating a cycle of uncontrolled asthma symptoms, review and therapy escalation (75-77).
Several factors may influence therapeutic adherence and persistence, like personal and individual factors, psychological issues, health beliefs and behaviors, the clinician-patient relationship, factors linked to the disease (progression, stability, exacerbations), to the treatment (complexity of current medications, difficult-to-use inhaler, frequency of dosing, side-effects), or to costs and access (**figure 3**) (5, 78, 79).
The simplification of the therapeutic regimen, with prescription of once daily medications and easy-to-use inhalers, are important factors for achieving good compliance (5, 80).
On the other hand, several studies suggest that one of the determinants of poor adherence is the perception that the medication should be used in response to symptoms more than on a regular basis (81-83). Not surprisingly, treatment discontinuation is significantly higher in those who seek medical assistance for symptom worsening. The findings reflect an incongruence between the medical perspective, emphatizing proactive control through prevention of symptoms and exacerbations, and the patient's perspective, where to some extent symptoms are regarded as part of having asthma, rather than a sign that their asthma is poorly controlled (84).
In the case of allergen immunotherapy (AIT), a period of repeated administration for at least 3 years is required for achieving sustained symptom relief and potentially altering the disease course. This long-term commitment can be challenging for patients to maintain. Indeed, despite long term benefits, real life studies on

Figure 3 *- Key barriers to medication in chronic disease.*



Adapted from Kvarnström *et al.* 2021 (79).

patients with allergic rhinitis and asthma showed that at 3 years the overall adherence to AIT was below 40% (85, 86). Adherence was higher in the first year of treatment, in children and, in some studies, with the subcutaneous formulation (SCIT) *versus* the sublingual formulation (SLIT) (85, 86). Reasons for treatment discontinuation are due to factors like long duration of treatment, need for regular injections or daily sublingual administration, perception of poor efficacy, costs, and potential side effects (85, 86). In conclusion, evidence on the adherence to medication regimens in patients specifically affected by PIA is poor. On the other hand, therapeutic adherence in asthma remains a recurrent problem, regardless of the trigger factor.

Risk of (severe) exacerbations: the unpredictability of exposure

Pollen exposure is one of the factors associated with worsening of the symptoms of allergic rhinitis and asthma (87). The impact of pollen on respiratory health can be particularly significant in children, given that more than half of pediatric asthma cases are thought to have an allergic component (40, 88).
In the study by De Roos *et al.* (89) on subjects aged < 18 years followed over a 5-year period, an increased odd of asthma exacerbation was found in association with the exposure to tree pollen. Even low pollen levels (≤ 5 grains/m^3) were associated with small risk, with an exposure-response pattern of increasing odds with higher pollen level. A 64% increased risk was observed at pollen levels > 1,000 grains/m^3; for grasses, asthma exacerbations were associated with exposure to 52 grains/m^3 of pollen, while no correlation was shown with exposure to ragweed pollen and other pollen.
An Australian study by Shrestha *et al.* (90) assessed the role of ambient levels of different pollens on hospital admissions for asthma over a 5-year period in 2,098 children and adolescents. The results showed a significant correlation between Plantago and Parietaria pollen peaks and the rate of hospitalization for bronchial asthma, especially in younger children of 2-5 years of age; specifically, an increase in pollen concentration of 50 grains/m^3 was strongly associated with the risk of hospitalization. Similarly to other studies, a trend toward a greater pollen effect was observed in boys. The correlation was higher in colder seasons, but this finding could also be related to viral infections, so it is unclear whether pollen stimulation was the primary trigger.
The association between outdoor pollen and childhood asthma hospitalizations was examined in a systematic review (91). Although there was a substantial heterogeneity among studies related to pollen species, geographical areas, method of analysis used to estimate the effect size and differences in lagged day effects considered for the analysis, the results showed that globally grass and birch pollen were important triggers of childhood asthma hospitalization: an increase in 10 grass pollen grains/m^3 was associated with a 3% increase in admissions for asthma and an extreme pollen day (> 100g/m^3) could lead to a 30% increase in hospitalizations for asthma.
Interestingly, a study on a large cohort of 47,456 children admitted to hospital for asthma showed that grass pollen exposure was associated with higher readmission rates for asthma, supporting

the importance of target interventions for asthmatic children prior the pollen season (92).
In the study by Lappe *et al.* (93) covering a 26-year period of observation, a strong association was found between 9 of the 13 pollen varieties analyzed (grasses, nettle, pigweed, birch, maple, pine, oak, willow, sycamore, mulberry) and Emergency Departement (ED) visits for asthma and wheeze, with a 1-8% increase in ED admissions per standard deviation increases in pollen, which is consistent with the results from other studies (94). In general, the strongest association was observed in younger people and in Afro-Americans subjects, although the data varied by pollen taxa. Birch pollen was shown to be associated to asthma exacerbations especially in Northern European countries and North America. A Swedish study found an increase in respiratory symptoms and use of respiratory drugs alongside a reduction in lung function parameters during the pollen season (95). Moreover, pollen exposure increased the susceptibility to adverse respiratory effects induced by pollutants (particulate matters and O_3).
The epidemiological prospective study by Dominiguez-Ortega (96) compared clinical, functional and pathophysiological outcomes during and outside the pollen season in 101 adults diagnosed with allergic asthma and rhinitis who manifested exclusively seasonal symptoms caused by grasses and/or olive tree. The results show that most patients experienced symptoms, lung function abnormalities and airway-inflammation (as reflected by measurement of FeNO) exclusively during the pollen season, although a few continue to experience abnormalities outside the exposure period.
The occurrence of thunderstorms during pollen season of some taxa may lead to the so called "thunderstorm asthma", an epidemic of allergic asthma outbreaks, sometimes also severe asthma attacks, as reported in many areas of the world (97). The Melbourne thunderstorm asthma epidemic during the peak grass pollen season in November 2016 was unprecedented in scale and impact, with a large number of people having breathing difficulties and about 9900 patients' presentations at hospital emergency departments (98, 99). A systematic analysis of hospital's patients in Melbourne aged ≥16 years with thunderstorm asthma was conducted by Lee *et al.* (98), to identify key risk factors. Of 85 adult patients assessed, the majority (60%) had no prior diagnosis of asthma. However, allergic rhinitis during the grass pollen season was almost universal (99%), as were ryegrass pollen sensitization (100%) and exposure to the outdoor environment during the thunderstorm (94%). Airborne pollen levels on the thunderstorm day were extreme (102 grains/m^3) (98). The results suggest that ryegrass pollen sensitization and clinical allergic rhinitis define the adult population at risk for thunderstorm asthma, with acute allergen exposure as a trigger factor. The size of ryegrass pollen grains is > 35 μm in diameter, but stormy moisture may cause their rupture into respirable 3 μm granules that can easily penetrate deeply into the airways and elicit respiratory symptoms in predisposed subjects.
Based on this evidence, thunderstorm asthma can be considered a model of PIA and a risk factor of severe exacerbations in patients with mild asthma, often undiagnosed, allergic asthma.

The management of pollen-induced asthma: a model of regular treatment?

The aim of asthma management should be to achieve the best possible long-term outcomes for the individual patient. This may include significant reduction (possibly the complete absence) of asthma daytime and nocturnal symptoms, to improve lung function, to prevent/minimize the risk of acute deterioration of asthma symptoms (exacerbations) and asthma-related death, provide optimal pharmacotherapy with a simple dosage schedule and minimal or no adverse effects and to allow the patients to have a normal or almost normal life. According to that, asthma may be considered under control when all these outcomes are achieved (5, 100-104).
Poor symptom control of asthma is associated with an increased risk of exacerbations, but even people with good symptom control or seemingly mild asthma can still be at risk of severe exacerbations (105), and even death (106). Thus, most guidelines recommend that asthma control should be assessed in two domains: 1) current symptom control and 2) risk factors for future poor asthma outcomes, particularly exacerbations (*e.g.*, smoke, history of exacerbations, blood eosinophilia or high FeNO, environmental exposure) (5, 100-104).
The definition of asthma control mostly refers to the stability of clinical and functional parameters. However, some authors suggest that the inflammatory profile of an asthmatic patient should also be considered in the evaluation of asthma control (107). In this regard, within populations of patients with allergic rhinitis or intermittent asthma, some subjects show evidence of ongoing bronchial inflammation, *i.e.*, low pH and high IL-5 concentrations in the exhaled breath condensate, as well as increased FeNO levels (107, 108).
The question whether subclinical airway inflammation may determine the risk of relapse later in future was addressed in a large population-cohort study (109). The results demonstrated that a number of inflammatory biomarkers was independently associated with future respiratory outcomes or accelerated lung function decline. In this respect, GINA document points out that increased levels of type 2 inflammatory markers are risk factors for poor asthma outcomes (5).
It should be also underlined that each bronchoconstrictor event determines epithelial and bronchial muscle stress (mechanotransduction), which translates into the release of cytokines and growth factors that accelerate bronchial remodeling and inflammation, generating positive feedback mechanisms that tend to

perpetuate the persistence of asthma (110-114). These findings have potential implications for asthma management, as the prevention of bronchoconstriction itself could be an important target, contributing to the reduction of inflammation.

