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Longitudinal patterns of allergic sensitization and its impact on allergic multimorbidities management

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Contents

List of Scientific Publications

This thesis is based on the following publications which are referred to in the text by Roman numerals:

I Farraia M, Mendes FC, Sokhatska O, Severo M, Cavaleiro Rufo J, Barros H, Moreira A. Sensitization trajectories to multiple allergen components in a population-based birth-cohort. *Pediatr Allergy Immunol*. 2023;34(6):e13963. doi: 10.1111/pai.13963.

II Farraia M, Mendes FC, Sokhatska O, Rama T, Severo M, Custovic A, Cavaleiro Rufo J, Barros H, Moreira A. Component-resolved diagnosis in childhood and prediction of asthma in early adolescence: a population-based birth cohort study. (Submitted to a scientific journal)

III Farraia M, Paciência I, Castro Mendes F, Cavaleiro Rufo J, Shamji M, Agache I, Moreira A. Allergen immunotherapy for asthma prevention: A systematic review and meta-analysis of randomized and non-randomized controlled studies. *Allergy*. 2022; 77(6):1719-1735. doi: 10.1111/all.15295.

IV Farraia M, Paciência I, Mendes FC, Cavaleiro Rufo J, Shamji MH, Agache I, Moreira A. Cost-effectiveness analysis of house dust mite allergen immunotherapy in children with allergic asthma. *Allergy*. 2022. doi: 10.1111/all.15321.

V Farraia M, Paciência I, Castro Mendes F, Cavaleiro Rufo J, Delgado L, Moreira A. Cost-effectiveness analysis of grass pollen specific immunotherapy in children with allergic rhinitis compared to the standard of care symptomatic treatment in Portugal. *Eur Ann Allergy Clin Immunol.* 2023;55(5):212-228. doi: 10.23822/EurAnnACI.1764-1489.240.

Abstract

Allergic diseases are a growing public health problem worldwide, significantly impacting patients' lives. Several factors contributed to this landscape, with a significant focus on environmental and lifestyle changes affecting individuals' allergic sensitization. Allergic sensitization is a decisive risk factor for several allergic multimorbidities, including asthma, rhinitis, atopic dermatitis, food allergy and anaphylaxis. Research has a significant focus on uncovering risk factors for the development of allergies and has given rise to the "biodiversity hypothesis", an approach focused on the One Health concept. One of the main mechanisms among the complex pathophysiology of allergic multimorbidities is related to immunoglobulin E (IgE) and intensified by exposure to allergens. However, allergen type and its allergenicity vary by geographic region and their impact on individuals' health. Therefore, understanding these differences and their effects on specific populations is critical to improving the management of these conditions and developing effective and appropriate health plans focused on the three levels of disease prevention. Ultimately, it may decrease the clinical and economic burden for patients, families and society.

This thesis aimed to identify the trajectories of allergic sensitization in childhood and to evaluate their impact on the development and management of allergic diseases, focusing on the prevention of allergy progression.

This work was based on different types of studies: observational, systematic review and meta-analysis, and economic evaluation. Data from the Generation XXI (G21) birth cohort was used to characterise childhood sensitization patterns, estimate their association with allergic diseases, and assess the performance of a personalized, predictive algorithm for asthma in early adolescence. The comprehensive systematic review and meta-analysis aimed to update knowledge about the preventive role of allergen immunotherapy (AIT) in the onset of asthma. Finally, economic evaluation studies compared the costs and health effects of allergen immunotherapy and standard-of-care treatment in children with allergic asthma and allergic rhinitis using a mathematical modelling approach.

About 40% of children in the G21 cohort were allergically sensitized by age ten years. Most children were sensitized to house dust mites (HDM), specifically to the Der p 23 component, followed by grass, animal, tree, and weed pollen. Mites are the leading cause of allergic sensitization in the North of Portugal, while in other European countries, birch and grass pollens are the major contributors. Five allergic trajectories were identified in a subsample of IgE-sensitized children, and different associations with allergic diseases were revealed, supporting clinically significant sensitization phenotypes and their impact on the risk of developing allergic disorders. The trajectory "early persistent HDM" was strongly related to asthma and allergic rhinitis, and "HDM and grass pollen", a broader trajectory characterized by multiple sensitizations

to individual components, was associated with allergic rhinitis only. The molecular characterization of allergic sensitization at 10 years helped predict asthma at 13 years old. Like other asthma risk scores, the addition of clinically relevant risk factors, such as early wheezing and a family history of allergy, improved the accuracy of the prediction model, and the sensitivity reached 75%, a superior performance compared to other published risk scores.

The meta-analysis suggested a preventive effect of AIT on the development of asthma. The preventive effect of AIT was remarkable in children, mono-sensitized patients, and when administered for at least three years, regardless of allergen and administration route. Lastly, AIT's cost-effectiveness in treating HDM-driven allergic asthma and grass pollen-driven allergic rhinitis, respectively, was evaluated using a Markov model for the Portuguese context. AIT, both for subcutaneous and sublingual administration, appears to be cost-effective in children with moderate HDM-driven allergic asthma. The core parameters that contributed to these results were the effects of AIT in reducing exacerbations and asthma medication. AIT also seems cost-effective in children with grass pollen-induced allergic rhinitis, despite the higher incremental cost-effectiveness ratio compared to the previous model focused on asthma treatment. The most relevant parameter in the model was the reduction of asthma-related costs due to the prevention of asthma cases. In both studies, sensitivity analysis demonstrated the robustness of the results.

With rising costs, the burden of allergic diseases is increasing for patients and society. Disease prevention is the ultimate goal of epidemiology and is essential for developing and evaluating public health-related policies. Altogether, this thesis provides further support and evidence to develop adequate health prevention plans by targeting children at high risk of developing allergic outcomes earlier. Allergic sensitization profiles differ by country and region and their role as a risk factor for the development of allergic diseases. Furthermore, it can be argued that secondary asthma prevention using AIT has the best chance of stopping the progression of allergic multimorbidities when prescribed early in life. Identifying high-risk children can be relevant to improving the targeting and cost-effectiveness of secondary asthma prevention using AIT. Finally, cost-effectiveness studies allow the comparison of strategies as health resources are limited, enhancing the choice of the most effective approaches for patients and health systems and being a unique tool to improve decisions in health.

Resumo

As doenças alérgicas são um problema crescente de saúde pública em todo o mundo, com um impacto significativo na vida dos doentes. Diversos fatores contribuem para este aumento, tais como as mudanças ambientais e de estilo de vida que, por sua vez, afetam a sensibilização alérgica dos indivíduos. A sensibilização alérgica é um fator de risco importante para a multimorbilidade alérgica, incluindo asma, rinite, dermatite atópica, alergia alimentar e anafilaxia. A investigação científica tem-se centrado, sobretudo, na identificação de fatores de risco para o desenvolvimento de alergias e contribuiu para desenvolver a "hipótese da biodiversidade", uma abordagem focada no conceito "One Health". Apesar da fisiopatologia complexa da multimorbidade alérgica, um dos principais mecanismos está relacionado com a imunoglobulina E (IgE), sendo intensificado pela exposição a alergénios. No entanto, o tipo de alergénio e a sua alergenicidade podem variar com a região geográfica, bem como o seu impacto na saúde dos indivíduos. Assim, entender estas diferenças e o seu impacto em determinadas populações é fundamental para melhorar a gestão destas doenças e desenvolver planos de saúde eficazes e adequados, focando-se nos três níveis de prevenção da doença. Em última análise, poderá contribuir para diminuir o impacto ao nível clínico e económico para os doentes, famílias e sociedade.

Esta tese teve como objetivo identificar as trajetórias de sensibilização alérgica durante a infância e avaliar o seu impacto no desenvolvimento e gestão da doença alérgica, com especial foco na prevenção da progressão da alergia.

Este trabalho baseou-se em diferentes tipos de estudos: observacional, revisão sistemática e meta-análise, e avaliação económica. Os dados da coorte de nascimento Geração XXI (G21) foram usados para caracterizar os padrões de sensibilização alérgica na infância, estimar a sua associação com doenças alérgicas, e avaliar o desempenho de um algoritmo preditivo e personalizado para o diagnóstico de asma no início da adolescência. A revisão sistemática e meta-análise teve como objetivo atualizar o conhecimento sobre o papel da imunoterapia específica (AIT) na prevenção de asma. Finalmente, os estudos de avaliação económica compararam os custos e efeitos na saúde da imunoterapia específica com alergénios e do tratamento padrão em crianças com asma alérgica e rinite alérgica, usando um modelo matemático.

Cerca de 40% das crianças da coorte G21 apresentaram sensibilização alérgica aos dez anos de idade. A maioria das crianças apresentava sensibilização aos ácaros da poeira doméstica (HDM), especificamente ao componente Der p 23, seguindo-se o pólen de gramíneas, animais, e pólen de árvores e plantas. Os ácaros são a principal causa de sensibilização alérgica no Norte de Portugal, enquanto nos outros países europeus o pólen de bétula e gramíneas são os principais contribuintes. Numa subamostra de crianças com sensibilização a alergénios foram identificadas cinco trajetórias alérgicas e diferentes associações com doenças alérgicas, suportando os fenótipos

de sensibilização alérgica clinicamente significativos e seu impacto no risco de desenvolver doenças alérgicas. A trajetória "early persistent HDM" foi relacionada com asma e rinite alérgica, e a trajetória "HDM and grass pollen", uma trajetória mais ampla e caracterizada por múltiplas sensibilizações a componentes alergénicos individuais, foi associada apenas com rinite alérgica. A caracterização molecular da sensibilização alérgica aos dez anos foi benéfica para prever o diagnóstico de asma aos 13 anos. Assim como outros índices preditivos de asma, a adição de fatores de risco clinicamente relevantes, como a sibilância precoce e a história familiar de alergia, melhorou a precisão do modelo preditivo e a sensibilidade atingiu 75%, um desempenho superior em comparação com outros índices publicados.

A meta-análise evidenciou um efeito preventivo da AIT no desenvolvimento de asma. Este efeito foi notável em crianças, doentes monossensibilizados, e quando administrada por pelo menos três anos, independentemente do alergénio e da via de administração. Por fim, a relação custo-efetividade da AIT para o tratamento de asma alérgica causada por HDM e rinite alérgica causada pelo pólen de gramíneas, foi avaliada usando um modelo de Markov tendo em conta o contexto português. A AIT, por administração subcutânea ou sublingual, demonstrou ser custo-efetiva em crianças com asma alérgica causada por HDM. Os principais parâmetros que contribuíram para estes resultados foram os efeitos da AIT na redução das exacerbações e da medicação para asma. A AIT também mostrou ser custo-efetiva em crianças com rinite alérgica induzida pelo pólen de gramíneas, apesar de ter evidenciado uma razão incremental de custo-efetividade maior quando comparada com o modelo anterior focado no tratamento da asma. O fator chave neste estudo foi a redução dos custos associados à asma uma vez que a prevenção de novos casos de asma foi considerada no modelo. Em ambos os estudos, a análise de sensibilidade reforçou a robustez dos resultados.

O impacto das doenças alérgicas tendo vindo a aumentar para os doentes e a sociedade, refletindo-se também em custos crescentes. A prevenção de doenças é o objetivo principal da epidemiologia e é importante para o desenvolvimento e avaliação de políticas públicas relacionadas com saúde. No geral, esta tese reforça e fornece evidência científica para o desenvolvimento de planos de prevenção de saúde adequados e direcionados a crianças com risco elevado de desenvolverem alergia. Os perfis de sensibilização alérgica diferem entre países e regiões, bem como o seu papel como fator de risco para o desenvolvimento de doenças alérgicas. Além disso, pode ser argumentado que a prevenção secundária da asma usando AIT tem a sua melhor oportunidade de interromper a progressão de multimorbidades alérgicas quando prescrita numa fase inicial. A identificação de crianças em risco de desenvolverem doenças alérgicas pode ser relevante para melhorar o direcionamento e o custo-efetividade da prevenção secundária de asma usando a AIT. Finalmente, os estudos de custo-efetividade permitem a comparação de diferentes estratégias uma vez que os recursos em saúde são limitados, melhorando a escolha das

abordagens mais eficazes para os doentes e sistemas de saúde, sendo uma ferramenta única para melhorar as decisões em saúde.

List of Figures

List of Tables

Abbreviations

AD	Atopic dermatitis
ademAPI	Asthma Detection and Monitoring API
AIT	Allergen immunotherapy
ALSPAC	Avon Longitudinal Study of Parents and Children
API	Asthma Predictive Index
APT	Asthma prediction tool
AQL-5D	Asthma quality of life utility index – 5 dimensions
AR	Allergic rhinitis
ARIA	Allergic Rhinitis and its Impact on Asthma
AUC	Area under the curve
BAMSE	Children, Allergy, Milieu, Stockholm, Epidemiology study
BIC	Bayesian information criteria
CAPS	Childhood asthma prevention study
CCD	Cross-reactive carbohydrate determinants
CDC	Centers for Disease Control and Prevention
CE	Cost-effectiveness
CEA	Cost-effectiveness analysis
CEAC	Cost-effectiveness acceptability curve
CI	Confidence interval
COPSAC	Copenhagen Prospective Studies on Asthma in Childhood
CRD	Component-resolved diagnostics
CUA	Cost-utility analysis
DALYs	Disease-adjusted life-years
DES	Discrete event simulation
DSA	Deterministic sensitivity analysis
EAACI	European Academy of Allergy and Clinical Immunology
EBC	Exhaled breath condensate
ED	Emergency department
FcεRI	High-affinity IgE receptor
G21	Generation XXI
GA^{2}LEN	Global Allergy and Asthma European Network
GBD	Global Burden of Disease study
GDP	Gross domestic product
GINA	Global Strategy for Asthma Management and Prevention of the Global Initiative of Asthma
GUSTO	Growing Up in Singapore Towards healthy Outcomes
GWAS	Genome-wide association studies
HDM	House dust mites
HTA	Health Technology Assessment
ICD-10	International Classification of Diseases - 10th Revision
ICER	Incremental cost-effectiveness ratio
ICS	Inhaled corticosteroid
ICS/LABA	Inhaled corticosteroid/Long-acting beta-agonists
IgX	Immunoglobulin (X refers to istotype: A, E, or G)

IL	Interleukin
ILC2	Induce type 2 innate lymphoid cells
IOW	Isle of Wight
ISAAC	International Study of Asthma and Allergies in Childhood
ISCED	International standard classification of education
ISU-E	ISAC standardized units
LC	Latent components
LCA	Latent Class Analysis
mAPI	modified Asthma Predictive Index
MAS	German Multicentre Allergy Study
MAAS	Manchester Asthma and Allergy Study
MeDALL	Mechanisms of the Development of Allergy study
MHC-II	Major histocompatibility complex class II molecules
NHS	National health service
NICE	National Institute for Health and Care Excellence
NK	Natural killer
NPV	Negative predictive value
NRSI	Non-randomized studies of interventions
OR	Odds ratio
PAPS	Persistent asthma predictive score
PARS	Paediatric Asthma Risk Score
PASTURE	Protection against allergy: Study in Rural Environments
PCA	Principal component analysis
PIAMA	Prevention and Incidence of Asthma and Mite Allergy
PLS	Partial Least Square
PPV	Positive predictive value
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analyses
PSA	Probabilistic sensitivity analysis
QALYs	Quality-adjusted life-years
RCT	Randomized controlled trial
RMSEP	Root mean square error of prediction
ROB	Risk of bias
RoB2	Revised Cochrane Risk of Bias tool
ROBINS-1	Risk of Bias tool for non-randomized studies of interventions
ROC	Receiver operating characteristic
RR	Risk ratio
RWE	Real world evidence
SCIT	Subcutaneous immunotherapy
sIgE	Allergen-specific immunoglobulin E
SES	Socioeconomic status
SLIT	Sublingual immunotherapy
SMD	Standardized mean differences
SOC	Standard-of-care
SPT	Skin prick test
TGF-β	Transforming growth factor β
Th	T helper
TNF-α	Tumour necrosis factor α
TSLP	Thymic stromal lymphopoietin

ucAPI	University of Connecticut Asthma Predictive Index
UK	United Kingdom
VAS	Visual analogue scale
VOCs	Volatile organic compounds
WHO	World Health Organization
WHS	World Health Survey
WTP	Willingness-to-pay