As a consequence, ideal treatment strategies should be also aimed at controlling underlying airway inflammation and possibly prevent or slow down remodeling processes.

Inhaled corticosteroids (ICS), alone or in single inhaler combination with long acting beta2 agonists (LABA), are the mainstay of asthma treatment and are recommended in several national guidelines as regular preventive therapy approach, in which the dose of ICS is appropriate to the severity of disease and can be increased as necessary, and decreased, when possible, to achieve and maintain disease control (100-104). The frequency of rescue medication use, such as the short acting beta2 agonists (SABA) to relieve symptoms, is considered a reliable measure of asthma control.

In mild-moderate asthma, the guidelines also consider the use of a single combination inhaler of ICS/LABA for maintenance and reliever therapy (MART), which might suit some individuals (5). It relies on the rapid onset of reliever effect with formoterol and by including a low dose of inhaled corticosteroid it ensures that, as the need for a reliever increases, the dose of preventer medication is also increased.

The analysis of MART clinical trials demonstrated that this strategy was at least as effective as a regular treatment with other ICS/LABA combinations plus SABA as needed in the prevention of severe exacerbations, but it is associated with a significant level of symptoms (54% of the days) and frequent use of rescue medication, that may be considered as a sign of an incomplete asthma control, particularly when these events are frequently reported (115-119). Notably, Pavord *et al*. (120) showed that sputum eosinophils and endobronchial biopsy eosinophils were significantly lower following a regular treatment with ICS/LABA plus SABA compared to MART strategy, where a trend towards increased cellularity was observed.

Interestingly, three surveys have been conducted in 16 countries all over the world to understand current treatment approaches for patients with asthma and how these align with the latest GINA recommendations in real-world clinical practice. Altogether 2,482 physicians (mainly pulmonologists and general practitioners) and 4,266 asthmatic patients have been enrolled (121-123). The results show important rates of poor asthma control and SABA use across all participating countries. Patients appear to overestimate their level of asthma control, that is not aligned with their reporting of symptoms/limitations. Physicians generally rated symptom control over exacerbation reduction as their main treatment goal for patients with mild to moderate asthma. This was consistent with prioritization of symptoms over exacerbations when prescribing daily maintenance medication. The consolidated proactive treatment with ICS/LABA and as-needed SABA remains the preferred initial approach. Furthermore, the co-prescription of MART therapy and SABA (frequently requested by the patients themselves) suggests confusion between reliever strategies in real world or alternatively is suggestive of patients who may remain uncontrolled on MART therapy and feel the need for a reliever to manage their asthma symptoms (122).

Another aspect to be considered is the hypoperception of airway obstruction by the patients that was reported in approximately 26% of asthmatics; these patients are poor judges of their clinical conditions, and this under-estimation may lead to poor adherence to maintenance therapy, inadequate treatment of airway inflammation and airway hyperresponsiveness and increased risk for exacerbations and episodes of near-fatal asthma.

However, the model of pharmacological treatment proposed in the guidelines, largely based on a similar type of therapeutic response for all patients, does not consider, in mild-moderate asthma, the possible different phenotypes that may require a personalized approach. In this respect, the PIA phenotype is pathognomonic, as the assessment of the impairment domain (symptoms), on which the control assessment is largely based, varies considerably depending on the exposure period to pollen, given that the questionnaires (such as ACT) often investigate the symptoms relating to the previous few days or weeks.

Furthermore, unlike other clinical phenotypes, in PIA the main future risk factor, the seasonal exposure to pollen, is known and partly predictable. This consideration is, however, still insufficient for a rational therapeutic approach, which cannot necessarily be the same in all periods of the year and in all subjects.

For this reason, we have proposed the need to carry out a seasonal risk stratification, based on the risk factors of exacerbation previously described and shown in **figure 2**, using the considerations summarized in **table I**.

Consequentially, the proposed therapeutic algorithm that considers the risk stratification model is schematized in **figure 4**.

In subjects at low risk, ICS/formoterol as needed or low dose ICS whenever SABA is taken can be considered. In the event that the use of the rescue medication is > 2 days/week or in case of symptoms ≥ 2 days/week, it is recommended to switch to a fixed daily therapy.

In subjects stratified as high risk, we propose a maintenance daily therapy with ICS/LABA and SABA as needed, or daily maintenance ICS and as needed SABA or MART with ICS/formoterol from the beginning of the exposure period, determined on the basis of the pollen calendar. The strength of ICS (medium or high) is determined by the healthcare professional based on risk stratification; generally, in patients with PIA a medium strength is sufficient. In any case, the rapid variability of pollen exposure conditions can make it difficult to obtain a maximal bronchoprotective effect using a symptom-driven approach, as this achievement requires therapeutic continuity. In addition, the persistence of risk factors for the loss of asthma control, including comorbidities and increased biomarkers of airway inflammation, even in

Table I *- Seasonal risk stratification.*

	Indicators	High risk	Low risk
Symptoms during pollen exposure	Severe exacerbations in the previous 12 months	≥ 1	None
	Frequency of respiratory symptoms	≥ 1 time/week	None
	Use of reliever	Regularly > 1 time/week	No, a few times
Symptoms before pollen season	Persistent (> 1 week) respiratory symptoms* after airway viral infection	Yes	No
	Respiratory symptoms* after hyperventilation (running, singing…)	Yes	No
	Respiratory symptoms* in the current and previous months	Yes	No
Biomarkers assessed before pollen season	FeNO	≥ 40 ppb	< 25 ppb
	Eosinophils	≥ 400 /mmc	< 150 /mmc
Lung function before pollen season	Spirometry: airway obstruction	FEV1/FVC < LLN or < 75%	FEV1/FVC ≥ LLN or ≥ 75%
	Spirometry: FEV1	< 80% predicted or > 10% fall *versus* previous control	Normal or ≥ 80% predicted unchanged from personal best
	Spirometry: bronchial responsiveness test	≥ 12% and 200 ml	< 12% and 200 ml
	Direct bronchial challenge (PC20)	High AHR: PC20 < 1 mg/ml Moderate AHR: PC20 ≥ 1 ≤ 4 mg/ml	Mild AHR > 4 ≤ 8 mg/ml AHR borderline > 8/mg/ml
	Indirect bronchial challenge (PD15)	Positive to mannitol test: PD15 ≤ 635 mg mannitol	Negative to mannitol test: PD15 > 635 mg mannitol
Other clinical features to consider	^Moderate-severe allergic rhinitis, rhinosinusitis, gastroesophageal reflux, obesity Impaired perception of bronchoconstriction (hypo-perceptors); perception reduced also in patients with high AHR		

*Respiratory symptoms: cough, shortness of breath, wheezing, chest tightness; ^the risk increases if multiple comorbidities are present; AHR: airway hyperresponsiveness.

a patient with apparently minor daily symptoms, should be also considered for treatment optimization, to prevent negative outcomes. Therapy will be withheld or reduced based on the progression of symptoms and the resolution of triggering factors, supported by the pollen data.

In patients with PIA receiving seasonal therapy it is advisable to use a principle of maximum precaution, in particular in those considered at high risk, as they may experience severe exacerbations or even episodes of near-fatal asthma due to the rapid changes in the allergenic load to which they are exposed, in the presence of a high degree of AHR not previously highlighted and undertreated with ICS (35-37). In this respect, modeling studies based on published experimental and clinical data showed that a different degree of asthma control and bronchoprotection (*i.e.*, suppression of the AHR) as well as systemic activity can be achieved depending on the adherence to the therapeutic regimen and the type of ICS used (124).

None of the above-mentioned pharmacological therapies address the pathogenetic mechanism of allergic asthma. Conversely, AIT is the only therapeutic intervention able to induce both immune modifying effects and long-term efficacy.

Different efficacy results have been reported in relation to heterogeneity in terms of products used, routes of administration (subcutaneous – SCIT and sublingual – SLIT), study populations, and study designs compared to those commonly employed in pharmacological clinical trials of asthma (125, 126).

The efficacy of AIT in seasonal allergic asthma caused by grass pollen allergy and tree pollen allergy (the most frequently studied pollens considering their epidemiological load) has been proven in clinical trials and real-word studies, especially with SCIT (127-129).

The large retrospective cohort study REACT analyzed German health insurance data from 2007 to 2017: the analysis showed that AIT prescription in patients with allergic asthma (compared

with a control group without AIT prescription) led to a lasting improvement in asthma control, lower medication consumption, and a decrease in the exacerbation rate (127). In addition, these effects even increased over time after the end of AIT and there was also an advantage for patients with asthma with regard to the occurrence of pneumonia and hospitalizations.

A population-based Danish study compared patients with asthma who received an AIT prescription with patients who did not receive an AIT prescription: in the 3 years following completion of the AIT prescription, there was a sustained reduction in the exacerbation rate (on average by 74% in patients with seasonal allergies and on average by 57% in patients with house dust mite allergies) compared with patients without an AIT prescription (128).

The results of a real-world study involving a large sample of patients showed that sublingual AIT was associated with a significant reduction in the risk of new asthma events for up to eight years and also in the risk of asthma onset or worsening, for all ages and allergens evaluated (129). The results support the long-term effectiveness of sublingual AIT treatment of patients with allergic rhinitis with and without pre-existing asthma, as a relevant causal option for patients with respiratory allergies.

AIT is currently recommended for allergic asthma, if it is well documented that allergens elicit asthma symptoms and if asthma is controlled (130). Thus, AIT is considered as an additional therapy for allergic asthma, and carried out after the initiation of adequate drug therapy for asthma. Ideally, inhaled therapy can be reduced during AIT or even stopped completely once AIT has been completed.

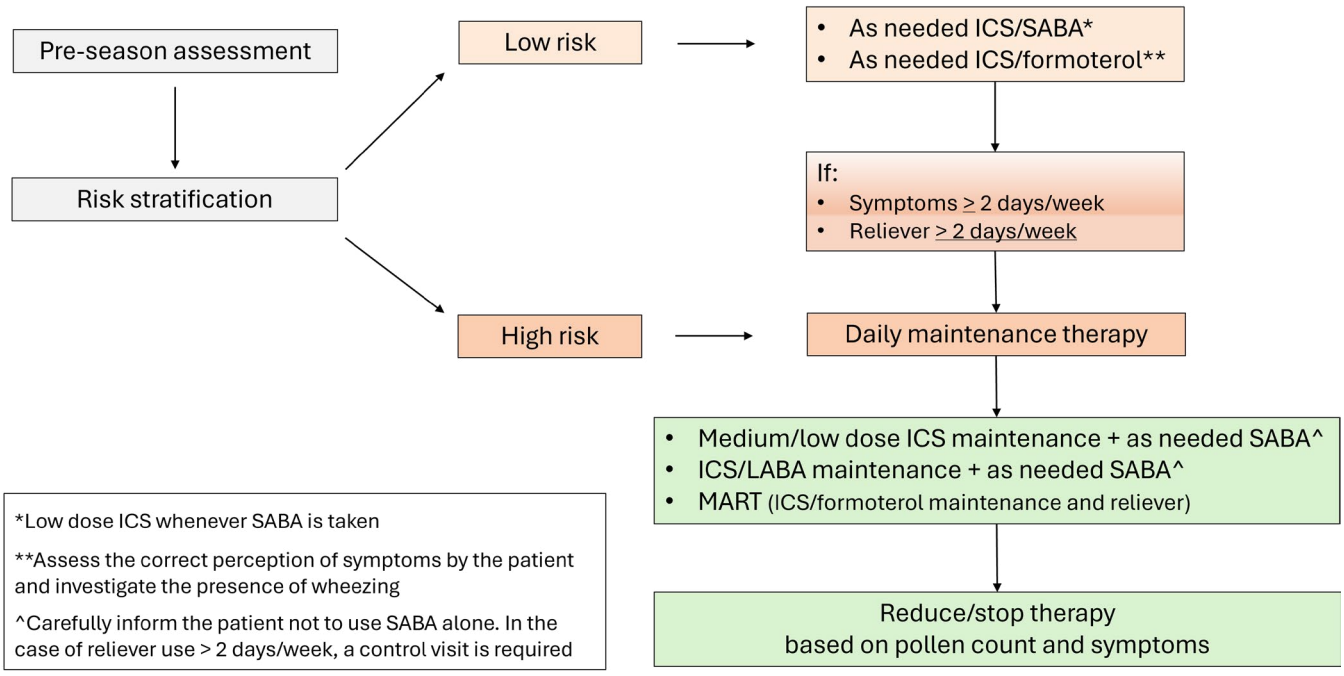
However, in case of patients with symptoms limited to the pollen season, AIT should be associated to a proper treatment according to the PIA therapeutic algorithm (**figure 4**).

Conclusions

Evidence supports the hypothesis of PIA as a specific asthma phenotype, characterized by substantial asymptomatic periods in which patients are not exposed to triggers, with allergic rhinitis being one of the most common comorbidities.

Although the pollen season represents the key factor affecting the risk of asthma outbreaks, pollen count, aerobiological data, the presence of polysensitivity that can overlap, and the meteorological conditions can also influence the clinical picture of the patients in different directions (38). In this context, a careful assessment of the clinical manifestations in the previous year and in the period before the pollen season, as well as the measurement of objective markers (FeNO, AHR, FEV1, circulating eosinophils), make it possible to stratify the risk of symptoms and exacerbations, allowing the therapeutic approach to be tailored in a rational manner during the seasonal exposure period.

Figure 4 *- Therapeutic algorithm for pollen-induced asthma.*



Effective disease control can be achieved through the use of therapeutic regimens containing ICS. Depending on patients' characteristics and risk factors, healthcare professionals and patients can share the decision on the best therapeutic strategy (131), considering effective bronchoprotection and the simplicity of regular once-daily administration of ICS/LABA, that favors the therapeutic adherence (132), and the flexibility of the MART strategy, which however may require more careful education and collaboration from the patient (133). In patients who, in previous years, have shown a loss of asthma control only in the season when they are exposed to sensitizing allergens, a seasonal therapy (*i.e.*, therapy prescribed during periods of seasonal exposure) may be considered (133). In any case, the poor predictability of exposure to pollen, with its variations in concentration and allergenicity, highlights the importance of a preventive approach to reduce the risk of asthma outbreaks. Thus, starting daily therapy with low-dose ICS/LABA before the period of maximum allergic exposure could be advisable to increase the level of bronchoprotection (133).
Allergen-specific immunotherapy is the only curative treatment that can be used in association with standard pharmacological therapy in PIA, that may provide benefit, especially in subjects with comorbidities, such as allergic rhinitis, and may reduce drug burden (134).
Educating patients on proper symptom perception and adherence to treatment is also crucial for optimal disease control.

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Contributions

All authors equally contributed to the manuscript draft and critical revision for important intellectual content, to data collection, analysis and interpretation.

Conflict of interests

LC reports personal fees from Chiesi Farmaceutici (consulting), Thermofisher (consulting, talks), GlaxoSmithKline (consulting, talks), Astra Zeneca (consulting, talks), Menarini (talks, consulting), Novartis (consulting, talks), and Sanofi (consulting, talks). AM reports personal fee from Sanofi (talks) and GSK (consulting). KJ reports personal fee from Sanofi (talks) and GSK (consulting). AMM reports personal fees from GlaxoSmithKline (consulting). MM reports personal fees from Chiesi Farmaceutici (consulting), GlaxoSmithKline (consulting, talks), and AstraZeneca (consulting). AV reports personal fees from Chiesi Farmaceutici (talks), GlaxoSmithKline (talks), AstraZeneca (talks), and Menarini (talks). MBB reports personal fees from ALK (consulting), AstraZeneca (talks), BluePrint (advisory board, talk), Gentili (talk), GSK (talks), Menarini (talks), and Sanofi (advisory board, talks). FP, GV, and BY report no conflict of interest.