1. Introduction

Allergic diseases are recognized as a global public health problem due to their rapid increase in prevalence worldwide.[1] Also, allergic diseases usually develop during childhood and may persist for life, contributing to a cumulative decrease in quality of life and an increase in costs. The severity of allergic conditions can evolve but can also affect other organs and tissues since their coexistence is common and distinct profiles of allergic multimorbidities have been demonstrated.[2] Distinct mechanisms or endotypes may explain the same or different phenotypes and may also change throughout life.[3] This variability is underpinned by complex gene-environmental interactions driven by changes in lifestyle and environment. Understanding clinical and mechanistic profiles and trajectories of diseases, within and between individuals, will improve prevention, diagnosis, treatment, and prognosis.[3, 4]

Allergy is an "immune-mediated hypersensitivity" that can result from but is not limited to, immunoglobulin E (IgE)-mediated pathways.[4, 5] Similar to allergic diseases, the rates of allergic sensitization are also increasing, posing significant challenges for personalized medicine, as sensitization profiles differ from country to country and within regions in the same country.[6] Distinct patterns and trajectories of allergic sensitization have been associated with various allergic multimorbidities, and a deep understanding of the evolution of IgE pathways may identify high-risk subpopulations among sensitized patients.[7, 8] One of the significant recent developments in the field of allergy was the advent of molecular allergology.[9, 10] Nowadays, we can identify which specific molecules within the allergen an individual is sensitized using component-resolved diagnostics (CRD).[10] The immune response against particular molecules varies considerably and may partly explain the diversity of clinical responses or why some sensitized people do not develop any form of allergy.[4] CRD provides accurate, complete, and specific information that would be over-aggregated using traditional diagnostic methods but requires a high degree of expertise to interpret the results. However, incorporating CRD into everyday practice is a challenge, and additional research is needed to unlock its potential use in personalized medicine, specifically in the prevention, diagnosis and treatment of allergic diseases.[9]

All information provided by molecular allergology can also be used to improve specific treatments, such as allergen immunotherapy (AIT).[9, 11] AIT is the only disease-modifying treatment available for allergic asthma, allergic rhinoconjunctivitis, food allergy, and insect venom allergy.[12-17] Allergen avoidance measures have been ineffective in preventing and managing allergies, while adopting an immune resilience approach has been pointed as a better option, as demonstrated by the first national public health programme in Finland (2008-2018).[18, 19] The programme was based on practical advice and actions to improve immune resilience by endorsing health and immunity, and increasing connection with nature and biodiversity. AIT was also considered within this programme's scope as it can induce immune tolerance through the

gradual administration of a targeted allergen, expanding the potential of personalized interventions. However, some challenges persist, such as evaluating preventive effects in allergy progression, identifying patients who may benefit the most, and characterizing mechanisms involved and why some patients do not develop long-term tolerance.[9]

The rapid evolution of health technologies and medicines poses significant challenges to the access to innovation by the population. Healthcare innovation is infinite, but resources are finite, which demands high-quality evidence to prove and compare clinical efficacy, economic effects, and social benefits between different interventions.[20] AIT has been used for decades and has been part of the allergy field since its inception.[21] Developments in molecular biology and recombinant DNA technology have enabled improvements in the effectiveness and safety of AIT.[9, 21] However, generating evidence from an economic and social point of view is becoming crucial to ensure access to innovation. This evidence is essential to show the effects on healthcare resources, such as the number of clinical appointments, emergency department (ED) visits, and hospitalization episodes, in addition to the costs of symptomatic treatment and the new intervention.[9, 22] Finally, evaluating the effects on quality of life allows the comparison of different therapies, supporting policymakers in health decisions.[20]

Managing the progression of allergic diseases is a public health challenge. IgE-mediated mechanisms are an essential feature of allergic diseases that need further clarification. The advent of CRD has opened up new opportunities to study these mechanisms, and the potential is promising. The molecular characterization of populations, especially well-characterized people such as birth cohorts, will allow the identification of high-risk patients, improve diagnosis and treatment, and contribute to the design of better prevention programmes. This work aimed to characterize trajectories of allergic sensitization in childhood and evaluate their impact on the development and management of allergic diseases. Furthermore, we aimed to uncover the potential effects of AIT on secondary and tertiary prevention and generate economic evidence in the Portuguese context.

2. Literature Review

2.1. The importance of allergic disease in public health

2.1.1. Epidemiology and disease burden

Allergic diseases are a reaction of hypersensitivity, described as "objectively reproducible symptoms or signs initiated by exposure to a defined stimulus at a dose tolerated by a normal person", characterized by specific immunological mechanisms.[23] The term allergic disease includes allergic asthma, allergic rhinitis, atopic dermatitis, food allergy, and anaphylaxis, but it is not limited to these conditions. Allergic asthma is mainly characterized by recurrent or persistent symptoms in the airways such as wheezing, coughing, dyspnoea and chest tightness.[24] Symptoms of allergic rhinitis are related to the nose and include sneezing, itching, rhinorrhoea, or blocked nose. Allergic rhinitis is frequently accompanied by conjunctivitis with symptoms like itching and redness of the eyes and lachrymation.[25] Atopic dermatitis comprises symptoms such as red rashes, swelling, and itchy, dry scaly, leathery, crusting skin.[26]

Several epidemiological studies have been carried out to determine the prevalence of allergic diseases.[27-29] In 2019, asthma and atopic dermatitis were estimated to affect 262 million (95% uncertainty interval, 224 to 309 million) and 171 million (165 to 178 million) people worldwide, respectively, according to the Global Burden of Disease (GBD) study published by The Lancet.[1, 30] The percentage change in prevalence between 2010 and 2019 was 15.7% for asthma and 7.2% for atopic dermatitis. The same study revealed asthma was responsible for 21,6 million disease-adjusted life-years (DALYs), which represented 20.8% of total DALYs from chronic respiratory disease. Atopic dermatitis contributed for 7,48 million DALYs.[1, 30] This International Initiative did not consider data and estimates for allergic rhinitis. Nevertheless, the Allergic Rhinitis and its Impact on Asthma (ARIA) update by *Bousquet et al.* 2008[25] estimated a prevalence of 25%, ranging from 17% and 29%, for allergic rhinitis in Europe. The same group estimated that allergic rhinitis affects 500 million people worldwide.

A recent review reported an overall median prevalence of allergic rhinitis worldwide of 18%, ranging from 1% to 55% depending on the region.[31] Oceania presented the highest median prevalence (38%), followed by Europe (19%), Asia (15%) and America (9%). In Portugal, the estimated prevalence was 26% in individuals over 16 years of age according to a population-based cross-sectional study.[32] In Portuguese children aged 13-14 years, included in the International Study of Asthma and Allergies in Childhood (ISAAC) phase III (2001-2002), the prevalence of allergic rhinitis ever was 37% and allergic rhinitis in last 12 months was 27%.[29] The overall prevalence obtained in the ISAAC phase III for allergic rhinitis ever was 42% and in last 12 months was 33%.[29] The prevalence of allergic rhinitis varied between centres and regions

when results were compared to ISAAC phase I; in general, the prevalence of allergic rhinitis increased except in Oceania and Western Europe. In Portugal, the prevalence increased 1.06% per year.[29]

The prevalence of diagnosed asthma was evaluated in 2002-2003 by the World Health Organization (WHO) through the World Health Survey (WHS) and the results ranged from 1.8% in Vietnam to 32.8% in Australia, with an estimated overall mean of 6%.[33] In 2020, the Centers for Disease Control and Prevention (CDC) reported a 7.8% prevalence of asthma in the United States, based on the National Health Interview Survey.[34] In Europe, the prevalence of asthma in adults was estimated at 6.2% in 2018, but only five countries were included in the National Health and Wellness Survey.[35] In Portugal, the prevalence of current asthma reached 6.8% and 7.2% of adults and children, respectively.[36] The results of the ISAAC phase III showed the prevalence of asthma symptoms in 12.9% and 12.0% of Portuguese children aged 6-7 and 13-14 years, respectively.[37]

The worldwide prevalence of atopic dermatitis symptoms in children ranged from 1.4% to 22.3%, according to the ISAAC phase III study, with a mean estimate of 7.3% at 13-14 years of age.[37] In Europe, it is estimated to affect 10% to 25% of children and 1% to 10% of adults.[38, 39] Epidemiological data on atopic dermatitis in Portugal are scarce; two studies conducted in adults estimated a prevalence of atopic dermatitis ranging between 0.61% and 2.64%.[40, 41] From the ISAAC phase III study, the prevalence of atopic dermatitis-related symptoms at 6-7 years old and 13-14 years old was 9.7% and 5.1%, respectively.[37]

2.1.2. A new framework: allergic multimorbidities

Atopic dermatitis has been considered a risk factor for the development of other allergic conditions, such as asthma and allergic rhinitis, driving the concept of atopic march.[42] The atopic march is a model that proposes the sequential development of allergic conditions. Atopic dermatitis has been described as the first manifestation of the atopic march in infancy or early childhood, followed by asthma and allergic rhinitis in late childhood and/or adolescence.[42, 43] Food allergy can also precede respiratory allergies in some children and increase the risk of developing other allergic diseases.[44] This concept emerged because atopic dermatitis has been considered an important risk factor for developing asthma and allergic rhinitis in different cohort studies.[45-47] In Australia, the Tasmanian Longitudinal Health Study (TAHS) presented the association of childhood eczema and preadolescence asthma that was extended into middle age asthma.[47] IgE sensitization and type-2 inflammation have been considered a strong link between these allergic diseases as well as genetic factors.[48-51] Barrier impairment has been pointed as the cause of increased epicutaneous sensitization followed by airway sensitization, being considered a plausible link between atopic dermatitis and other allergic diseases.[51, 52] Nevertheless, despite some shared risk factors and mechanisms, the development of allergic diseases varies between

individuals as the majority of atopic children will not develop asthma, and other underlying mechanisms may be involved.[53] Therefore, the atopic march model may oversimplify the connection and mechanisms underlying allergic diseases as it focuses on the temporal progression of allergic symptoms from skin to airways and/or nose.[3, 54]

A multimorbidity framework has been suggested as a more appropriate approach to understanding the mechanisms and improving the management of allergic multimorbidities in which "no single condition holds priority over any of the co-occurring conditions from the perspective of the patient and the healthcare professional".[3, 55] Allergic diseases are complex, multifactorial disorders caused by various mechanisms that ultimately comprise multiple heterogeneous phenotypes.[3] The method suggested by Belgrave and colleagues[2] to define and assess the heterogeneity of clinical phenotypes was a Bayesian machine learning modelling framework. The study used two population-based birth-cohorts from United Kingdom, Avon Longitudinal Study of Parents and Children (ALSPAC) and Manchester Asthma and Allergy Study (MAAS), pooling data from 9,801 children at five follow-ups (1, 3, 5, 8, and 11 years of age). The results suggested a solution of eight latent classes classified as: no disease (51.3%), atopic march (3.1%), persistent eczema and wheeze (2.7%), persistent eczema with later-onset rhinitis (4.7%), persistent wheeze with later-onset rhinitis (5.7%), transient wheeze (7.7%), eczema only (15.3%), and rhinitis only (9.6%) (Figure 1).[2] Later, *Custovic et al*.[3] highlighted that having an allergic disorder may influence physicians to diagnose another disorder due to the concept of atopic march; thus, we must cautiously interpret longitudinal profiles in observational studies and account for some degree of misclassification. Recent studies carried out in different population-based cohorts demonstrated that atopic march is a phenotype present in a low proportion and other classes have emerged to explain the heterogeneity of phenotypes despite the variation in the number of optimal classes.[56-60] Specifically, *Kilanowski et al*.[60] explored and identified seven allergic disease trajectories up to adolescence in two German birth cohorts. The "multimorbid" trajectory was the most similar to the atopic march concept and only affected 9% of children with at least one allergic condition. *Haider et al*.[59] reported five allergic disease trajectories from infancy to early adulthood using Latent Markov modelling. In this study, only one in five participants with eczema transitioned to the "multimorbid" trajectory, the most similar to the atopic march progression. Therefore, most children with eczema did not evolve to any multimorbidity pattern.

In addition to other allergic and non-allergic mechanisms, IgE-sensitization has been considered an important hallmark and risk factor for the development of allergic conditions.[4, 61] The Mechanisms of the Development of Allergy (MeDALL) study showed the multimorbidity of asthma, rhinitis and eczema, and highlighted that almost 40% of those cases were attributed to allergic sensitization.[4] Therefore, type-2 inflammation has been considered an important immunological mechanism in allergic diseases, especially in children with allergic

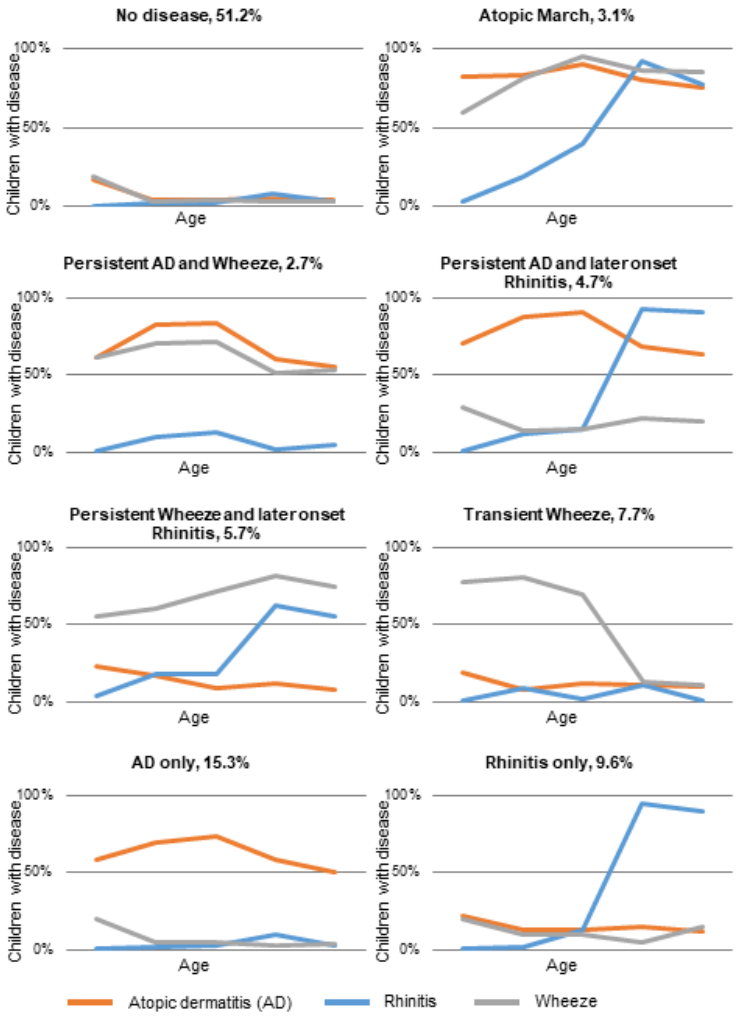

Figure 1 – Allergic disease profiles presented in *Belgrave et al. 2014* using Bayesian machine learning joint modelling of atopic dermatitis (AD), rhinitis and wheeze. Figure adapted from *Belgrave et al. 2014*

multimorbidities. Allergic multimorbidity might also be partially explained by shared genetic factors that are likely to disrupt immune mechanisms.[49] The previous work focused on unravelling distinct phenotypic trajectories using a Bayesian approach also showed differential genetic associations across the eight latent classes; namely, the filaggrin *locus,* which is a skin barrier protein responsible for compaction of keratin filaments and barrier formation, was positively associated with latent classes of atopic dermatitis symptoms and was even stronger among those with comorbid asthma and rhinitis.[2, 62]

The adoption of a multimorbidity framework emphasizes the complex and heterogeneous mechanisms underlying allergic diseases within and between individuals. The linear temporal progression of skin and airway/nasal symptoms is not a general framework capable of capturing the natural development of allergic diseases, as it occurs in a small fraction of individuals. Instead, allergic diseases may be characterized by several phenotypic groups. Different profiles of allergic diseases differ in genetics, sensitization, and lung function, highlighting the biological and clinical

relevance of latent classes.[58, 62] The mechanisms underlying these patterns are not fully elucidated, but this framework aims to disambiguate the complexity and interactions of allergic symptoms and diseases. Ultimately, it may facilitate the design of future studies and improve the development of personalized prevention and treatment strategies for allergic diseases.

2.1.3. Unmet needs in allergic health and care

The MeDALL study was created to improve the knowledge of the mechanisms of allergy onset by defining IgE-mediated phenotypes using unsupervised data-driven approaches to overcome the limitations of classical hypothesis-driven epidemiology.[63] This study demonstrated that allergen-specific IgE (sIgE) and non-allergic mechanisms may coexist in the same individual and that allergic multimorbidities can share common mechanisms partially mediated by sIgE in 40% of the cases.[4] The same study highlighted that many individuals developed sIgE sensitization without symptoms, but it is still unclear which individuals will remain asymptomatic.[64] Furthermore, it has been shown that classification of allergic sensitization as a single phenotype of atopy based on a positive sIgE response alone is not sufficient to explain the role of sIgE in the development of allergic diseases.[7, 64] Instead, recognizing the heterogeneity of sIgE sensitization allowed to identify distinct atopic vulnerabilities and a multiple early sensitization phenotype was able to predict asthma.[7]

Molecular allergology using component-resolved diagnostics (CRD), an allergen-specific IgE molecular test, was a major development in recent years and introduced the possibility of improving the characterization of allergic sensitization and partially explained clinical heterogeneity in allergy.[9, 10] Allergen extracts differ in composition between and within companies and do not allow the identification of cross-reactive components which may lead to a less precise diagnosis and management of disease.[10] CRD allow to overcome these limitations by enabling precise diagnosis and supporting personalized medicine, but is expensive and the results are difficult to interpret at the clinical level.[10, 65] CRD has many relevant advantages and implications, such as helping in the selection and targeting of allergen immunotherapy, monitoring allergy progression, identifying children at higher risk of developing allergic diseases, and discriminating between cross-reactive molecules and "true sensitization" markers compared to allergen extracts, allowing the discrimination of less clinically relevant allergens/components.[8, 10, 11, 66] Recent studies highlighted the importance of assessing allergic sensitization according to subtypes, based on the specific allergens to which a person is sensitized and time of onset, rather than using a single categorical variable (IgE-sensitized vs non-IgE sensitized).[8, 67] However, geographic location influences allergic sensitization profiles, compromising the generalization of patterns and trajectories; thus, the assessment of different populations remains an unmet need, as CRD is not widely used in birth cohorts or in clinical settings. Despite the increase in diagnostic specificity, the potential use of CRD continues to be explored due to the amount and complexity

of the data provided that requires an adequate interpretation; for example, expertise to distinguish genuine sensitization from sensitization due to cross-reactivity.[9, 68]

One of the urgent challenges is to identify which individuals are at higher risk for allergy progression to prescribe and design better targeted therapies or preventive actions/programmes. The atopic march does not explain the heterogeneity of allergic comorbidities, as it is estimated that only 7% of children with atopic dermatitis symptoms follow this trajectory.[2] Additionally, about 50% of children with atopic dermatitis do not develop other atopic airway diseases.[2] Therefore, efforts to design studies and prevention strategies focused on children with atopic dermatitis may be quite ineffective, and selecting children based on this hypothesis to integrate asthma prevention programs may also be inappropriate.[2, 3] Several predictive algorithms have been proposed to identify children at high risk of developing asthma, but their use is limited and the predictive performance needs to be optimized, as a large proportion of children cannot be appropriately classified.[69]

Finally, studies on immune tolerance mechanisms are rising and implementing good and effective allergy prevention measures and programs is becoming a priority in some countries.[70] The WHO defines primary, secondary and tertiary allergy prevention as the "prevention of immunological sensitization", "prevention of development of allergic disease following sensitization", and "treatment of asthma and allergic diseases", respectively.[71] However, identifying relevant preventive measures based on mechanisms of immune tolerance remains a challenge, as well as evaluating the cost-effectiveness of these preventive measures to bring innovation to real-life. Specifically, AIT has been used for more than a century in clinical practice to treat allergic diseases, but its role in allergy prevention remains unclear.[21, 72] In combination with molecular allergology, AIT holds great promise for precision and personalized medicine.[9]

Unmet needs in allergic diseases cover several aspects. There are essential gaps on the mechanisms, prevention, curative therapies, biomarkers and the necessity to guarantee equity in access to drugs and patient care.[73] The next steps include the improvement and use of molecular diagnostic methods to fill mechanistic gaps and improve the target of therapies and prevention strategies. It is also expected to uncover immune tolerance mechanisms to allergens and new ways to induce them for curative and/or prevention purposes.

2.2. Mechanisms of the development of allergy: lessons from birth cohorts

2.2.1. The role of type-2 inflammation in allergy pathogenesis

Allergy is an "immunologically-mediated and allergen-specific hypersensitivity" manifested in allergic diseases such as allergic asthma, allergic rhinitis, atopic dermatitis and others.[5] Allergic diseases are characterized by immune dysregulation typically driven by type-2 responses, chronic inflammation, and remodelling of affected tissues, namely, skin and mucous membranes.[74]

Airway and/or skin epithelial cells are the first line of protection against allergens, toxins, pollution, and infectious agents. The impairment of this barrier has been demonstrated in individuals with allergic diseases and the disruption of its integrity allows the exposure and penetration of allergens, toxins and other particles through the tissues.[75] Mutations in the genes of filaggrin have been studied and are considered relevant genetic determinants of atopic dermatitis and subsequent development of allergic sensitization.[76, 77]

Briefly, damaged epithelial cells release pro-inflammatory chemokines and cytokines, such as TSLP (thymic stromal lymphopoietin), IL-25 and IL-33 (interleukins, IL) that activate innate lymphoid cells 2 (ILC2) and dendritic cells, which in turn induce a T helper 2 (Th2) response characterized by the release of type 2 cytokines (IL-4, IL-5, IL-9, IL-13, and IL-31).[74] These immunological mechanisms can be divided into two phases: sensitization phase and effector phase (Figure 2).[5] Dendritic cells are antigen-presenting cells that uptake antigens on mucosal surfaces and migrate to draining lymph nodes where processed antigens are presented, via major histocompatibility complex class II molecules (MHC-II), to naïve $CD4^+$ T cells inducing activation, expansion, and polarization towards Th2 cells.[74] Dendritic cells located in the epithelia of the airways, gut and skin are strategically disposed to capture allergens. The migration of dendritic cells is amplified by IL-13 and TNF-α (tumour necrosis factor α) produced by ILC2 and mast cells. $CD4^+$ T cells can generate different Th2 cells, such as effector Th2 cells that return to

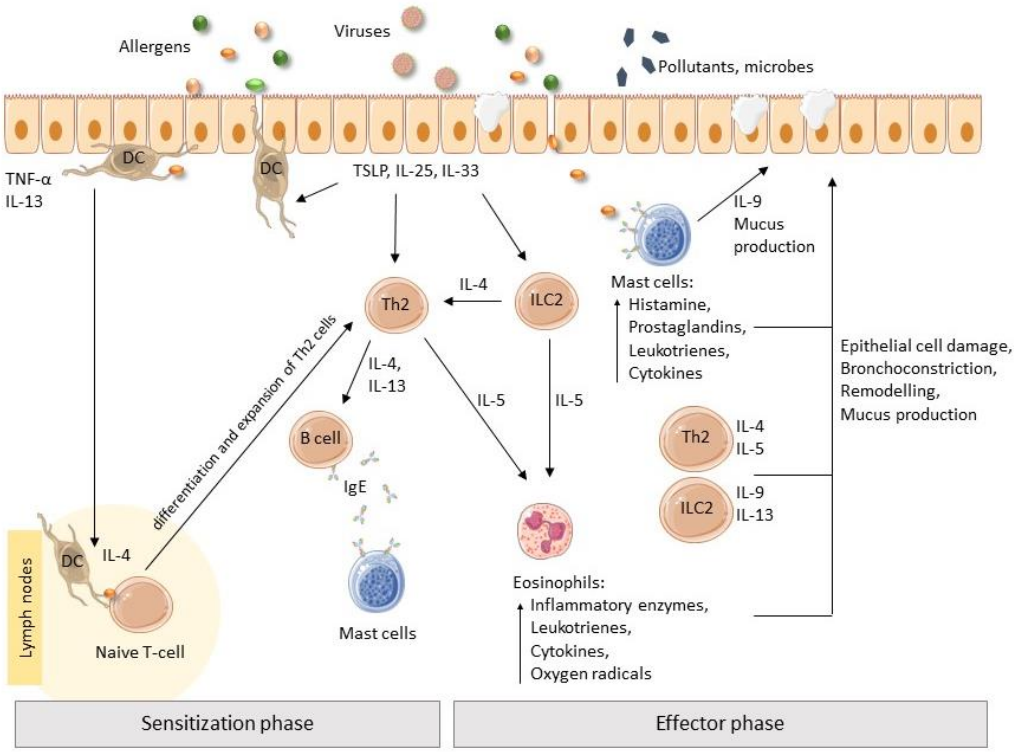

Figure 2 – Mechanisms of allergic mechanisms in asthma: sensitization and effector phases. Figure adapted from Akdis et al., 2020 and Global Atlas of Asthma. Parts of the figure were drawn by using pictures from Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

tissues, central memory Th2 ($Th2_{CM}$), and tissue-resident memory Th2 cells ($Th2_{RM}$).[74] These memory T cells are responsible for the immune memory that contributes to the chronicity of type-2 inflammation upon regular contact with that specific allergen.[5] IL-4 and IL13, produced by ILC2 and Th2 cells, are responsible for the IgE class-switching in B cells.[74] The sensitization phase is characterized by the development of memory T and B cells responses and IgE production. Allergen-specific IgE binds to mast cells through high-affinity IgE receptor (FcεRI) causing sensitization of the individual.

The effector phase is defined by tissue inflammation, injury and remodelling. Pro-inflammatory mediators such as histamine, leukotrienes, cytokines, proteases, and prostaglandins are released after a new encounter with the allergen through FcεRI in sensitized mast cells and basophils (immediate hypersensitivity reaction). Additionally, ILC2 and Th2 effector cells release type 2 cytokines, which are responsible for several effects in type-2 driven inflammation. IL-4 and IL-13 activate B-cells, induce IgE class-switching, proliferation and secretion, prolonging immunological memory and the allergic response. Both cytokines promote mucus secretion and mast cells activation. IL-4 is also responsible for Th2 cell differentiation. IL-5, which is mainly released by Th2 cells, stimulates eosinophils maturation, proliferation, and migration into the airways. Eosinophils produce, store, and release cytotoxic proteins, lipid mediators, and cytokines responsible for epithelial cell damage (barrier defects), bronchoconstriction, and tissue remodelling (fibrosis).

2.2.2. Longitudinal pathways of IgE sensitization and allergic multimorbidities

The immunoglobulin of isotype E was the fifth class of immunoglobulins in humans that was discovered and recognized by the WHO's International Reference Center in 1968.[78] Allergic sensitization defines the process by which a person becomes sensitized to an allergen. However, IgE sensitization does not cause allergic symptoms in all individuals, which means that a person can be sensitized without being allergic.[6]

Simpson et al.[7] demonstrated that allergic sensitization assessed as a dichotomous variable may over aggregate the underlying complexity of sensitization to multiple allergen components. The authors found that "multiple early sensitization" class was strongly associated with asthma compared to conventional definition of atopy, also demonstrating time of onset as a determining factor. The role of different dimensions of sIgE sensitization, such as time course, allergen specificity, and quantitative levels of sIgE, seems crucial to identify patterns of sensitization and its progression over time in relation to allergic diseases. A similar work has been published using allergen extract-based sIgE measurements techniques.[79-81] In a birth cohort representative of five cities in Germany (MAS, German Multicentre Allergy Study), *Hose et al.* 2017[79] identified five sensitization trajectories. The "severe atopy" class was associated with asthma, hay fever, eczema

and decreased lung function and was characterized by allergic sensitization to seasonal, food, and perennial allergens with an impressive increase of sensitized children before the age of five years. The results were replicated in a different cohort representative of rural areas from five European countries, including Austria, Finland, France, Germany, and Switzerland (PASTURE, Protection against allergy: Study in Rural Environments). The findings of the DIABIMMUNE study, which includes children from urban and suburban areas of Finland and Estonia, were similar to the MAS and PASTURE studies.[81] In a Danish clinical birth cohort (COPSAC) characterized by mothers with a history of asthma, the authors identified seven trajectories that were replicated in a Swedish population-based birth cohort (BAMSE, Children, Allergy, Milieu, Stockholm, Epidemiology study).[80] However, some minor differences between the two cohorts occurred when assessing associations with allergic outcomes. In the Danish cohort, sensitization to animals was associated with asthma, while rhinitis was related to sensitization against animal allergens, birch/grass pollen, molds or house dust mites (HDM). In the Swedish cohort, asthma and rhinitis were associated with almost all trajectories, except the one characterized by HDM.[80] These findings are summarized in Table 1.