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ORIGINAL ARTICLE

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The role of basophil activation test in venom immunotherapy: comparative evaluation with specific IgE and skin prick tests, innovative approaches

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Impact statement

The basophil activation test (BAT) offers high accuracy in diagnosing insect venom allergy and making immunotherapy decisions, providing significant insights for clinical practices.

Summary

Background. *In diagnosing insect venom allergy and making immunotherapy decisions, clinical history, skin tests, and specific serum IgE levels are commonly utilized. This study aims to emphasize the clinical significance of using the basophil activation test in accurately identifying sensitivities in individuals with insect venom allergy and to compare its effectiveness with other testing methods.* ***Methods.*** *This study included a total of 43 patients, who experienced at least one systemic allergic reaction following insect stings and were deemed suitable for immunotherapy. Basophil activation test, specific serum IgE levels, and skin prick test results utilized in making immunotherapy treatment decisions were recorded.* ***Results.*** *Our study determined that the overall clinical sensitivities of the basophil activation test (BAT), specific serum IgE (spIgE), and skin prick test (SPT) for Apis mellifera were 95.5%, 95.7%, and 48.4% respectively, while for Vespula vulgaris, they were 83.3%, 100%, and 33.3%. Based on these results, the prediction of systemic reactions to bee stings is ordered as spIgE > BAT > SPT. Additionally, early-stage skin prick tests showed a sensitivity of 67% and specificity of 50% at a cut-off value of 1.5 mm, and 33% sensitivity and 83% specificity at 2.5 mm.* ***Conclusions.*** *This study demonstrates that the basophil activation test (BAT) can provide a high positive predictive value in immunotherapy treatment decisions and offer significant insights in clinical practices.*

Introduction

Hypersensitivity to Hymenoptera stings, affecting a considerable segment of the population (56-94%), poses a potential life-threatening risk (1). Systemic allergic reactions to such stings have been reported in up to 7.5% of adults and 3.4% of children (2). Venom immunotherapy (VIT) remains the sole effective treatment for patients showing severe reactions following Hymenoptera stings, reducing the risk of a serious systemic reaction to a sting by approximately 90% (3, 4).

For the detection of hypersensitivity to Hymenoptera venom, skin tests and the measurement of specific IgE antibodies in serum are the commonly employed methods, proving effective

in confirming diagnoses in various cases. Nonetheless, there can be instances of test results not aligned with clinical histories. Precise identification of a patient's sensitivity before initiating VIT is critically important for the success of the treatment. In such scenarios, there arises a need for alternative testing approaches, like cellular *in vitro* tests, that can yield more definitive outcomes. With the limitations of traditional diagnostic methods such as specific IgE and skin prick tests in mind, the significance of the Basophil Activation Test in diagnosing venom allergy is increasingly being acknowledged (5).

Presently, the basophil activation test is utilized in determining clinical sensitivity at the commencement of venom immunotherapy, in patients with conflicting or negative skin test or specific IgE results, for allergen selection in patients with dual sensitivities for VIT, and in the monitoring and evaluation of VIT's efficacy (6, 7). To date, there has been a limited number of studies in literature on this subject and none from our country.

The main objective of our study is to showcase the applicability and effectiveness of the Basophil Activation Test in detecting hypersensitivity to Hymenoptera venom. In particular, this study provides a comparative evaluation of this test with skin prick tests and allergen-specific serum IgE measurements, in terms of both clinical sensitivity and positive predictive values. The study highlights the necessity for current approaches in the more accurate and effective detection and management of hypersensitivity conditions resulting from Hymenoptera stings.

In this regard, the role of the basophil activation test in the immunotherapy process for bee allergy is thoroughly compared with traditional approaches, such as specific IgE measurement and Skin Prick tests. The study delves into the advantages and limitations of these three distinct testing methods and evaluates the potential contribution of BAT in diagnosing Hymenoptera venom allergy.

Materials and methods

Study population and design

This study included a total of 43 patients who exhibited systemic allergic reactions following *Apis mellifera* and *Vespula vulgaris* were consequently treated with venom immunotherapy (VIT). Demographic data, clinical characteristics, and the severity of reactions of the patients were recorded (**table I**). Anamnesis information included details about the type of stinging bee and reaction characteristics. The severity of the patients' reactions was graded according to the Muller classification (2).

Skin prick test

Standardized purified venom antigens of *Apis mellifera* and *Vespula vulgaris* (ALK-Abello, Horsholm, Denmark) were used for skin tests. Application was performed at the recommended standard dosage of 100 μg/mL concentration. Patients underwent skin

Table I *- Demographic information of patients.*

	n	Percentage %
Gender		
Male	34	79.1
Female	9	20.9
Age		
Below 10 years old	6	14.0
Between 10-20 years old	24	55.8
Above 20 years old	13	30.2
Time to Initiate VIT after Bee Sting		
Below 1 month	4	9.3
Between 1-2 months	18	41.9
Between 2-6 months	5	11.6
Between 6 months to 1 year	12	27.9
Above 1 year	4	9.3
Age of Starting VIT		
Below 5 years old	3	7.0
Between 5-10 years old	18	41.9
Between 10-15 years old	7	16.3
Above 15 years old	15	34.9
Incidence of Bee Sting during VIT		
No	21	48.8
Yes	22	51.2
Incidence of Sting by Treated Bee Species		
No	1	4.5
Yes	21	95.5
Reaction Type		
None	14	63.6
Local	6	27.3
Systemic	2	9.1
Duration of VIT		
1 year	5	11.6
2 years	4	9.3
3 years	5	11.6
4 years	8	18.6
5 years	18	41.9
6 years	3	7.0

prick tests, and intradermal test methods were not employed. Positive test results were defined according to the recommendations of the European Academy of Allergy and Clinical Immunology. Intradermal test results were considered positive if the difference from the negative control was greater than 3 mm (2).

Specific IgE antibody determination

The levels of allergen-specific IgE in serum samples were measured using the ImmunoCAP 1000 system manufactured by Phadia (Sweden). For each serum sample, IgE levels against Honeybee (*Apis mellifera*, I1) and Wasp (Vespula Spp, I3) were measured using the ImmunoCAP test kit. A specific test (test code: 6759) for the Bee Venom Components IgE panel was applied. The levels of Allergen-Specific IgE (spIgE) were classified according to a predetermined evaluation scale. Values below 0.10 kU/L were considered negative, while values above 0.10 kU/L were considered positive.

Basophil activation test

BATs were conducted using Flow CAST (Bühlmann Laboratories AG). Venous blood was collected in 10 mL EDTA tubes and stored at 4 °C for no longer than 24 hours. For each patient and allergen, polystyrene tubes were prepared with different concentrations of allergens (bee and wasp venom) and diluted in stimulation buffer. The Flow CAST method was employed for *Apis mellifera* (BAG2-I1) and Vespula spp (BAG2-I3). The cut-off point for CD63 activation was determined as 11.5 ng/mL or higher concentrations at ≥ 10%. Positive controls included monoclonal anti-FcεRI antibody and N-formyl-methionyl-leucine-phenylalanine (2 mM), and the negative control used only the stimulation buffer. Cells were analyzed by flow cytometry using a FACSCalibur flow cytometer (Becton-Dickinson Biosciences GmbH, Heidelberg, Germany). Basophilic cells were selected from the lymphocyte population using anti-CCR3 and the upregulation of the activation marker CD63 was calculated as the percentage of CD63 cells in the total basophilic cell population. The cut-off point was set at 10% CD63cells, as recommended by the supplier.

Statistical evaluation

After encoding the data obtained from the research, it was transferred to the computer and analyzed using the SPSS (Statistical Package for Social Sciences) software package (Version 22 for Windows, SPSS Inc, Chicago, IL, USA). Frequency (categorical) data were expressed in numbers and percentages (%). The diagnostic decision-making characteristics (sensitivity, specificity, *etc*.) of SpIgE, BAT, and SPT results in predicting *Apis mellifera* and *Vespula vulgaris* stings were assessed through Receiver Operating Characteristic Curve (ROC) analysis. In the evaluation of Area Under the Curve (AUC) values in ROC analysis, a test was considered statistically significant when $p < 0.05$.

Ethical committee

The ethical approval for this study was obtained from the Clinical Research Ethics Committee of Ondokuz Mayıs University (number: 2021000609-1). Our study was conducted in accordance with the principles of good clinical practice based on the Helsinki Declaration. Ethical approval confirms that research studies are conducted in compliance with ethical standards and human rights, and that the rights of participants are protected.

Results

Of the 43 patients included in the study, 79.1% were male and 20.9% were female. The ages of the patients at the start of venom immunotherapy are presented in **table I**.

Based on anamnesis, 33 (70.2%) cases were attributed to *Apis mellifera* stings and 14 (29.8%) to *Vespula vulgaris*. Among the patients who reacted to *Apis mellifera* stings, 42.4% displayed Grade 3 reactions and 39.4% Grade 4, while for those reacting to *Vespula vulgaris* stings, 21.4% were Grade 3 and 71.4% Grade 4.

Skin Prick Test (SPT) was administered to all 43 patients. Immediately after bee stings, in the first presentation, only 19 skin prick tests were positive (14 *Apis mellifera*, 5 *Vespula vulgaris*). Therefore, those who tested negative among the patients who applied within the first 8 weeks were retested. Sensitivity and specificity were evaluated according to these results. Positive reactions to *Apis mellifera* were observed in 31 patients, while the remaining 12 showed positive reactions to *Vespula vulgaris*. Dual sensitivity was observed in 17 patients. The sensitivity of SPT in predicting *Apis mellifera* stings was 48.4% with a positive predictive value (PPV) of 65.2%. For *Vespula vulgaris* stings, the sensitivity was 33.3% with a PPV of 20.0% (**table II**).

SpIgE assessment was conducted in 31 patients. Among 23 patients who showed systemic reactions to *Apis mellifera* stings, 22 had positive SpIgE results, while all 8 patients with systemic reactions to *Vespula vulgaris* stings had positive results. The sensitivity of SpIgE for systemic reactions caused by *Apis mellifera* and *Vespula vulgaris* stings was determined as 95.7% with a PPV of 100.0% for *Apis mellifera*, and 100.0% with a PPV of 88.9% for *Vespula vulgaris* (**table II**). In 17 patients, dual sensitivity was detected in the DPT test, while in 7 patients, dual sensitivity was detected in the spIgE test.

BAT assessment was carried out in 28 patients. Of the 22 patients stung by *Apis mellifera*, 21 were confirmed by BAT results, and 5 of 6 patients stung by *Vespula vulgaris*. The sensitivity of BAT in predicting *Apis mellifera* stings was 95.5%, with a PPV and Likelihood Ratio (LR) of 95.5% and 5.72, respectively. For *Vespula vulgaris* stings, the sensitivity of BAT was 83.3%, with a PPV and LR of 83.3% and 18.51, respectively (**table III**). Dual sensitivity was not detected.

In terms of diagnostic efficacy in identifying systemic reactions to *Apis mellifera* and *Vespula vulgaris* stings, the diagnostic supe-

Table II *- Diagnostic values of diagnostic tests in predicting stings from the Apis mellifera and Vespula vulgaris.*

	Diagnostic test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR
Apis mellifera	SPT	48.4	33.3	65.2	80.0	0.73
	SpIgE	95.7	100.0	100.0	11.1	NA
	BAT	95.5	83.3	95.5	16.7	5.72
Vespula vulgaris	SPT	33.3	48.3	20.0	34.8	0.64
	SpIgE	100.0	95.7	88.9	NA	23.26
	BAT	83.3	95.5	83.3	4.5	18.51

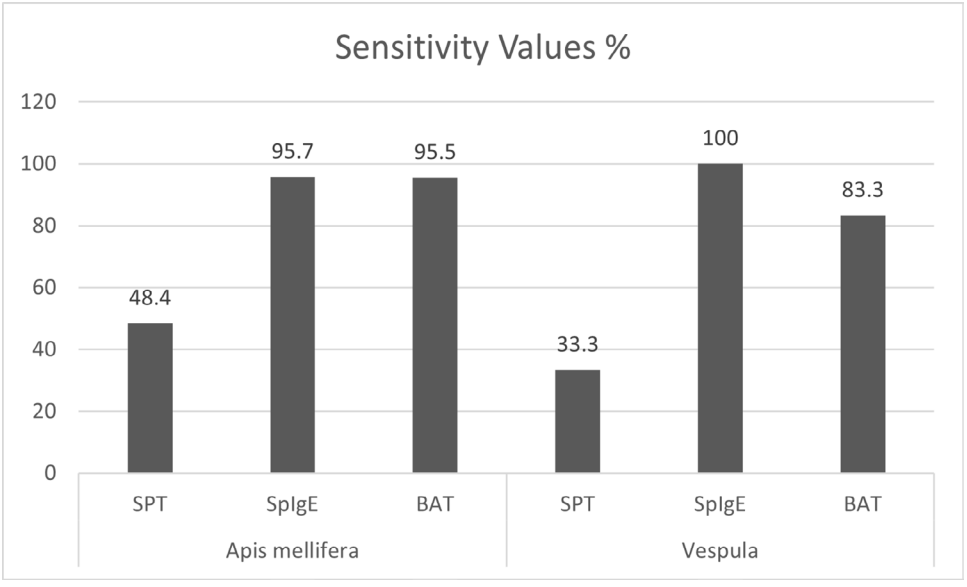
Positive predictive value; negative predictive value; likelihood ratios.

Table III *- Comparison of basophil activation test sensitivity results with skin prick test and specific IgE results by bee species.*

Variables			Sensitivity results of BAT		P-value*	P-value**
			Positive n (%)	Negative n (%)		
Apis mellifera	SPT	Positive	8 (38.1)	0 (0.0)	NA	NA
		Negative	13 (61.9)	0 (0.0)		
	SpIgE	Positive	16 (94.1)	1 (100.0)	1.00	0.817
		Negative	1 (5.9)	0 (0.0)		
Vespula vulgaris	SPT	Positive	3 (60.0)	0 (0.0)	NA	NA
		Negative	2 (40.0)	0 (0.0)		
	SpIgE	Positive	5 (100.0)	0 (0.0)	NA	NA
		Negative	0 (0.0)	0 (0.0)		

*Fisher's Exact test; **Spearman correlation analysis; NA: no analysis done.

Figure 1 *- Sensitivity values for Apis mellifera and Vespula vulgaris: comparing Basophil Activation Test (BAT), Specific Serum IgE (spIgE), and Skin Prick Test (SPT).*



riority ranking was established as Specific IgE (SpIgE) > Basophil Activation Test (BAT) > Skin Prick Test (SPT) (**figure 1**).

Determining the optimal cut-off value for SPT through ROC analysis (figure 2)

Patients were categorized based on the time intervals following bee stings for conducting the skin prick test: within the first 4 weeks, between 4 and 8 weeks, and beyond 8 weeks. ROC analyses were performed on the measurements in millimeters obtained from the skin prick test results according to this categorization. Diagnostic power of SPT was evaluated considering the patients' time of presentation. For cases presented ≤ 4 weeks, a cut-off = 1.5 mm resulted in a sensitivity of 67% and specificity of 50%. When the cut-off was set at 2.5 mm under the same conditions,

Figure 2 - *Diagnostic evaluation of SPT in distinguishing Apis mellifera from Vespula vulgaris using ROC analysis.*