The characterization of allergens at the molecular level introduced a new era of high-resolution molecular diagnostics.[10] *Hatzler et al*.[82] showed that early sIgE sensitization to timothy grass pollen can start before the first symptoms of seasonal allergic rhinitis and predict the future onset of disease in children from the German MAS cohort. The authors also explained the process of molecular spreading by verifying the increase in serum sIgE concentration and molecular complexity over the years. In the sensitization process, Phl p 1 was the initiating molecule in the majority of cases that was followed by sensitization to Phl p 4 and Phl p 5, then Phl p 2, Phl p 6 and Phl p 11, and finally Phl p 7 and Phl p 12. This process of molecular spreading was reinforced by other studies carried out in the MAAS cohort and was not limited to seasonal allergens.[8, 67, 83] Later, a broader spectrum of HDM components was tested and a "spreading" pattern was also observed as in grass pollen molecules.[67, 83] Participants on the broader sensitization stage were at higher risk of asthma.[67, 83] For HDM, sensitization spreading was presented as followed: Der p 2, Der p 1 and Der p 23 in the first stage, then Der p 5, Der p 7, Der p 4 and Der p 21, and finally Der p 11, Der p 18, Der p 14 and Der p 15, which only affected less than 10% of individuals.[83]

A recent work on the sIgE trajectories of molecular allergen components demonstrated that different patterns can predict specific allergic outcomes later on.[8, 67, 84] In the MAAS cohort, the number of sIgE clusters increased with age, showing the spread of allergic sensitization and distinct patterns of evolution in early and late childhood.[8] Sensitization to HDM was already established at three years of age and was consistent over time, while sensitization to grass/cat was relevant after five years of age. A previous work by *Custovic et al*. 2015[67] confirmed early

Table 1 – Summary of studies reporting the associations between allergen-specific IgE sensitization trajectories and allergic outcomes.

Study	Country	Participants	IgE test	Traj.	Results
Simpson et al. 2010.[7] MAAS cohort.	UK	1,053 children. sIgE assessed at 1, 3, 5, 8 years old.	SPT, Phadiatop	5	"Early multiple sensitization" had the strongest associations with asthma at 8 years old.
Garden et al. 2013.[85] CAPS cohort.	Australia	509 children. sIgE assessed at 1½, 3, 5, 8 years old.	SPT	4	All trajectories were associated with asthma, rhinitis and eczema at 8 years old.
Custovic et al. 2015.[67] MAAS cohort.	UK	235 children. sIgE of grass and HDM assessed at 5, 8, 11 years old.	ISAC	Grass: 3; HDM: 4	Grass: "early-onset" associated with asthma, "late-onset" associated with rhinitis at 11 years old. HDM: "complete sensitization" had the strongest association with asthma, rhinitis, eczema, and allergic multimorbidity.
Hose et al. 2017.[79] MAS cohort.	Germany	680 children. sIgE assessed at 1, 2, 3, 5, 6, years old.	Phadiatop	5	"Severe atopy", "seasonal", "mites" associated with lifetime asthma and hayfever at 6 years old. "Severe atopy" and "mites" associated with atopic dermatitis. Results were replicated in the PASTURE cohort.
Hose et al. 2017.[79] PASTURE cohort.	Austria, Switzerland, France, Germany, Finland	766 children. sIgE assessed at 1, 4½, 6 years old	Other*	5	"Severe atopy", "inhalant" associated with hayfever. "Severe atopy" associated with lifetime asthma and atopic dermatitis at 6 years old.
Schoos et al. 2017.[80] COPSAC 2000 cohort.	Denmark	398 children (familiar history of asthma). sIgE assessed at ½, 1½, 4, 6 years old.	Phadiatop	7	"Animals" associated with asthma; "animals", "HDM", "peanut/soybean" associated with rhinitis; "animals", "molds", and food clusters associated with atopic dermatitis, at 7 years old. Results were replicated in the BAMSE cohort.
Schoos et al. 2017.[80] BAMSE cohort.	Sweden	3,045 children. sIgE assessed at 4, 8 years old.	Phadiatop	7	"Animals", "grass" associated with asthma, rhinitis, atopic dermatitis. "Molds" and food clusters associated with asthma and rhinitis, at 8 years old.
Howard et al. 2018.[8] MAAS cohort.	UK	922 children. sIgE assessed at 1, 3, 5, 8,	ISAC	6	"HDM" associated with asthma at 16 years old. "Profilin" and "broad sensitization" associated with rhinitis.

		11, 16 years old.			
Schmidt et al. 2019.[81] DIABIMMUNE cohort	Finland, Estonia	2,289 children. sIgE assessed at 3, 5 years old.	Phadiatop	5	"Severe atopy" had the strongest association with wheeze, rhinitis, atopic dermatitis at 5 years old.
Gabet et al. 2019.[86] PARIS cohort.	France	1,080 children. sIgE assessed at 1½ and 8 years old.	Phadiatop	5	All trajectories were associated with asthma at 8/9 years old. The associations for rhinitis and eczema differed according to the trajectory.
Lau et al. 2022.[87] GUSTO cohort.	Singapore	997 children. sIgE assessed at 1½, 3, 5, 8 years old.	SPT	3	"Early food and mite sensitization" was associated with eczema and wheezing at 8 years old. "Late mite sensitization" was associated with eczema at age 8 years.

*Semiquantitative Allergy Screen test panel for atopy (Mediwiss Analytic, Moers, Germany). Serum specific-IgE tests included: Phadiatop (Phadiatop Infant ImmunoCAP™) and ISAC (ImmunoCAP™ ISAC). IgE: Immunoglobulin E; sIgE: specific-IgE; SPT: skin prick test; Traj.: number of trajectories.
BAMSE: Children, Allergy, Milieu, Stockholm, Epidemiology study; CAPS: The childhood Asthma Prevention Study; COPSAC 2000: Copenhagen Prospective Studies on Asthma in Childhood 2000; GUSTO: Growing Up in Singapore Towards healthy Outcomes; MAAS: Manchester Asthma and Allergy Study; MAS: German Multicentre Allergy Study; PASTURE: Protection against Allergy: Study in Rural Environments.

sensitization to HDM and highlighted that sensitization against a greater number of HDM components led to an increased risk of asthma and multimorbidity of asthma, rhinitis and atopic dermatitis. Finally, Der p 1 and Der p 23 at age five, two of the first HDM components that a person is sensitized, predicted the development of asthma at school age.[83] Recently, a French study reported four trajectories from childhood to adulthood.[84] Seasonal and animal allergens clustered together and were associated with rhinitis, while HDM cluster was associated with asthma, and a complex pattern composed of HDM, seasonal, and animal allergens was related to asthma, rhinitis, and decreased lung function.

The development of allergic sensitization and diseases usually occurs during childhood and can persist throughout life, causing a significant burden on the daily lives of patients.[88] Allergic sensitization varies greatly by age group and geographic region, including between and within countries, which can explain the different associations with the development of allergic conditions.[6, 89] Overall, assessing the heterogeneity of sIgE sensitization using CRD and considering its trajectories may improve clinical practice and explain the disparities in studies evaluating allergic sensitization as a single homogenous entity. Complex patterns comprising a higher number of molecules from different allergenic sources and early onset of sensitization were frequently associated with allergic multimorbidities. In combination with the clinical history and

symptomatology, these tests are useful to support the diagnosis and improve the management of allergic diseases.

2.2.3. Predictive scores of allergic diseases

The increasing prevalence of allergic diseases and multimorbidities requires the development of prediction models to identify high-risk groups and assist in effective primary, secondary, and tertiary prevention. Prediction scores combine data on risk factors into a single index, informing how likely an outcome may occurs.[90] Identifying children at high risk of developing allergic diseases is an important public health priority to decrease morbidity and health-related costs. These models have the potential to be applied in general populations or at the clinical level to target the individuals who will most benefit from allergy prevention therapies or programs, contributing to meaningful discussions about prognosis and, ultimately, to sustainable health systems. Many efforts have been made to predict asthma, food allergy, and atopic dermatitis as individual diseases and as multimorbidities, but the accuracy and validity of such instruments vary widely and clinical utility is still to be demonstrated.[91, 92]

Several indexes, presented in Table 2, have been proposed for the prediction of asthma based on characteristics or predictors that are easily evaluated in the clinical setting, such as the Asthma Predictive Index (API) and its variations, and the Paediatric Asthma Risk Score (PARS), which includes parental history of asthma/allergy, characteristics of wheezing, and diagnosis of eczema or rhinitis.[92, 93] The original API is currently the most used because it is simple to apply, less expensive, and was externally validated.[94] These models have relevant limitations due to poor sensitivity and positive predictive values.[93] Some of those models included allergic sensitization data assessed as a binary response using skin prick test (SPT) and the predictive accuracy increased compared to the original API, which is only based on symptoms and clinical history.[95, 96] The use of relevant allergen components may help to improve the definition of atopy and contribute to a better understanding of the development of asthma. However, the implementation and interpretation of CRD is complex and efforts are needed to simplify its use at the clinical level.[10] CRD has been studied to predict peanut, tree nut, cat, and dog allergies.[97-99] In these studies, the combination of molecular components demonstrated higher predictive accuracy compared to IgE extracts techniques. Predictive scores for the onset of atopic dermatitis and allergic rhinitis are limited compared to evidence to predict asthma and food allergy as described.[91, 100, 101]

The rapid developments in the -omics field have led to recent publications of scores including these complex data. The ademAPI is a modification of the original API that includes -omics data such as exhaled volatile organic compounds (VOCs) and gene expression (31 genes).[93, 102] The performance metrics for asthma prediction have greatly improved, but the possibility to be use in a clinical setting is compromised due to the expensive and complex data required to feed the

Table 2 – Summary of Asthma Prediction Tools published in the literature.

Study	Tool	Participants	Age*	Sens.	Spec.	Validation	Predictors
Castro-Rodríguez et al. 2000.[94]	API	1,246 children, from US general population	6, 8, 11, 13[+]	15%	97%	External (Switzerland, Norway, Colombia, UK)	Wheezing, parental history of asthma/allergy, atopic dermatitis, rhinitis, blood eosinophilia
Chang et al. 2013.[95]	mAPI	289 children, from a high-risk US population	6, 8, 11[++]	19%	99%	No	Wheezing, parental history of asthma, atopic dermatitis, blood eosinophilia, SPT
Amim et al. 2014.[96]	ucAPI	589 children, from a high-risk US population	7	44%	94%	No	Wheezing, parental history of asthma, atopic dermatitis, rhinitis, SPT
Klaassen, et al. 2015.[102]	ademAPI	202 children, from Dutch general population	6	88%	90%	Internal	Wheezing, parental history of asthma, atopic dermatitis, rhinitis, sIgE (Phadiatop), VOCs, gene expression
Kuruku-laaratchy, et al. 2003.[103]	IOW	1,034 children, from a high-risk UK population	10	53%	85%	No	Parental history of asthma/allergy, rhinitis, SPT, chest infections
Caundry et al. 2009.[104]	PIAMA	2,171 children, from a high-risk Dutch population	7, 8	60%	76%	External (Netherlands, Colombia)	Sex, wheezing, atopic dermatitis, chest infections, parental education and inhaled medication, post-term delivery
Vial Dupuy et al. 2011.[105]	PAPS	200 children, from a high-risk French population	6	42%	90%	External (France)	Familiar history of asthma, atopic dermatitis, polysensitization
Pescatore et al. 2014.[106]	APT	1,226 children, from a high-risk UK population	6, 8	72%	71%	Internal, External (Germany, UK)	Age, sex, wheezing, parental history of asthma/allergy, atopic dermatitis, rhinitis, dyspnoea, activity disturbance

Biagini-Myers, et al. 2019.[107]	PARS	762 children, from a high-risk US population	7	68%	77%	External (UK)	Wheezing, parental asthma, early eczema, race, SPT+ to ≥2 allergens

*Age considered for asthma prediction in years. [+]Sensitivity and specificity values reported in this table are for asthma prediction at 13 years old using the stringent index. [++]Sensitivity and specificity values reported in this table are for asthma prediction at 11 years old. ademAPI: Asthma Detection and Monitoring API; API: Asthma Predictive Index; APT: Asthma Prediction Tool; EBC: exhaled breath condensate; IOW: Isle of Wight; mAPI: modified API; PAPS: Persistent Asthma Predictive Score; PARS: Paediatric Asthma Risk Score; PIAMA: Prevention and Incidence of Asthma and Mite Allergy; Sens.: sensitivity; Spec.: specificity; SPT: skin prick test; ucAPI: University of Connecticut API; VOCs: volatile organic compounds.

model.[102] Atopic dermatitis has been studied in relation to genetic risk variants through genome-wide association studies (GWAS), especially since the discovery of filaggrin loss-of-function variants, and a polygenic risk score was recently published.[91] Despite the good performance of these models, clinical applicability in everyday life is difficult due the data and analysis required.

Recently, due to the availability of large real-world datasets through mobile platforms, machine learning can be used to explore the characteristics of patients and improve the prediction of allergic diseases by adding understanding of the natural history and progression of allergy-related symptoms.[101] However, the limitations of predictive scores remain despite the availability of large datasets and diverse predictors, including immunological biomarkers, allergen sensitization, genetics, and extensive clinical history. These models need to be reliable, simple, easy to use, and validated in different populations.[90]

2.3. A call to action: preventing allergic diseases

2.3.1. Immunological resilience and biodiversity for prevention

Allergic diseases are influenced by genetic determinants.[108, 109] However, genetics cannot be used alone to determine the individual asthma risk and does not explain the increased prevalence of allergic diseases.[5, 108] Changes in lifestyle factors, urbanization, and environmental triggers have promoted an increase in allergic diseases, especially among susceptible and genetically predisposed individuals.[5] The interaction between genes and environment plays a major role and epigenetic studies are increasingly common.[5, 110] Gene activity can be modified by DNA and histone methylation or by changes in microRNAs.[109, 110] These environmental-driven mechanisms can be inherited with each cell division and transmitted from parent to child, with a possible cumulative effect across generations.[5] Differential methylation in the DNA of new-borns has been associated with an increased risk of childhood asthma.[111] Pollution, diet, lifestyle, time spent

indoors, and hygiene habits may also drive these changes and more studies are needed to elucidate the role of external factors and epigenetic changes in the development of allergic diseases. [5, 110]

Outdoor air pollution, increased by industrial activities and traffic, is associated with respiratory diseases and explained by different mechanisms, such as epithelial barrier impairment, oxidative stress, DNA methylation, and immune dysfunction caused by pollutants (such as, sulfur dioxide, ground-level ozone, nitrogen oxides, particulate matter, VOCs, carbon monoxide, and others).[112] Exposure to tobacco and second-hand smoke at different times, including prenatal and postnatal exposure, has also been linked to asthma development[113] and a linear dose-response effect has been suggested based on data from eight European birth cohorts.[114] Poor indoor air quality also has harmful effects on respiratory health and is a relevant risk factor, as populations spend more time inside buildings, especially in developed countries.[115, 116] Chemicals and allergens may arise and increase in concentration inside buildings due to poor ventilation, construction problems and materials, dampness, dust, smoke, cleaning/disinfection products and indoor activities that promote the release of gases and VOCs.[115] Finally, changes in life-style and related to diet also impact respiratory health as a poor diversity of diet and consumption of less vegetables as well as an increased consumption of high fat, sugar and salt have been associated to an increase of airway inflammation and asthma.[117, 118]

Urbanization, climate change, pollution, and reduction of green spaces in developed countries have affected the macroscopic diversity of plants and animals, influencing the diversity of microbial species in the soil and air; consequently, exposure of humans to environmental microbial diversity has substantially decreased and compromised immune resilience.[112, 119] Immune resilience is the capacity of our body to recover and adapt to change by balancing useful and harmful response to maintain homeostasis and health.[18] The lack of immune resilience to respond to these changes can lead to disease due to immune dysfunction and impaired tolerance.[18] The "biodiversity hypothesis" explains the increase in allergic and inflammatory diseases due to the loss of macro- and micro-biodiversity, which is reflected in a poor biodiversity of the inner and outer layers of the microbiota in humans.[119] An enriched microbiome has important roles in the development and maintenance of barrier function, immune resilience and, ultimately, homeostasis.[18, 119] In urban populations, the decrease of contact with microbial diversity has been highlighted to be the main reason for the disruption of immune resilience.[18, 120, 121]

In parallel with the poor biodiversity in urban environments, the skin and airways of individuals are also deprived and less diverse compared to individuals in rural areas. [121, 122] The lack of diversity of the nasal, skin, and gut microbiota early in life has been related to increased allergy in children.[121, 122] Regular and more diversified exposure to natural green environments has been considered a protective factor, but there are still some inconsistencies in the literature.[123-125] The reasons may be related to the heterogeneity of the environments, as well as how, where, and when green spaces were evaluated.[126] In addition, other factors such as pollution, fungal

spores, endotoxins, soil microbial diversity, vegetation type and its variety, may play different roles and hinder positive or negative associations.[126] Still, regular contact with plants, animals, and soils appears to diversify human microbiota, but interventional studies are needed to test microbial exposure strategies in promoting a balanced immune regulation.[18]

The concept of "One Health" supports a "holistic view on the health of people, animals, plants, and our environment and recognizes that human health is closely related to a healthy state of the environment, vegetation, and animals".[126] Allergic diseases need to be addressed in light of this multidisciplinary concept when evaluating causes, prevention, and treatment strategies. The Finnish Allergy Program 2008-2018 was the first national prevention program that included a comprehensive plan based on the One Health concept aimed at reducing the burden of allergic diseases.[70] Table 3 presents the main goals, measures implemented, and results achieved at the end of the programme. Several actions supported by the concepts of immune resilience and biodiversity were carried out to improve primary and secondary prevention, specifically, increase connection with natural environments to strengthen immune regulation, shifting the paradigm from avoidance to tolerance.[70] The 10-year Finnish Allergy Programme resulted in the stabilization of the prevalence of allergic diseases and decrease in allergic symptoms.[19] The prevalence of food allergy in schools was reduced by at least 43% and occupational allergies by 45%. Asthma-related emergency department visits were reduced by 53% in children and, globally, healthcare and disability costs were reduced by 30%, exceeding the goal set at the beginning of the program and highlighting the potential global savings resulting from prevention.[19]

Table 3 – Summary of measures implemented in the Finnish Allergy Programme 2008-2018.[19, 70, 127]

Prevention	Measures	Goals	Results
Primary	• Support breastfeeding; • Increase contact with nature; • Use antibiotics only when necessary; • Avoid parental smoking; • Include probiotic bacteria in some food/preparations.	Prevent allergy; Improve immune tolerance; Improve allergy diagnostics; Reduce occupational allergies; Avoid severe allergies by improving treatment in time; Reduce costs associated with allergic diseases.	Steadiness of allergic rhinitis and asthma prevalence; Decrease by 43-65% of food allergy diets in day care and launch of new recommendations; Education and certification of allergy diagnostic centres; Decrease by 45% of occupational allergy; Decrease of asthma-related ED visits by 53% in children; Decrease by 30% of healthcare and disability costs.
Secondary and tertiary	• Promote physical exercise and healthy diets (Mediterranean or Baltic); • Include probiotics in food; • Early management and treatment of airways/skin inflammation; • Improve management of exacerbations; • AIT for more severe symptoms; • Avoid smoking.		

AIT: allergen immunotherapy; ED: emergency department.

In conclusion, despite the significant role of genetic determinants that predispose to the onset of allergic diseases, the effects are modest and likely to be explained by environmental exposures and lifestyle changes. Both the loss of biodiversity due to urbanization and the sedentary indoor lifestyle pattern contributed to microbial deprivation and dysbiosis, consequently, lack of immune resilience and increase of allergic and inflammatory diseases. Current prevention strategies are not effective, and Finnish experience has shown that a more comprehensive approach promoting immune tolerance and resilience is needed to effectively decrease the burden of allergic diseases.

2.3.2. The role of allergen immunotherapy

Allergen immunotherapy (AIT) was first introduced and described in 1911 using grass pollen extracts and since then many innovations have occurred to improve both efficacy and safety.[21, 128] Current pharmacotherapy interventions in patients with allergic diseases are only able to control and reduce allergic symptoms and are not effective in all patients.[24, 25] Consequently, AIT remains the only disease-modifying treatment available for some allergic diseases, demonstrating the capacity to reduce symptoms and medication use during and after therapy.[12] AIT consists on the gradual and regular administration of allergens in high doses for at least three years to maximize clinical effects by inducing immune tolerance. The most common routes of administration are subcutaneous injections (SCIT) and sublingual preparations such as tablets or drops (SLIT tablets or SLIT drops), but new routes are emerging, for example oral mucosal, epicutaneous, and intralymphatic, which may also target other IgE-mediated hypersensitivities in addition to aeroallergies while meeting patient expectations of tolerability, effectiveness, and adherence.[129, 130] The European Academy of Allergy and Clinical Immunology (EAACI) has published clinical guidelines recommending the use of AIT in allergic asthma driven by HDM, allergic rhinoconjunctivitis to seasonal and perennial allergens, IgE-mediated food allergy, and insect venom allergy.[12, 14-17]

In addition to symptomatic treatment, AIT is an effective long-term option because it can alter the natural course of the disease, inducing allergen-specific immune tolerance and suppressing allergic inflammation and tissue remodelling.[131, 132] Immune tolerance is a complex immunological process that requires the interaction of immune cells, tissues, and mediators to limit the reactions to allergens (Figure 3).[12] On the one hand, exposure to high doses of allergens as in AIT increases allergen-specific regulatory T and B cells (Treg and Breg) and decreases Th2 cells.[133, 134] Treg cells demonstrated prolonged suppressor functions due to the production of IL-10, IL-35 and TGF-β (transforming growth factor β) that were maintained after three years of subcutaneous AIT against HDM and correlated with improved clinical response.[133] Breg cells produce IL-10 in AIT responders promoting IgG4 release from B cells, improving inflammation and symptoms even after AIT end.[134] Regulatory natural killer (NK) cells and ILC2 cells may also have inflammation-inhibiting properties by releasing IL-10, but their role in AIT needs to be

addressed.[12] IL-10 has immunosuppressive properties on Th2 cells, inhibits the production of proinflammatory mediators, and induces IgG4 production in B cells.[135, 136] On the other hand, memory B cell responses are skewed from IgE to IgG4, which in turn reduces mast cell and basophil actions.[137, 138] IgG4 has neutralizing properties to inhibit basophil and mast cell responses mediated by allergen-specific IgE in patients with allergy, preventing the release of pro-inflammatory mediators.[139] Briefly, IgG4 isotype may act as a blocking antibody and compete with IgE for allergen binding, preventing the crosslinking of allergen-IgE complexes to FcεRI in mast cells and basophils and inhibiting their effects.[138, 140] IgG4 antibodies might also negatively regulate FcεRI signalling through low affinity IgG receptors, inhibiting the activation of mast cells and basophils.[141, 142] IgA may also play a role as it may interfere with allergen binding in mucosal surfaces and maintain treatment effects after therapy termination, especially in the sublingual route.[143, 144] The mechanisms of action involved are not well established and a blocking effect similar to IgG4 was not demonstrated, but induction of IL-10 release by monocytes was reported.[145, 146]

Several studies have demonstrated the clinical benefits of AIT, especially in adults, since studies in children are in a smaller proportion.[12] A meta-analysis demonstrated a significant decrease in standardized mean differences (SMD) of symptoms, medication, and combined symptom and medication scores among patients with allergic rhinoconjunctivitis under SLIT or SCIT, in the short-term.[147] Evidence for long-term effects was modest, but the number of studies

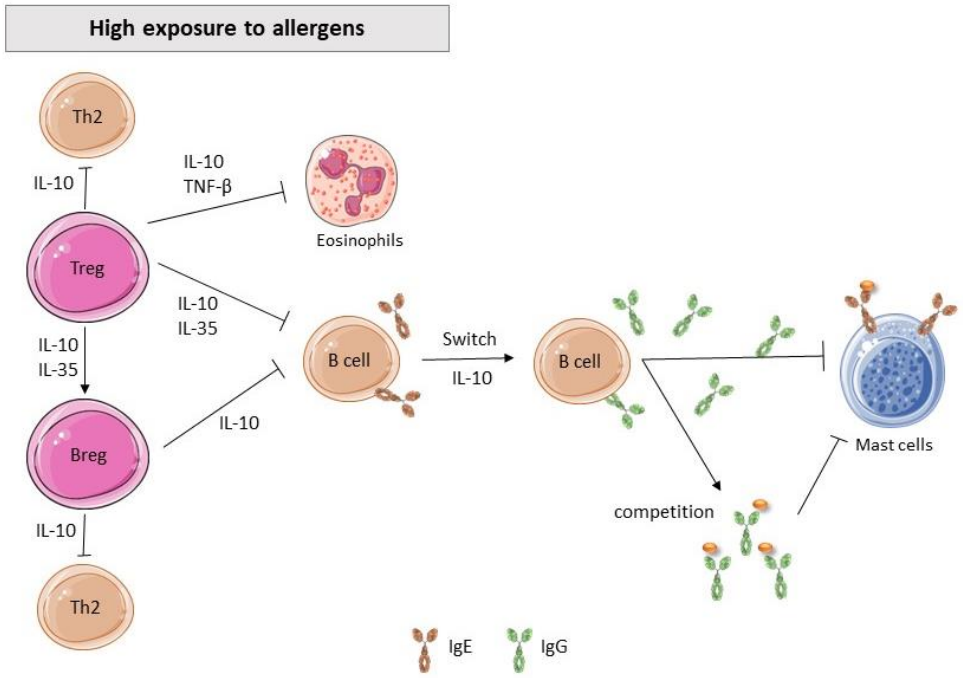

Figure 3 – Immune mechanisms of allergen immunotherapy, specifically, the role of Breg and Treg cells in the suppression of allergic inflammation. Figure adapted from Akdis et al., 2014 and Shamji et al., 2020. Parts of the figure were drawn by using pictures from Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.

was scarce. A similar effect on SMD of symptoms and medication was observed in patients with allergic asthma, but not for the combined score, in the short-term.[72] In this meta-analysis, only one study considered long-term beneficial effects. Overall, these meta-analyses have some limitations, such as the potential for publication bias, the heterogeneity of specific AIT products used in each individual study, and the lack of evidence of long-term effects.[72, 147] The mechanisms of immune tolerance mentioned above have been reported during and after SCIT and SLIT administration and include modulation of ILC2s, deletion of Th2 cells, deviation of type-2 inflammation toward type-1, and induction of Treg and Breg cells.[148]

Recent evidence has highlighted the importance of pregnancy and breastfeeding in protecting IgE sensitization in offspring.[139] IgG, including maternal IgG to inhalants and food allergens, and IgA antibodies can be transferred from the mother during pregnancy via the placenta or breast milk.[149, 150] Allergen-specific maternal IgG was associated with fewer allergic symptoms and may protect against sensitization in the offspring.[149, 150] An experimental study found IgG glycosylation to be an important factor when assessing mother-to-child IgG transmission as IgG glycosylation patterns induce different inflammatory effects.[151] A different study revealed that IgE may also be transported across the placenta via IgG anti-IgE/IgE immune complexes.[152] However, more research is needed to elucidate the mechanisms of sensitization in infancy and the role of maternal antibodies.

Despite remarkable advances in allergen immunotherapy, additional research is still needed at different levels. First, safety and effectiveness parameters can be improved. The rising of molecular allergology may improve the target of patients and AIT formulations to ensure personalized medicine by delivering the most suitable therapy to each patient according to their molecular sensitization profile.[10, 153] In addition, studies confirming that CRD may improve AIT clinical outcomes are limited, but they have demonstrated good insights.[153, 154] Stratification of patients with HDM allergy according to molecular allergen components and follow-up of IgG responses demonstrated AIT to be more effective in patients sensitized to Der p 1 and Der p 2 compared with patients sensitized to additional components.[153] However, the sample size was small and the allergen extracts administered were not analyzed for molecular components present in the formulation. Second, biomarkers research is still needed to evaluate the prognosis of AIT.[138] Despite the advances in mechanisms of efficacy and tolerance, this knowledge has not been translated into robust, reliable, and accessible biomarkers of AIT effectiveness.[155] Third, high-quality, long-term studies are needed to investigate the effects of AIT on maintaining immune tolerance and its potential role in preventing new sensitizations and allergic multimorbidity, especially asthma onset.[13, 132] Fourth, the access to these therapies needs to be addressed at regional, national and global levels; AIT is a long and expensive treatment that might not be available to all eligible patient.[12, 22, 156] Low adherence rates are mainly justified by the high costs of this therapy, which may increase the gap between socioeconomic groups.[157] However,

geographical differences are also relevant as access to these therapies requires contact with specialist allergy clinics, which are higher in bigger urban centres, but SLIT may overcome this problem.[158] Lastly, there are other barriers for AIT that should be noted as highlighted by the international consensus document of the International Collaboration in Asthma, Allergy and Immunology such as regulatory issues, standardization of allergenic products, and methodological concerns related to economic analyses.[22]

2.3.3. Health Technology Assessment as a tool to improve health systems

The rapid evolution of medicines and technologies to improve the quality of life of patients with different diseases requires a system capable of evaluating and comparing interventions to guarantee sustainable health systems, since resources are finite and innovation is infinite. Health technology assessment (HTA) refers to "the systematic and multidisciplinary evaluation of the properties of health technologies and interventions covering both their direct and indirect consequences to inform and guide on how these technologies can be used in health systems around the world", according to the WHO.[159] HTA aims to summarize evidence on different aspects of a given health technology, including medical, economic, social, and ethical issues, in a systematic, transparent and unbiased way to guide better health policies and reach the best value for health technologies.[160] The concept of HTA emerged in the 1960s to respond to a high demand of innovation in health technology and to ensure its introduction in the market in a sustainable, effective and competitive way, guaranteeing the broad public and private interests.[161] Health technologies are "any practical application of knowledge to improve or maintain individual and population health", comprising drugs, biologics, medical devices, surgical procedures, public health programs, and support/organizational systems for prevention, screening, diagnosis, treatment, rehabilitation or palliation purposes.[161] The main objective of HTA is to inform and guide policymaking for health technologies at the patient/individual, institutional, regional, and/or national levels, including direct and indirect consequences. [20, 161]

HTA requires multidisciplinary teams and the use of several methods to assess safety, efficacy, effectiveness, economics, and ethical/social issues to guarantee transparent, consistent, and robust evidence.[20] Primary data methods are useful for assessing the safety, efficacy and effectiveness of health technologies and include randomized controlled trials (RCTs), observational and other studies.[161] The quality and validity of these studies are critical for determining the value of health technology. Integrative or secondary data methods include the combination of data from primary sources such as systematic reviews, meta-analysis, modelling studies, and expert opinions.[161] Methods of economic analyses used in HTA can use data from primary and secondary methods and include different types, such as cost-of-illness analysis, cost-minimization analysis, cost-effectiveness analysis (CEA), cost-utility analysis (CUA), cost-benefit analysis, and budget-impact analysis.[161] The main difference between the previous

analyses is related to the valuation of outcomes as none, equal for both interventions, natural units, utilities, and monetary units.[161] There is great interest in economic analyses due to concerns about rising costs in health systems and pressure on policymakers to allocate resources. [162]