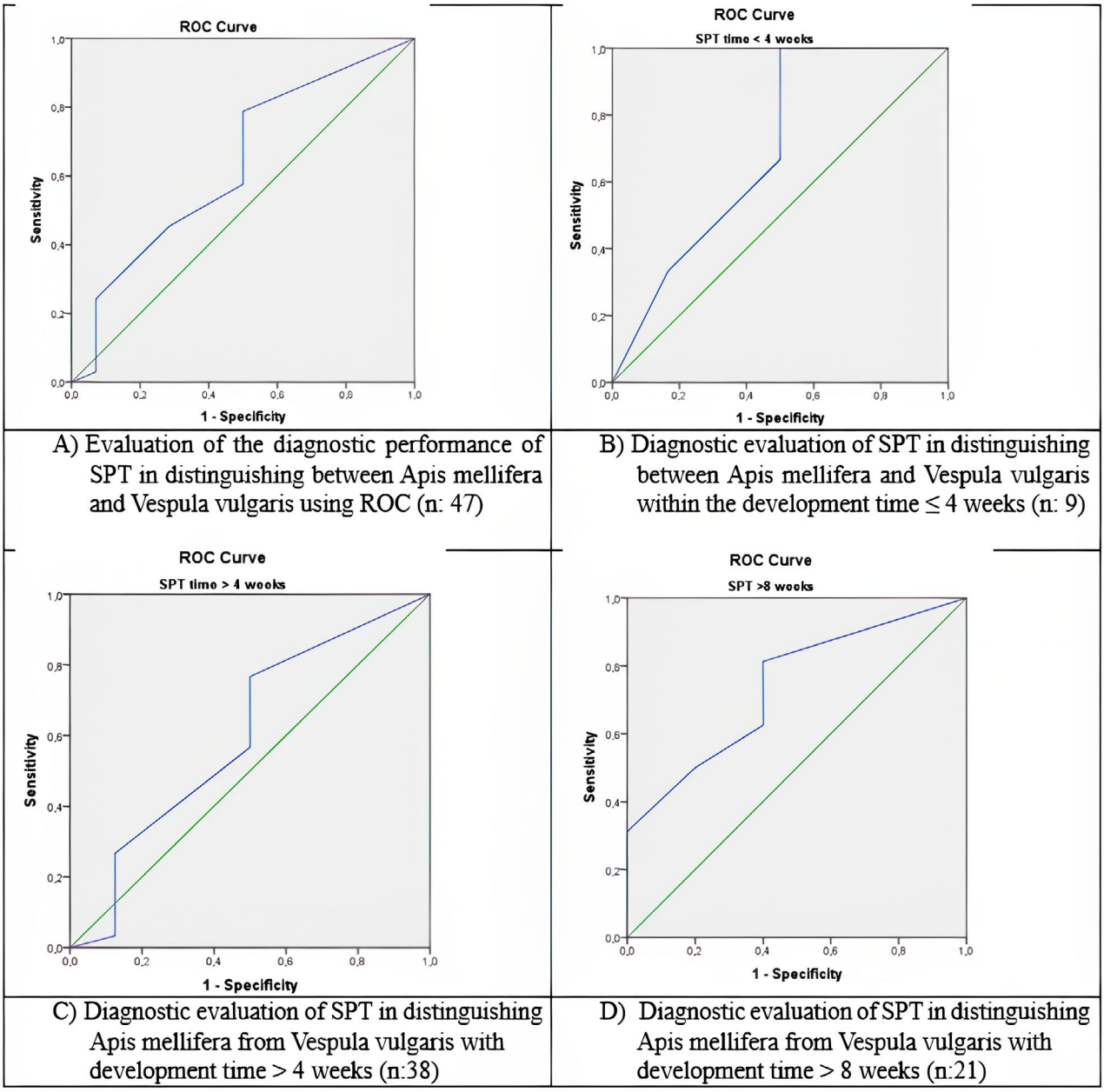


Table IV *- Diagnostic values of SPT in distinguishing Apis mellifera from Vespula vulgaris.*

Application time	Cut off value	Sensitivity	Specificity	AUC	SE	P-value	95% Confidence Interval (Minimum - Maximum)	
≤ 4 weeks	1.5	67%	50%	0.69	0.18	0.36	0.33	1.00
	2.5	33%	83%					
> 4 weeks	1.5	57%	50%					
	2.5	47%	62%	0.60	0.12	0.39	0.36	0.84
	3.5	27%	87%					
> 8 weeks	1.5	63%	60%					
	2.5	50%	80%	0.73	0.12	0.11	0.49	0.97
	3.5	31%	100%					

the sensitivity was 33% and specificity was 83% (AUC = 0.69; p = 0.36; 95%CI 0.33-1.00). In cases presented > 4 weeks, the statistical evaluation yielded a sensitivity of 57% and specificity of 50% for a cut-off = 1.5 mm (AUC = 0.60; p = 0.39; 95%CI 0.36-0.84). The calculated cut-off values and diagnostic values for SPT according to the patients' presentation times are shown in **table IV**.

In our study, irrespective of the patients' time of presentation, ROC analysis was conducted to determine the most appropriate cut-off point for distinguishing between *Apis mellifera* and *Vespula vulgaris* species based on the reaction diameters measured in SPT. In this assessment, the area under the curve (AUC) was calculated as 0.633 (95%CI 0.45-0.81), and a sensitivity of 86% and specificity of 93% were found for a 3.5 mm cut-off value (p = 0.153)

Discussion and conclusions

Venom immunotherapy (VIT) is highly effective, with 77% to 84% of patients protected from anaphylaxis after bee venom VIT, and this rate increases to 91% to 96% following wasp venom VIT (2, 8). However, VIT is expensive and time-consuming, requiring a treatment duration of at least 3 to 5 years. According to the guidelines of the European Academy of Allergy and Clinical Immunology for specific immunotherapy for Hymenoptera venom allergy, allergen-specific immunotherapy is recommended for children and adults who have systemic allergic reactions exceeding general skin symptoms, with documented sensitivity to the culprit insect's venom, determined by skin tests and/or specific serum IgE (sIgE) tests and/or basophil activation test (BAT) (9, 10). All three test methods can provide valuable information in the immunotherapy process for bee allergy. While the basophil activation test offers a sensitive approach to determining cellular response, specific IgE tests and Skin Prick tests are more commonly used methods with quicker results. The combination of these tests and consideration of clinical symptoms are important in determining the treatment plan. The advantages and limitations of each test method should be considered to select the most appropriate diagnostic approaches for individual patients. The bee species identified in a patient's history may not always align with the results from diagnostic tests, or there may be sensitivities to multiple bee species. This can be due to a person's sensitivity to multiple bees or cross-reactivity between bee venom allergens or both (11). Basophil activation test (BAT) helps in diagnosing clinically relevant venoms in cases where routine tests (specific IgE, skin tests) are inconclusive in Hymenoptera venom allergy. A study in our country identified bee venom as the most common cause of anaphylaxis in adults, accounting for 60.8% of cases. However, the basophil activation test (BAT) is not yet widely used as a first-line test in venom allergy diagnosis and is only available in a few centers in our country and worldwide. Nevertheless, the role of BAT in the diagnosis of Hymenoptera venom allergy (HVA) is well known. When basophils are activated, surface markers such as CD63 and CD203C increase. Measurement of these markers by flow cytometry is a reliable method in allergy diagnosis.

In our study, the sensitivity of BAT for *Apis mellifera* was found to be 95.5%, with a PPV of 95.5% and an LR of 5.72. Similarly, for *Vespula vulgaris*, the sensitivity was 83.3%, PPV 83.3%, and LR 18.51. A study in the literature found the sensitivity of BAT to be between 83-92% and specificity between 80-100% (12, 13). In our study, dual sensitivity was detected in 39.5% of patients in the DPT test and in 22% of patients in the spIgE test, while no dual sensitivity was observed in the BAT test. Therefore, BAT plays a crucial role in treatment decisions in cases of dual sensitivity. While additional diagnostic tests are not mandatory when SpIgE and skin prick test results are definitive and consistent, in "difficult cases" where SpIgE and DPT results are negative or contradictory, the use of BAT is recommended. This is especially true in cases of double positivity to wasp and bee venoms (14). In a previous study, 19 out of 26 patients (73%) who

had systemic allergic reactions, with negative skin prick tests and undetectable specific IgE, had positive BAT results for a single venom (6 for bee venom, 7 for wasp venom), and six were positive for both venoms (15). BAT has been found applicable in patients with very low sIgE levels where inhibition tests are not possible. It offers additional advantages over specific IgE tests, as basophils are not activated by clinically insignificant IgE antibodies. 75% of 47 sIgE negative patients had a positive reaction in the basophil activation test (15).

In our study, 39.5% of participants had positive Skin Prick Tests (SPT) for both bee species. For patients with double sensitivity, basophil activation test results were considered before starting immunotherapy treatments. Moreover, 4 patients had both skin test and sIgE results negative. Nevertheless, based on clinical history and basophil activation results, venom immunotherapy against *Apis mellifera* was initiated.

A study in the literature indicated that the general clinical sensitivities of the basophil activation test, specific serum IgE, and skin test were 90%, 76%, and 64%, respectively. The same study found the PPVs for these three tests for bee venom were 79%, 73%, and 78%, for wasp venom 86%, 59%, and 43%, and for both venom types 84%, 77%, and 22%, respectively (16).