CEA and CUA are recognized approaches to estimate short- and long-term consequences on the costs and health outcomes of a specific intervention.[163] CEA compares costs with outcomes quantified in natural units, such as mortality, exacerbations, medication change, hospitalization, or emergency visits.[164] CUA compares costs with outcomes quantified in terms of utilities, such as quality-adjusted life-years (QALYs). QALYs are used to quantify, on a scale from zero to one, the health outcome in economic evaluations allowing comparisons between interventions as it can be applied in all models regardless of the type of interventions or diseases.[163] CEA and CUA studies are important to inform costs and health gains of an intervention, in a specific time horizon, reflecting the effects obtained in clinical trials and real-world studies.[164] The results provided by these studies allow to drive health decisions, such as the reimbursement of therapies, and to implement policies that contribute to improving patients' lives, reducing health inequalities regarding access and adherence to therapies.[157, 165]

QALYs are a summary measure of health that combine both quantity and quality of life and it was first introduced in 1970.[166] Therefore, QALYs are calculated by multiplying the length of time in the given health state and health-related quality of life that is measured as utilities.[167, 168] Utilities are a measure of preference obtained using preference-based instruments derived from generic (SF-6D and EQ-5D)[169, 170] or disease-specific (AQL-5D, for example)[171] quality of life questionnaires. The score of these preference-based questionnaires ranges between 0 (death) and 1 (perfect health) and is combined with survival data to obtain QALYs associated to a particular health state.[168] This measure of health allows the comparison and prioritization of health technologies within or between different clinical areas. However, some limitations and concerns have been raised, namely, ethical considerations, methodological issues, methodological assumptions, and disease specific considerations.[172] Disease-specific preference instruments have demonstrated to be more sensitive to changes of disease control or severity over time than generic instruments, but comparison of health technologies across different therapeutical areas may be compromised.[172-174]

Decision analytical modelling comprises decision trees and Markov models to assess cost-effectiveness of health technologies using multiple data sources to fed the model, handling different assumptions and uncertainty.[164, 175] These types of models use hypothetical cohorts to simulate what would happen in real-life; decision trees are unidirectional whereas in Markov models individuals can transition between health states (partially cyclic directed graphs).[163] Decision trees are used for transitory events; that is, when the effects of the treatment happen (or not) quickly and preferably once in a short period of time.[164] This type of model is composed of decision strategies, decision nodes, and outcomes nodes characterized by specific health and cost

effects, while input probabilities are assigned to each node to represent the natural course of disease in both interventions.[163] Markov models are most appropriate when health events occur one or more times and treatment has long-term effects.[164] These models are also useful when considering more than one health effect and for diseases that evolve over time. In this case, each intervention arm is characterized by Markov health states and changes happen over time according to distribution probabilities retrieved from the literature. Each health state is characterized by health effects and costs; specifically, in the CUA health effects are measured as QALYs.[164] However, Markov models are memoryless, which means that patient history is not retained and the risk profile is homogenous in time.[176] Discrete event simulation (DES) can overcome the previous limitations of Markov models, but this analysis is more complex and time-consuming, requiring more input data.[176]

The previous models can handle uncertainty in estimating the expected future cost and clinical results of a given treatment choice, contributing to transparent, reproducible, and robust analysis to inform decision making.[164] Uncertainty can derive from sampling variation, extrapolation and generalization of data, model structure, and methodological issues. Deterministic and probabilistic sensitivity analysis can handle uncertainty, as well as analysis of extremes and thresholds.[163, 164] Deterministic sensitivity analysis (DSA) is used to assess the uncertainty of each individual parameter in the model according to 95% confidence intervals or other measure of variance found in literature, resulting in the range of the incremental cost-effectiveness ratio (ICER) due to parameter uncertainty that can be graphically represented in a Tornado plot.[163] Probabilistic sensitivity analysis (PSA) is applied to assess how changes of several variables affect the ICER by using random values from the distribution of the parameters that are uncertain and Monte Carlo simulations. The results are presented in a cost effectiveness plane and cost-effectiveness acceptability curve that shows the probability of an intervention being cost-effective compared to the other for different threshold values.[163, 164] At the end, both health technologies are compared using the ICER, which is a relative measure calculated by dividing the difference between the cost by the difference between the health gains/effects.[175] The ICER is used by several HTA, regulatory and insurance agencies to make recommendations on a given health technology to be considered within the health system, namely, the National Institute for Health and Clinical Excellence (NICE), one of the most relevant and recognized agencies worldwide.[177] In this example, the ICER is compared to a cost-effectiveness threshold generally accepted of £20,000 to £30,000 during the appraisal process to establish if the new technology represents and efficient use of resources.[177]

Pharmacoeconomics is highly important in health policy making because it allows the comparison and analysis of the value of a particular health technology with another. HTA is an important tool for different stakeholders, including patients, health professionals, policy makers, industry, governments, healthcare institutions, investors, regulatory agencies, and society.[20] The

assessment of safety, therapeutic, economic and social benefits/risks is essential to make health decisions, assess the cost-opportunity of different interventions, build sustainable health systems, and guarantee the access of population to scientific innovation and, ultimately, improve quality of life. The implementation of health technologies, including medicines, devices, or public health programs, requires the best presentation of evidence provided by different sources in a transparent and robust way.

2.3.4. Challenges and opportunities

The diagnosis and treatment of allergic diseases is traditionally focused on symptom-based criteria and phenotypes, which are defined by the clinical characteristics of the disease without considering the underlying mechanisms.[3] A mechanism-based framework considering endotypes has been claimed to be essential to improve the management of allergic multimorbidities.[3] Furthermore, the concepts of regiotype and theratype emerged and are defined as the regional differences in allergens and environment, and the characterization of clinical responders to a specific therapy option, respectively.[12] These terms highlight the need to better describe patients by considering the underlying pathogenesis to prescribe adequate treatment, as well as to develop new and effective therapies, such as biologicals that target a specific disease-inducing molecule.[5] *Custovic et al*.[3] highlight "allergic airways disease" as an example of an endotype characterized by allergic sensitization (mechanism) that can be assessed through sIgE (biomarker) and treated by AIT (prescription).

Allergic diseases and sensitization have climbed in recent decades.[178, 179] The cause for this increase has been attributed to changes in environmental exposures and lifestyle.[180] However, the effects of environmental exposures have generated inconsistent results; namely, the effect of green spaces and air pollution on developing allergic sensitization.[123, 181] These disparities can be explained by differences in geographic location, time or method of exposure/outcome assessment, study participants, outcome definition, or even diet, which may modulate the effects of air pollution on asthma, but the definition and diagnostic accuracy of atopy also play a role in this phenomenon.[6, 7, 81, 182] The development of CRD techniques has been decisive to exploring the characteristics and evolution of IgE sensitization and identifying patterns and trajectories that bear different associations with allergic diseases.[8, 67, 79] CRD studies using birth-cohorts found distinct atopic vulnerabilities over time or to specific allergens instead of using a simple dichotomous variable to define atopy based on SPT or total sIgE. Therefore, the definition and diagnostic accuracy of atopy using CRD should be further studied, as well as the heterogeneity of allergic sensitization and its relevance to the development of allergic diseases, considering different populations because sensitization rates and allergens involved in allergic sensitization of individuals are different and vary by geographic region.[6]

Research focused on mechanisms of immune tolerance revealed important findings with clinical implications, such as the Finnish Allergy program 2008-2018 focused on strengthening immune tolerance instead of the traditional approach of allergen avoidance. This national program was revolutionary and other countries can adapt and implement a similar national health program according to each reality to maximize the health benefits. One of the measures suggested for secondary and tertiary prevention was the recommendation of AIT for more severe symptoms.[127] However, there are no biomarkers to identify ideal candidates for AIT and to assess the prognosis after initiation of AIT to optimize the use of healthcare resources. In Portugal as in other countries, AIT is an expensive therapy that is not reimbursed, causing high rates of nonadherence and health inequalities in the access to therapies.[157, 158, 165] The results provided by hypothetical economic models are essential to guide policy decisions and allow a transparent and robust assessment of health technologies to identify the cost-opportunity among different therapies and maximize the use and allocation of resources within health systems. HTA aims to optimize the rapid access to health technologies by eligible patients regardless of socioeconomic situation, minimizing health disparities.

These unmet needs can be overcome by (1) optimizing and developing research focused in prevention, biomarkers, and novel treatments; (2) increasing public awareness and knowledge, especially, on modifiable risk factors; (3) improving patient care and equity in access to specialists and treatments; (4) including allergy as a relevant domain in the political agenda and reinforce the importance of a national allergy programme encouraging health.[5] Finally, the "One Health" concept must be integrated in all the aforementioned aspects because is the key for equity and human sustainable development.

3. Aims of the thesis

The general aim of this thesis was to identify the trajectories of allergic sensitization in childhood and evaluate its impact on the development and management of allergic diseases, focusing on the prevention of allergy progression.

Study-specific aims:

(1) To identify and characterize sensitization trajectories of multiple allergen component-specific IgE in childhood and to evaluate the association with allergic diseases such as asthma, rhinitis, and atopic dermatitis. (**Study I**)

(2) To develop a clinical friendly prognostic tool for the onset of asthma in early adolescence. (**Study II**)

(3) To conduct a systematic review and meta-analysis on the efficacy of allergen immunotherapy to prevent the onset of asthma. (**Study III**)

(4) To evaluate the cost-effectiveness of allergen immunotherapy for allergic asthma (**Study IV**) and allergic rhinitis (**Study V**), from the perspective of the Portuguese healthcare system, using a mathematical modelling approach.

4. Material and methods

4.1. Study design and participants

This thesis was based on different types of studies: observational, systematic review and meta-analysis, and economic evaluation. **Studies I** and **II** used data of participants from the Generation XXI (G21) birth cohort to characterize childhood allergic sensitization trajectories and estimate their association with allergic diseases (**Study I**), and to assess the performance of a personalized predictive algorithm for asthma in early adolescence (**Study II**). The systematic review and meta-analysis (**Study III**) included RCTs and non-randomized studies of interventions (NRSI) and aimed to investigate the preventive role of AIT in the onset of asthma. Economic evaluation studies compared the costs and health effects of AIT plus standard-of-care (SOC) treatment and SOC alone in children with allergic asthma (**Study IV**) and allergic rhinitis (**Study V**) using a mathematical modelling approach.

4.1.1. Allergic sensitization trajectories and prediction of asthma (Study I, II)

Studies I and **II** used data of participants collected as part of the evaluations of G21 birth cohort. G21 birth cohort includes 8,495 mothers and 8,647 newborns from the Porto metropolitan area, in Northern Portugal. The recruitment took place between April 2005 and September 2006 in all five public maternity units, where 95% of the region's births occurred. During the hospital stay, women delivering live births with more than 23 gestational weeks were invited to participate in the G21 cohort study (92% of participation rate). All participants were invited to be re-evaluated at four (2009/11; N= 7,459), seven (2012/14; N= 6,889), ten (2015/17; N= 6,397), and 13 (2018/20; N= 4,640) years of age, corresponding to a participation of 86%, 80%, 74% and 54%, respectively. The assessment at age 13 was interrupted in March 2020 due to the COVID-19 pandemic, explaining the low participation in this follow-up (Figure 4). More details about the cohort were previously published.[183]

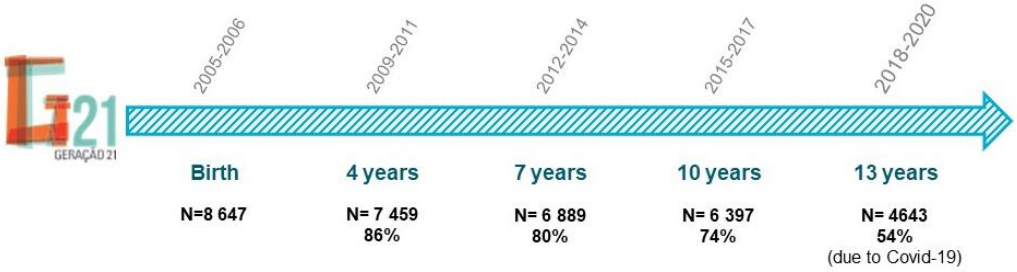

Figure 4 – Generation XXI birth-cohort. Follow-up visits after birth (2005-2006) and number/percentage of participants assessed in each visit.

During follow-up visits, participants completed a standardized questionnaire and were subjected to a physical examination by trained personnel, which included taking blood samples. Among 6,397 children evaluated at 10-years old follow-up, 4,047 participants had blood samples collected and 1,125 were randomly selected by a computer-generated randomization sequence and screened for measurement of sIgE levels using the ImmunoCAP Phadiatop Infant test. Of this subsample, 452 children (40%) were sIgE positive and 376 of them were further characterized using the ImmunoCAP™ ISAC test to identify sIgE antibodies against 112 aeroallergens and food components (**Study II**). ImmunoCAP ™ ISAC was also performed at 4- and 7-years old follow-ups, on the aforementioned sample of participants with available serum samples; at the end, 186 children had complete data for the ImmunoCAP ™ ISAC test at 4-, 7-, and 10-years old (**Study I** and **II**). The flowchart of participants included in both studies is presented in Figure 5.

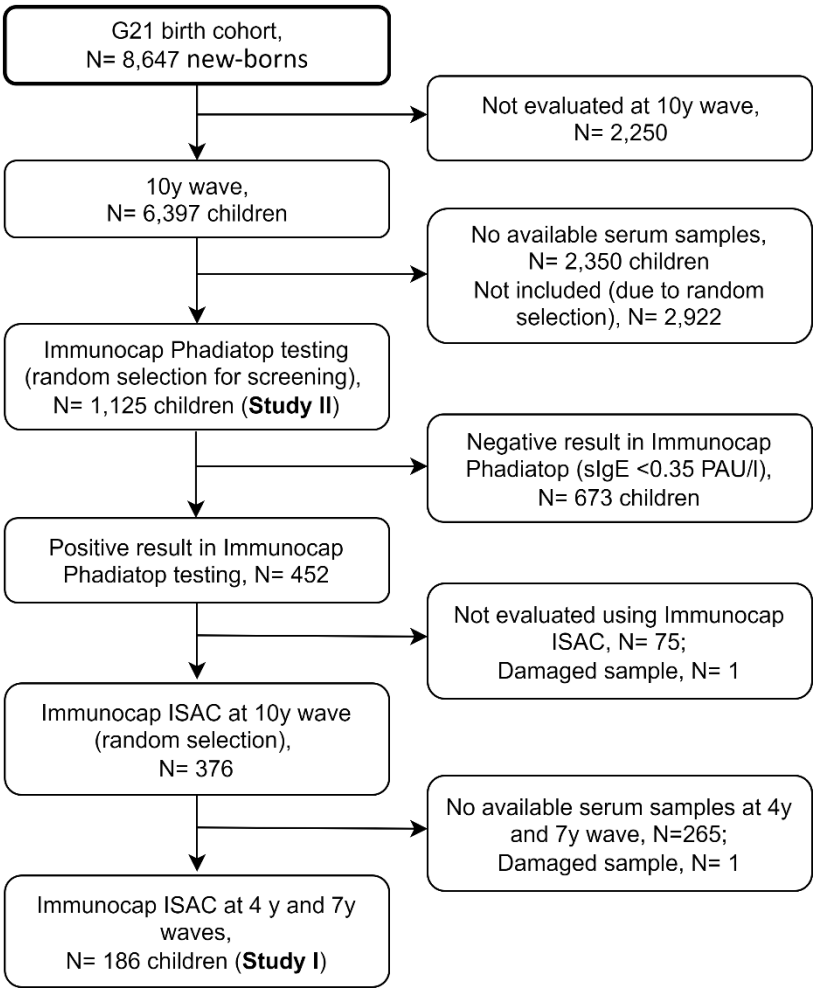

Figure 5 – Flowchart of participants of the G21 birth cohort included in Studies I and II. Participants with available serum samples at 10 years old were randomly selected for a screening test (ImmunoCAP Phadiatop) to assess IgE sensitization status. CRD using ImmunoCAP ISAC® test was performed in 376 participants that were positive in the screening test and in participants with available serum samples collected at 7- and 4-years old follow-ups.

4.1.2. AIT and prevention of asthma (Study III)

In **Study III,** original studies evaluating the effect of AIT on the development of asthma in healthy subjects or in subjects with other allergic diseases were assessed. A detailed description of the systematic review procedures is presented in the original article (chapter "Original Publications"). This systematic review was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) checklist 2020[184] and the Cochrane Handbook for Systematic Reviews of Interventions Version 6.1[185]. The systematic review protocol was registered under PROSPERO registration number: CRD42021251166.

The search was performed in Pubmed, Scopus and Cochrane databases using the following search terms: (immunotherapy OR vaccine OR allergy shot) AND asthma* AND (prevent* OR risk). Duplicates were removed, and two independent reviewers screened the search results based on the title and abstract. Two independent reviewers read the full text of the previous selected studies and those accomplishing the inclusion criteria were selected for data extraction. A manual search of the reference lists of primary original studies was performed to identify additional eligible studies. References were managed with EndNote X9 Software (Clarivate, Philadelphia, PA). The inclusion criteria were defined following the PICOS framework. Studies were included if conducted on subjects of any age, either healthy or with allergic conditions such as rhinitis, atopic dermatitis, or rhinoconjunctivitis. Studies including subjects with comorbid asthma were included if data were available for the outcome of interest in those without asthma. The intervention included any type of allergen administered as SLIT or SCIT. The comparator group included placebo, SOC or no intervention (control group). The outcome of interest was the development of asthma. Different asthma definitions were considered to guarantee a comprehensive analysis: asthma symptoms, asthma medication, asthma symptoms and medication, and medical diagnosis and/or following GINA guidelines. Both RCTs and NRSI were considered for inclusion and data extraction.

After abstract and full-text screening, the main characteristics of the included studies (study design, population characteristics, intervention, comparator, follow-up, baseline allergic conditions, asthma definition and outcome of interest) were extracted into a customized data extraction worksheet in Microsoft Excel by one reviewer. The revised Cochrane Risk of Bias tool (RoB2) for randomized trials[186] was used to assess the risk of bias (ROB) of the included RCTs. The ROB was judged as low, moderate, high or unclear for each domain: randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome, selection of the reported result and overall bias. The Risk of Bias tool for non-randomized studies of interventions (ROBINS-I)[187] was used to assess the ROB of the included observational studies. The domains included in this tool are divided according to the stage of the intervention: pre-intervention (bias due to confounding, bias in selection of participants into the study), at

intervention (bias in classification of interventions) and post-intervention (bias due to deviations from intended interventions, bias due to missing data, bias in measuring outcomes and bias in a selection of the reported result). The domains and the overall ROB were judged as low, moderate, high (included serious and critical judgements) or unclear. These assessments were performed independently by two reviewers and discussed if results differed across the evaluations. For the meta-analysis, the outcome of interest was extracted as the absolute number of subjects developing asthma. When not reported, this number was estimated if the percentage of subjects developing the outcome was available or by contacting the authors of the study. If none of the previous data were reported, the study was only considered for the qualitative synthesis.

4.1.3. Cost-effectiveness analysis of AIT (Study IV, V)

In **Studies IV** and **V,** a hypothetical Markov model was developed. **Study IV** evaluated the costs and health outcomes of AIT plus SOC in children with HDM-driven allergic asthma compared with SOC alone. The inclusion criteria of patients were a diagnosis of moderate-persistent asthma according to GINA guidelines[24] (corresponding to Step 3) and sensitization to HDM with clinically relevant symptoms. **Study V** evaluated the cost-effectiveness of AIT plus SOC in children with grass pollen-induced allergic rhinitis compared with SOC alone. The inclusion criteria included a diagnosis of moderate persistent allergic rhinitis eligible for AIT[188], and a positive SPT to grass pollen. In both studies, a Markov model was developed to compare three different strategies: SOC, SLIT tablets, and SCIT. All the input parameters (transition probabilities, costs, and other effectiveness parameters such as exacerbations) were retrieved from the literature and discussed with clinical experts. A detailed description of each model and parameters is described in the original articles (chapter "Original Publications").

The model structure adopted in **Study IV** used the GINA[24] stepwise approach to define health states reflecting differences in symptomatic treatment and was based on a previous cost analysis study conducted by *Parra-Padilla et al.*[189] Therefore, each health state represented a specific level of asthma severity or disease remission. Patients were allowed to move across five health states according to the intervention prescribed (SOC or AIT that could be SCIT or SLIT tablets according to each strategy): GINA Step 3 ("AIT+G3" or "SOC+G3"), all patients started at this health state in the first Markov cycle defined as a low dose of inhaled corticosteroid plus long-acting beta-agonists (ICS/LABA); GINA Step 4 ("AIT+G4" or "SOC+G4"), characterized as an increase in asthma severity requiring a medium dose of ICS/LABA; GINA Step 2 ("AIT+G2" or "SOC+G2"), described an improvement of asthma by ICS step-down protocol (low dose of ICS); remission of asthma ("AIT+R" or "SOC+R"), defined as the withdrawal of asthma SOC medication for the remaining time horizon; and any-cause death ("death"), every participant was at risk of death during all the time horizon independently of the health state (Figure 6). Each health state was characterized by specific costs and utility values.

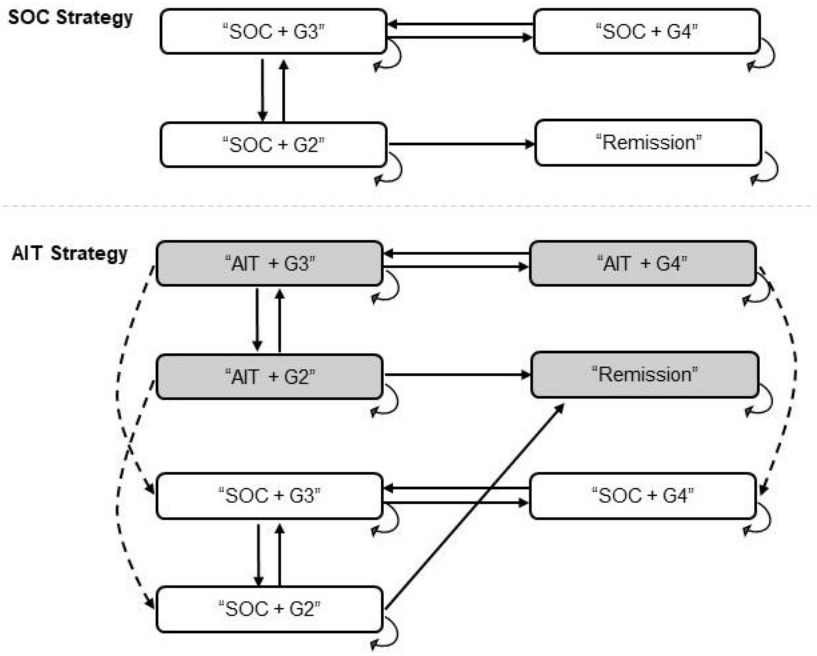

Figure 6 - Basic structure of the Markov model (Study IV). The risk of death is not shown (in order to simplify representation). Patients under AIT may discontinue treatment at any time (dashed arrows); at that time, patients follow the transitions and health states represented for the SOC arm. Patients achieving asthma remission do not return to any other health state (single arrow). AIT represents SCIT and SLIT strategies. AIT, allergen immunotherapy; Gx, GINA steps; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; SOC, standard-of-care treatment.

The Markov model simulated a hypothetical cohort of 12-year-old children, 1,000 per strategy, over a 10-year horizon divided into 6-month cycles, following the perspective of the Portuguese healthcare system. The cycle length was defined to represent the average number of medical appointments scheduled in a year and allow adjustments in asthma therapy like in real-life. AIT was ideally administered for 3 years as recommended [14], and in both SLIT and SCIT strategies, patients were allowed to discontinue AIT as in real-life [165]. The effectiveness of AIT was captured through changes in (a) health-related quality of life, quantitively measured as utilities; (b) asthma exacerbations, included as disutilities (negative scale, opposite of utilities); and (c) medication requirements, as patients moved along health states according to treatment-specific probabilities of step-up or step-down of the controller medication.

In **study V**, the Markov model was characterized by three health states according to each strategy (SOC or AIT that could be SCIT or SLIT tablets): allergic rhinitis ("SOC + AR" or "AIT + AR"), allergic rhinitis plus asthma ("SOC + AR + AA" or "AIT + AR + AA"), and any cause death ("death") (Figure 7). At inclusion, none of the children had a diagnosis of asthma, however, throughout the model, the probability of developing allergic asthma was considered and accounted as an effectiveness parameter. Based on literature findings, we assumed that AIT would generate a decrease in AR medication and symptoms, and a reduction of asthma onset when compared to the SOC strategy; thus, each health state was characterized by different utilities. AIT

was ideally administrated for three years, but we considered the possibility of therapy discontinuation as it happens in real world.[190] The Markov model simulated a hypothetical cohort of 8-year-old children, 1,000 per strategy, over a 10-year horizon divided into cycles of one year, following the perspective of the Portuguese healthcare system.

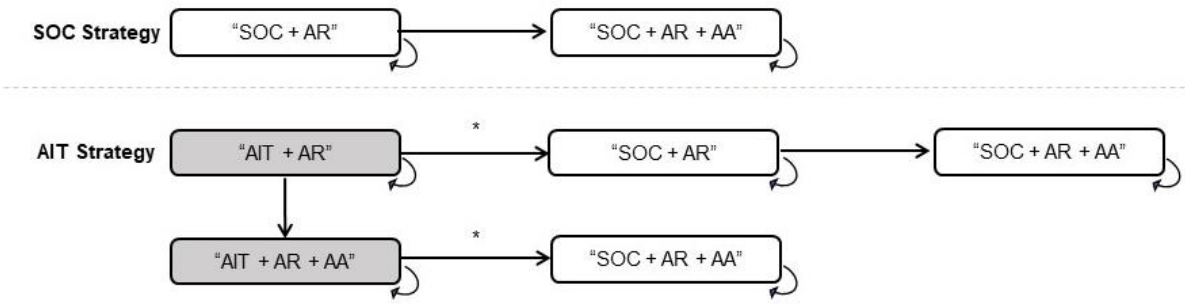

Figure 7 - Basic structure of the Markov model (Study V). The risk of death is not shown (in order to simplify representation). Patients underAIT may discontinue treatment at any time; at that time patients follow the transitions and health states represented for the SOC arm. The same happens when treatment ends (after 3 years). The previous situations are explained in the scheme with an asterisk (*). AIT represents SCIT and SLIT strategies. AA: allergic asthma, AIT: allergen immunotherapy, AR: allergic rhinitis, SOC: standard-of-care treatment, SCIT: subcutaneous immunotherapy, SLIT: sublingual immunotherapy.

4.2. Assessments

A summary of the study's assessments and instruments is presented in Table 4.

Table 4 – Summary of the study's assessments and instruments.

Assessment	Instrument	Age (years)	Study				Ref.
			I	II	IV	V	
Demographic							
SES	G21 questionnaire	birth	x	x			[191]
Allergic diseases (self-reporting of medical diagnosis)							
Parents (allergy)	G21 questionnaire	na	x	x			[191]
Children							
Asthma	G21 questionnaire, based on ISAAC standardized questionnaire	4, 7, 10, 13	x	x			[191, 192]
Rhinitis		4, 7, 13	x	x			
Eczema		4, 7, 13	x	x			
Allergic markers							
Screening sIgE	Phadiatop Infant, fx1 nut mix, and fx22 nut mix ImmunoCAP™ (Thermo Fisher Scientific, Uppsala, Sweden)*	10	x	x			[193]
Components sIgE	ImmunoCAP™ ISAC sIgE 112 (Thermo Fisher, Uppsala, Sweden)*	4, 7, 10	x	x			[194, 195]
QALYs							
Asthma	AQL-5D	12			x		[173, 196, 197]
Rhinitis	VAS	8				x	[198]
Costs of healthcare resources							
AIT	Pharmaceutical company	na			x	x	
SOC medication	Infarmed	na			x	x	[199]
Medical visits	Reference terms for the NHS (Portugal), 2020	na			x	x	[200]
SPT		na			x	x	
ED visit		na			x		
Hospitalization		na			x		

AIT: allergen immunotherapy, AQL-5D: Asthma Quality of Life Utility Index-5 Dimensions, ED: emergency department, G21: Generation XXI, na: not applicable, NHS: national health service, QALYs: quality-adjusted life-years, SES: socioeconomic status, SOC: standard-of-care, SPT: skin prick test, VAS: visual analogue scale. *According to manufacturer instructions

4.2.1. Demographic data and allergic outcomes (Study I, II)

Information on demographics (sex, age, socioeconomic status) and history of allergic disease of at least one of the parents were collected within 72h after delivery by a face-to-face interview using a standardized questionnaire.[201] All questionnaires of this project can be found in the G21 website (www.geracao21.com)[191]. Socioeconomic status was evaluated at birth using three different criteria, as it is suggested as a risk factor for allergic diseases.[202] Maternal education level was measured in years of schooling and categorized into two classes: primary (≤9 years of education, International Standard Classification of Education (ISCED) 2011 classes 0–2,

corresponding to the compulsory education in Portugal in the age cohort of the G21 cohort parents), and secondary/tertiary (>9 years, ISCED classes 3–6).[203, 204] Household crowding was measured as the ratio between the number of occupants and the number of rooms, and dichotomized as <1.5 or >1.5 occupants per room.[203, 204] The neighbourhood socioeconomic deprivation was assessed based on the weighted sum of standardized variables obtained from census data and was described in a previous study.[205] The index was categorized into quintiles; the first quintile corresponded to the least deprived and the fifth quintile to the most deprived category.

At each follow-up visit, the history of medical diagnosis of allergic conditions was completed by the participants' legal guardians, based on the ISAAC standardized questionnaire.[192] Asthma prevalence was defined as a positive answer to the question "*Has your child ever been diagnosed with asthma by a physician?*". Allergic rhinitis and eczema were defined as a positive answer to the questions "*Has your child ever been diagnosed with allergic rhinitis by a physician?*" and "*Has your child ever been diagnosed with eczema by a physician?*", respectively. The diagnosis of asthma was assessed at 4-, 7-, 10- and 13-years of age, while allergic rhinitis and eczema were evaluated at 4-, 7- and 13-years.