In our study, the clinical sensitivities for *Apis mellifera* were determined as 95.5%, 95.7%, and 48.4%; for *Vespula vulgaris* as 83.3%, 100%, and 33.3%, respectively. SpIgE demonstrated high sensitivity and PPV ranging between 95.7% and 100%. BAT similarly showed high sensitivity and PPV values. However, SPT had some limitations with lower sensitivity and PPV. These results should be considered significant factors in the selection of tests for the diagnosis of venom allergy and should guide future research in this field.

Following a systemic reaction to venom, a skin prick test may be conducted depending on the patient's clinical condition and stability. Skin prick testing can provide rapid, cost-effective, and clinically valuable results. Typically, reaction diameters below 3 mm are considered negative in the literature. However, it is generally advisable to wait for a certain period after a systemic reaction before performing a skin prick test. This waiting period usually ranges from 4 to 6 weeks but may occasionally yield negative results for up to 6 months.

Beekeeping is prevalent in our region, with many patients being beekeepers or their children. Patients prefer to commence treatment as soon as they seek medical attention. However, in our study, skin prick tests were negative in 55% of patients upon initial presentation. Hence, our aim was to establish a new cutoff value for early-stage skin prick tests. In cases with a history of systemic reaction, a cutoff of 1.5 mm was accepted for both bee species in instances lasting 4 weeks or less, with a sensitivity of 67% and specificity of 50%. Conversely, a cutoff of 2.5 mm resulted in a sensitivity of 33% and specificity of 83% (AUC = 0.69; p = 0.36; 95%CI = 0.33-1.00). The absence of these sensitivities in healthy individuals underscores a limitation of our study.

In contemporary medical practice, component-resolved diagnosis (CRD) methodologies represent a significant advancement in bee venom immunotherapy, employing molecular diagnostic techniques. CRD facilitates the identification of specific IgE sensitivities to bee venom components, enabling treatment processes to be more targeted and personalized. Compared to traditional skin prick tests or total IgE tests, CRD more effectively distinguishes cross-reactivity situations and simplifies the management of patients with sensitivities to multiple venoms. This method allows for the development of specialized immunotherapy formulations for individuals sensitive to specific venom components.

The implementation of CRD aids in predicting the response to immunotherapy and reduces the need for potentially dangerous allergen skin tests, thereby enhancing safety for patients at high risk of severe allergic reactions. However, the widespread adoption of CRD is hampered by challenges such as high costs and limited accessibility. These challenges are particularly pronounced in healthcare systems with limited resources, inhibiting the broad utilization of CRD. Therefore, further research into the cost-effectiveness and accessibility of CRD is necessary. In our country, the access to these tests is still not at the desired level, which constitutes one of the limitations of our study. This shortfall highlights the importance of strategic planning and resource allocation for future advancements (18,19). In our study, we evaluated the relationship between basophil activation test, specific serum IgE , and skin prick test in the diagnosis of Hymenoptera venom allergy. We emphasize that each of these tests has its own significant advantages and limitations. We concluded that sIgE could be superior to BAT and SPT in terms of sensitivity and specificity in some cases. However, our study demonstrated that BAT could play a significant role, especially in situations of diagnostic uncertainty and in decisions regarding immunotherapy. We proposed that SPT is critical in determining early-stage reactions and in immunotherapy decisions, yet its cutoff values need reevaluation. Consequently, we believe the combined use of these three testing methods is important for a more comprehensive and accurate diagnosis of Hymenoptera venom allergy. This study can be considered a critical step in advancing diagnostic and treatment methodologies.

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Contributions

ŞİKK, AY: data curation, writing – original draft, writing – review & editing. ŞİKK, EB, EA: conceptualization, data curation, writing – original draft. ŞİKK, DÖ, ÖT, RS, AY: conceptualization, writing – original draft, writing – review &editing. ŞİKK, RS, AY: conceptualization.

Conflict of interests

The authors declare that they have no conflict of interests.

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LETTER TO THE EDITOR

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Nasal challenge with ketorolac: utility and safety in clinical practice

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Key words

Aspirin exacerbated respiratory disease; nasal ketorolac challenge; asthma; nasal polyps.

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To the Editor,

aspirin and non-steroidal anti-inflammatory drugs (NSAIDs)-exacerbated respiratory disease (AERD-NERD) is characterized by an underlying Th2 airway disease exacerbated by the intake of this type of medication. The nasal challenge test with NSAIDs, specifically with lysine acetylsalicylic acid (NLC) or ketorolac (NKC), is indicated for its diagnosis as an alternative to oral/bronchial challenges when FEV1 < 70% or with uncontrolled asthma (1-3). NKC is also used as a first step in aspirin desensitization protocols for AERD-NERD patients (4, 5).

NKC has lower sensitivity, specificity, positive predictive value, and negative predictive value compared to OAC (gold standard) (6). This makes it necessary to perform an OAC to confirm AERD-NERD diagnosis when NKC is negative (1-3). Although NKC is considered a safe technique, some authors have reported extranasal symptoms during its performance (6, 7).

To evaluate the diagnostic utility and safety outcomes, we analyzed 19 NKC (intranasal increasing doses of ketorolac every 30 minutes up to 16.38 mg) performed at our institution in AERD-NERD patients. Negative tests were followed by a 500mg OAC. This work was approved by the Ethics Committee of our institution (PI-2860) and all patients gave their written informed consent. Six NKC were negative (32%) (**table I**). Of the patients who reacted, 1 (7.7%) presented isolated bronchial symptoms (chest tightness and FEV_1 decrease ≥ 15%), 5 (38.5%) developed rhinitis (nasal discharge, nasal congestion, sneezing) and 4 (30.7%) presented bronchial symptoms and rhinitis (chest tightness, cough, nasal discharge, nasal congestion, sneezing). Furthermore, there were three patients (23.1%) who developed an anaphylactic reaction (generalized urticaria, palpebral angioedema, ear pruritus, chest tightness, cough, nasal discharge, nasal congestion, sneezing and conjunctivitis): two with a cumulative dose of 16.38 mg and one with 8.82 mg of ketorolac. No significant differences

Table I *- Demographics, clinical characteristics and NKC outcomes.*

n total = 19	Positive NKC (n = 13)	Negative NKC (n = 6)	P-value
Gender			0.630
Male	7	3	
Female	6	3	
Age (mean ± SD) (range)	45.62 ± 14.13 (25-64)	45.40 ± 17.85 (29-74)	0.979
Smoking habit (n,%)			0.837
Non-smoker	7 (54%)	3 (50%)	
Smoker	1 (8%)	1 (17%)	
Ex-smoker	5 (38%)	2 (33%)	
Baseline eosinophilia (median, IQR)	430 (230-830)	435 (110- 1130)	0.868
Total IgE (median, IQR)	204 (105- 1472)	508 (211-881)	0.374
Previous diagnosis (n,%)			
Rhinosinusitis	1 (8%)	1 (17%)	
Asthma and Rhinosinusitis	2 (15%)	0	
Asthma and polyps	1 (8%)	0	
Rhinosinusitis and polyps	9 (69%)	5 (83%)	
n sinus surgeries (mean ± SD)	1.67 ± 2.06 (non anaphylaxis) 3.43 ± 2 (anaphylaxis)	1.67 ± 2.25	
Actual treatment			0.689
None	0	1	
Corticosteroids + Montelukast	13	5	
Baseline PNIF (mean ± SD) (range) L/min	130 ± 40.4 (60-200)	108.33 ± 41.2 (90-200)	0.568
Baseline FEV_1 (mean ± SD) (range) mL	3,259.23 ± 1,035.75 (1,870-5,270)	3,526.67 ± 1,022.89 (2,050-4,860)	0.606
NKC outcomes			
Asthma	1	-	
Rhinitis	5	-	
Asthma and Rhinitis	4	-	
Anaphylaxis	3	-	

NKT: Nasal ketorolaco challenge; FEV_1: forced expiratory volume in 1 second; PNIF: peak nasal inspiratory flow.

were found between the 3 patients who suffered an anaphylactic reaction compared to the other 10 patients with a positive NKC. The 6 patients with negative NKC underwent an OCA and two of them presented a positive challenge with bronchial symptoms and urticaria, respectively.

There were 15 patients in our cohort with a confirmed diagnosis of AERD-NERD: 13 with a positive NKC (86%) and 2 with a negative NKC followed by a positive OCA. Extranasal symptoms appeared in 61.5% of patients (38% asthma, 23% anaphylaxis).

To analyze possible associations SAS 9.3 software (SAS, Institute, Cary, NC, USA) was used.

The study by White *et al.* (6) found that 17% of patients with positive NKC had a decrease in FEV1 > 15% and the study by Quiralte-Castillo *et al.* (7) 4/21 patients presented with asthma symptoms although just 1 showed a decrease in FEV1 > 15%. When combined with OCA to desensitize AERD-NERD patients, NKC breakthrough reactions were associated with bronchospasm in 24% (5) to 39% (4) of cases and with extrapulmonary symptoms (ana-

phylaxis) in 7% (5) to 28% (4). If clinical signs appeared during the nasal or oral challenge, they were not specified.

Miller *et al*. (8) reported that 21/100 of positive NLC had bronchial and nasal symptoms but only 2 had decreased FEV1 > 15%. Seven patients also had urticaria. In positive NLC, Alonso-Llamazares *et al*. (9) and Casdevall *et al*. (10) reported exclusively nasal symptoms.

Inflammatory mediators migrate from the nasal mucosa to the lower airways after nasal challenge, causing bronchial inflammation (3). NKC has been proposed as a safer diagnosis challenge for patients contraindicated to bronchial or oral challenges. Despite not being statistically significant probably because of sample size, our findings suggest the technique may not be as safe in daily clinical practice as previously reported due to significant bronchial and systemic breakthrough reactions.

Differences in populations, drug-delivery techniques, and/or monitoring techniques may explain the disparity in results. A nasal nebulizer spray cannot provide us with information about where ketorolac tromethamine is being applied or how much can reach the lower airways (4). Contrary to this, administering L-ASA by means of a dosimeter allows accurate measurement of the dose and monitoring of the effective inspiratory volume at each step of the bronchial challenge (1). For all these reasons, we question the NKC indication in patients with FEV1 < 70% or with uncontrolled asthma.

In conclusion, in our cohort, NKC with 16.38 mg is a useful method for AERD-NERD diagnosis combined with an oral challenge. However, safety concerns have to be considered.

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Contributions

All authors: conceptualization, data curation formal analysis, writing – original draft, writing – review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

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LETTER TO THE EDITOR

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Online food allergen labeling: is it a matter of concern?

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To the Editor,

food allergies are increasingly common and represent a significant public health concern. The main way to manage food allergies is to avoid the involved foods (1). In order to do that, food-allergic patients are often advised to rely on food products labels (2). Therefore, it is vital for food labels to clearly indicate allergens, ensuring the safety of consumers with allergies. The rise of e-commerce, accelerated by COVID-19, means more people are buying groceries online (3), highlighting the need for clear labeling in online stores to support safe shopping for those with allergies. The main objective of this cross-sectional study was to assess the online availability and compliance of food product labels, focusing on the identification of allergens.

Food labels from 230 products across four categories (bakery products (36.1%), breakfast cereals (28.3%), vegetable drinks (18.3%), and 40 commercially available complementary foods (CACFs) (17.4%)) were collected both on-site and online from 4 Portuguese grocery retailers/companies between February and March 2022. The information on the companies' web pages was analyzed and then compared with that on the physical label.

All physical products used as a basis for comparison had a label available and an indication of allergens, in accordance with Regulation (EU) No. 1169/2011.

We have identified that 32.6% (n = 75) of products had no label available or readable online. The food category exhibiting the highest label unavailability was bakery products (n = 38; 45.8%), followed by breakfast cereals (n = 21; 32.3%) and commercially available complementary foods (n = 9; 22.5%). Our results also showed that, despite legal provisions, 50.4% (n = 116) of the online products had no allergen identification or declaration in the label when compared to the physical product. Bakery products presented the lowest compliance (39.5%) while the highest compliance was found in CACFs category (65%).

For the products that effectively had allergen identification in the ingredient list, 14.7% (34) also presented an allergen decla-

ration in the end (as "contains X"). However, for the majority of these products (93%), the information contained in the allergen declaration was not in accordance with the list of ingredients. Concerning the precautionary labeling of food allergens (as "may contain traces of"), we also found that, when compared to the physical label, 64% of the online products did not present it. The results of our study reveal a concerning number of products with either unavailable or incomplete/unreadable online labels on websites that offer e-commerce options. Furthermore, we also report errors in allergen identification and/or declaration and discrepancies in the trace declaration between physical and online products, posing a potential threat to the safety and inclusion of consumers with food allergies.

Despite legislative obligations arising mainly from Reg. (EU) 1169/2011, inconsistencies in allergen labeling persist, highlighting the need for continuous monitoring and stricter enforcement to safeguard consumers with food allergies, especially on the online setting. Our findings align with the challenges reported in recent investigations for both allergen labelling (4, 5) and general food products online labelling (6, 7), although these studies do not address online allergen labeling. Then, our study provides a sample for a pioneering descriptive analysis in the European context considering the digital food environment for a consumer with food allergies, reinforcing the importance of monitoring labeling compliance and extending it to all products. Nevertheless, our study has some limitations, particularly with regard to the number and diversity of products analyzed. The results presented emphasize the need for effective compliance with labeling legislation and underscores the importance of collaborative efforts by regulatory bodies, manufacturers, and retailers to ensure the online accessibility and clarity of food labels. Addressing these issues is crucial to ensure the safety and well-being of consumers in the rapidly growing landscape of online food commerce.

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Contributions

AT: conceptualization, methodology, formal analysis, investigation, writing – original draft. FC, MFL, MP: conceptualization, methodology, formal analysis, investigation. IP: conceptualization, methodology, writing – review & editing, visualization, supervision.

Conflict of interests

The authors declare that they have no conflict of interests.

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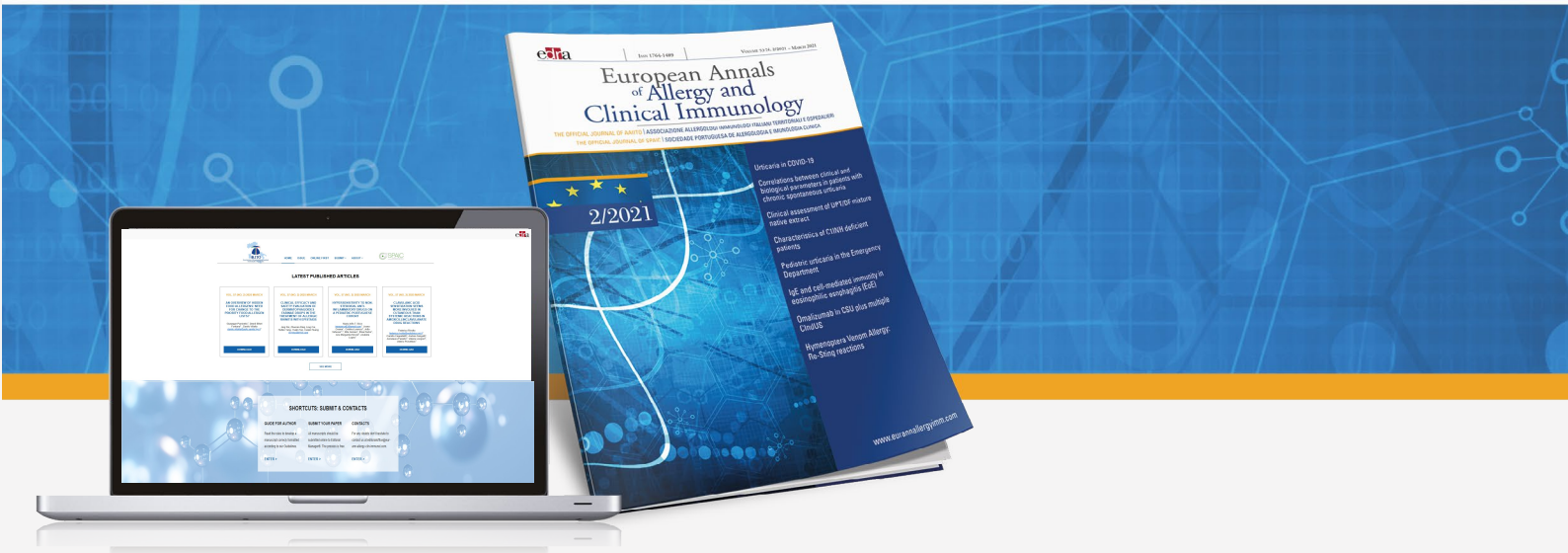

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