4.2.2. Screening and characterization of IgE sensitization (Study I, II)

During the 10-year follow-up, blood samples were collected following an overnight fast, centrifuged at 3,500 rpm for 10 min, aliquoted, analyzed or deep frozen (−80 °C) until analysis. Serum samples were tested for sIgE antibodies using the Phadiatop Infant ImmunoCAP™ (Thermo Fisher Scientific, Uppsala, Sweden) against hen's egg, cow's milk, peanut, shrimp, cat epithelium and dander, dog dander, house dust mite, common silver birch, timothy, ragweed, and wall pellitory (*Parietaria Judaica*). ImmunoCAP™ fx1 nut mix (peanut, hazel nut, Brazil nut, almond and coconut) and fx22 nut mix (pecan nut, cashew nut, pistachio and walnut) were also tested. Allergic sensitization was defined as an sIgE level of 0.35 (PAU/l) (Pharmacia Arbitrary Units per litre) or greater according to the manufacturer's instruction and were coded as positive in dichotomous analyses.[193]

The levels of 112 allergenic components from different allergen sources were assessed in serum samples (with sIgE≥0.35 PAU/l) using the multiplex array ImmunoCAP™ ISAC sIgE 112 (Thermo Fisher, Uppsala, Sweden). The results for each component were reported semi-quantitatively in ISAC standardized units (ISU-E) whenever the threshold was achieved (≥0.30 ISU-E).[194, 195] Allergenic components in this multiplex array encompass 48 allergen sources or species (28 aeroallergens, 19 food items, and latex), representing nine allergen groups (house dust mites, grass pollen, furry animals, tree pollen, weed pollen, fungi, cockroach, latex, and food). A detailed description of ImmunoCAP™ ISAC array is presented in the Annex 1.

4.2.3. Quality-adjusted life years and health outcomes (Study IV, V)

In **study IV**, the utilities were calculated from a study using the 5-dimension Asthma Quality of Life Questionnaire (AQL-5D) in adults.[173] However, utilities in children are generally lower.[198] Previous utilities were adjusted according to the methodology described by *Di Bona et al.*[206]; thus, a multiplicative function using the "perfect health" utility value for children, according to the AQL-5D questionnaire, was applied to the previous values (0.870±0.005).[196] Disutilities values for moderate and severe exacerbations were used based on the AQL-5D questionnaire.[197] QALYs was the outcome used to translate efficacy in health gains. QALYs for each GINA step were determined based on the corresponding utilities values and the subtraction of disutilities according to the probability of exacerbations[189]; disutilities due to moderate and severe exacerbations were considered in GINA steps 3 and 4 health-states while moderate exacerbations only were accounted for GINA step 2 health states.

In **study V**, QALYs were extrapolated from a study conducted in children with grass pollen rhinoconjunctivitis with or without well- to partially controlled allergic asthma.[198] The effect was assumed to be the same for SLIT and SCIT since we did not find specific data for children by the AIT administration route. Alternative QALYs values were retrieved from another study conducted in adults with a grass pollen allergy that stratified SLIT and SCIT effects on symptoms.[207] The authors applied the multi-attribute Rhinitis Symptom Utility Index (RSUI) to convert symptoms severity in utilities.[208] Symptoms severity was evaluated through the rhinoconjunctivitis total symptom scores (RTSS) reported in a meta-analysis.[209, 210] To adjust these data from a single condition, we further considered the patient's age and co-existing asthma utilities values to better describe our paediatric population using a multiplicative function.[207, 211] Thus, we used as reference a value for "perfect health" valid for children (0.960) and incorporated asthma comorbidity into utilities by attributing a utility of 0.737.[196, 207] These adjusted QALYs were applied in an alternative scenario analysis to strengthen the base case analysis.

4.2.4. Healthcare-related costs (Study IV, V)

In **study IV**, costs were defined for each health-state, per six months, to express differences in medication and healthcare resources used between strategies. The costs of asthma controller medication were calculated for each GINA step and according to the mean price of these drugs in the country; namely, for step 2 we considered a low dose of ICS while for steps 3 and 4 a combination of low or medium ICS/LABA, respectively.[24, 199] We assumed full-adherence to the symptomatic treatment in all strategies. We also considered costs related to moderate and severe asthma exacerbations according to the probability of ED visits and hospitalizations based on contracted values for Portuguese public hospitals.[200] The probability of ED visits and hospitalizations decreased by 74% and 65% for patients under SCIT and SLIT tablets strategies

in comparison to SOC.[212-214] One scheduled visit every six months and medical tests were considered for all strategies.[200] Acquisition prices of SCIT and SLIT tablets were retrieved from a pharmaceutical company; 255€ and 900€ for one year of intervention, respectively. SCIT strategy also included hospital administration costs for monthly injections.[200]

In **study V**, costs were defined for each health state, per year, expressing differences in medication and healthcare resources use between strategies. AR treatment followed ARIA recommendations[188] and the duration of the pollen season was estimated at four months (120 days).[215] We assumed full adherence to the AR symptomatic treatment in all strategies. The cost of symptomatic treatment was calculated based on total costs for four months; patients were under nasal corticosteroids and oral antihistamines.[188, 199] AIT costs for one year were based on the price list of a pharmaceutical company. In SCIT strategy, we considered administration costs as in **study IV**.[200] Asthma symptomatic medication costs were calculated according to the price of drugs in the country; patients were stratified by GINA guidelines in steps 2, 3, and 4 in equal proportions.[24, 199] We also considered costs related to asthma exacerbations as described in **study IV**. In all strategies, we considered two scheduled medical visits (including medical tests), per year, and SPT testing was considered only in SLIT and SCIT strategies, at the first visit. All costs are detailed in each corresponding manuscript (chapter "Original Publications").

4.4. Statistical Analysis (Study I – V)

The data analysis was performed using the IBM SPSS Statistics for Windows, Version 27 (IBM Corp, Armonk, NY, USA) (**study I and II**) and the RStudio Software versions 3.4.2 (**study III, IV and V**) and 4.1.3 (**study I and II**) (R Foundation for Statistical Computing, Vienna, Austria) with the following packages: "poLCA" in **study I**; "pls" and "proc" in **study II**; "meta" and "metafor" in **study III**; and "Heemod" in **studies IV** and **V**.

The baseline characteristics of G21 participants randomly selected for the screening test (N=1,125) and the remaining cohort were compared (**study I** and **II**) using chi-square tests for categorical variables and a P-value ≤0,05 was considered statistically significant. Comparisons of the characteristics of selected and non-selected children eligible for molecular characterization at the 10-year follow-up (**study II**) and the characteristics of sensitized children selected for the analysis of allergic sensitization trajectories (**study I**) were performed using chi-square tests. The prevalence of sIgE-sensitized children at 10 years of age was expressed as a percentage. The characteristics of sIgE-sensitized and non-sensitized children, namely, sex, socioeconomic status, family history of allergy, and diagnosis of allergic diseases were compared using chi-square tests. Quantitative results from the ImmunoCAP™ ISAC multiplex array were categorized according to the threshold and reported as a percentage, showing the proportion of positive results for each sIgE component among sIgE-sensitized children.

Latent class analysis (LCA) was used to identify clusters of individuals using component-specific IgE measurements to different allergens using of the data from the three follow-ups (**Study I**). Among the 112 allergenic components, 30 were never positive in any child at any time; therefore, 82 molecular components were included in this analysis. The optimal number of clusters or latent classes was defined based on Bayesian information criteria (BIC), starting from one class and increasing until the number of classes did not lead to a decrease in BIC. The rank of probabilities attributed to each allergen component, conditionally on class membership, was used to interpret and label the clusters (based on probabilities higher than 0.500). Children were assigned to each cluster according to the highest probability of class membership. At each follow-up, demographic and clinical characteristics were compared between clusters using Chi-square and Kruskal-Wallis tests for categorical and continuous variables, respectively. After the classification of the individuals in clusters at each follow-up, the number and proportion of transitions between clusters over time was obtained. Sensitization trajectories were determined based on the stabilization of individuals classified in the same cluster throughout the follow-up and according to the most prevalent transitions between clusters, regardless of the time at which they occurred. The trajectories were characterized and compared using Chi-square and Kruskal-Wallis tests, as appropriate. Associations between sensitization trajectories and allergic outcomes (asthma, allergic rhinitis, atopic dermatitis) at 13 years old were estimated by crude and adjusted odds ratios (OR) and respective 95% confidence interval (95% CI) using logistic regression models. Sex, parents' history of allergy, and maternal education were considered in the adjusted models.

The development of a predictive algorithm for asthma based on the molecular sensitization characteristics in childhood (**study II**) consisted of two steps: feature reduction through partial least squares (PLS) analysis and logistic regression for classification. Due to the large number of predictors at 10-years old (80 molecular allergen components were positive in at least one participant) as well as multicollinearity and multiple allergic outcomes, PLS was applied to reduce the dimensionality of correlated variables and to model the underlying information of those variables to find a dependence between predictors (molecular components) and outcomes (cumulative reporting of asthma, rhinitis, and eczema throughout childhood). The optimal number of latent components (LC) was selected based on the root mean square error of prediction (RMSEP) and R^2 metrics that were plotted into an elbow plot. Internal validation using leave-one-out method was applied to confirm the optimal number of LC. The PLS weights, percentage of explained variance and Y loadings were obtained for each LC. PLS weights equal/lower than -0.100 or equal/higher than 0.100 were identified as the variables which contributed the most to each LC. Logistic regression was fitted to estimate the associations between LCs and allergic outcomes at 13 years (asthma, rhinitis, and eczema) to reinforcing "y loading" results. PLS and regression analyses were applied independently to allergic predictors assessed at 4- and 7-years

old to check the consistency of the results. Other potential predictors used in previous asthma risk scores were considered; namely, parental history of allergy, early wheezing, and early eczema.[94, 107] Only statistically significant predictors were kept in the final model (p-value ≤0.05). The predictive ability of the model was calculated using Receiver Operating Characteristic (ROC) curve statistics.[216] Sensitivity and specificity were determined as the true positives divided by the total positives and the true negatives divided by the total negatives, respectively. Sensitivity and specificity were calculated at different thresholds and the optimal cut-off point was established using Youden's J statistic.[217] The area under the ROC curve [sensitivity/(1-specificity)] (AUC) was calculated as a measure of the predictive capacity of the model and acceptable discrimination was defined by an AUC of 0.70 to 0.79, good discrimination by an AUC of 0.80 to 0.89, and excellent discrimination by an AUC equal to or greater than 0.90.[216, 218]

In the systematic review (**study III**), the quantitative data from 18 studies were used to calculate a pooled estimate of the impact of AIT on asthma onset using random-effects meta-analysis. Event rate data were pooled as risk ratio (RR) with a 95% confidence interval using the Der Simonian-Laird estimator.[185, 219] The magnitude of heterogeneity between studies was estimated using the Higgins I^2 statistic, Cochran's Q, tau-squared and prediction interval.[185] Outlier and influential analyses were performed to demonstrate the robustness of the results. Publication bias was assessed by creating a funnel plot and using Egger's test.[220] The Thuval & Tweedie's trim-and-fill procedure was applied to estimate the effect size if missing small studies have been published.[221] Sensitivity analysis was performed by excluding studies classified as high ROB. Subgroup analyses were undertaken according to the type of study, AIT administration route, population, asthma definition, follow-up and therapy duration, type of outcome analysis, type of allergen, and history of sensitization.

The cost-effectiveness of AIT in children with HDM allergic asthma (**study IV**) and grass-pollen allergic rhinitis (**study V**) was established by calculating the ICER as incremental costs divided by incremental QALYs, assuming SOC strategy as the reference. Costs and QALYs were discounted at 3% per year as performed in previous studies.[163, 189, 222, 223] The cost-effectiveness threshold (CE threshold) was based on WHO recommendations as one to three times the gross domestic product (GDP) per capita of the country.[224] We considered the value corresponding to the lowest category suggested by WHO; thus, up to one time the GDP per capita.[224, 225] In Portugal, the GDP per capita in 2020 was set at 22,488,62USD (18,482,80€; conversion on June 7th, 2021). One-way deterministic sensitivity analysis (DSA) and probabilistic sensitivity analysis (PSA) were performed to assess uncertainty of the models. DSA was executed by varying the values of individual parameters while evaluating its impact on ICER, in comparison to the base case scenario, and summarized in a tornado diagram.[163, 164] PSA was performed by running 1,000 Monte Carlo simulations and graphically represented in a cost-effectiveness plane.[163, 164] These multiple repetitions of ICER calculations were drawn randomly according to the defined

distribution for utilities and transition probabilities parameters. Based on these results, a cost-effectiveness acceptability curve (CEAC) was created showing the probability of the intervention being cost-effective, compared to the symptomatic arm, for different values of willingness-to-pay (WTP).[164] Other possible scenarios were considered according to the circumstances that may happen in the real-world, to evaluate the robustness of the model and strengthen the recommendations provided by the base case results in **studies IV and V**. Some of the scenarios considered were related to AIT acquisition prices, time horizon, adherence rates, absence of AIT on asthma exacerbations or asthma remission, and others. The full description is available in the original articles.

4.5. Ethics

Ethical approval was obtained from the Ethics Committee of the University Hospital Center of São João (CES-01/2017) and signed informed consent was obtained from all participant's caregivers (**Study I** and **II**). All phases of the study followed the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki.

5. Results

5.1. Participants (Study I, II)

The characteristics of participants that were randomly selected for allergic sensitization screening and included in **studies I and II** are presented in Table 5. Except for maternal education and household occupation, there were no significant differences between the characteristics of the screened children (1,125) and the remaining participants of the cohort. The overall prevalence of asthma at 10 years of age was 8.3% and among screened children was 7.6%.

Table 5 - Characteristics of the included participants (selected for the allergic sensitization screening test) and the remaining participants of G21 cohort.

	Birth cohort	**Not screened**	**Screened**	**p-value***
N	8,647	7,522 (87.0%)	1,125 (13.0%)	
Sex				
Girls	4,237 (49.0%)	3,681 (48.9%)	556 (49.4%)	0.76
Boys	4,410 (51.0%)	3,841 (51.1%)	569 (50.6%)	
Type of birth				
Vaginal	4,269 (49.4%)	3,716 (49.4%)	553 (49.2%)	
Caesarean	3,154 (36.5%)	2,764 (36.7%)	390 (34.7%)	0.09
Other	1,224 (14.2%)	1,042 (13.9%)	182 (16.2%)	
Neighbourhood socioeconomic deprivation (distribution by quintiles, Q)				
Q1 (least deprived)	1,105 (12.8%)	957 (12.8%)	148 (13.2%)	
Q2	1,681 (19.5%)	1,455 (19.4%)	226 (20.1%)	
Q3	2,138 (24.8%)	1,858 (24.8%)	280 (24.9%)	0.18
Q4	1,924 (22.3%)	1,654 (22.1%)	270 (24.0%)	
Q5 (most deprived)	1,766 (20.5%)	1,565 (20.9%)	201 (17.9%)	
Household crowing (occupants per room)				
<1.5	6,868 (79.4%)	5,942 (79.0%)	926 (82.3%)	**0.04**
≥1.5	1,496 (17.3%)	1,327 (17.6%)	169 (15.0%)	
Maternal education				
≤9 years	4,253 (49.2%)	3,742 (49.7%)	511 (45.4%)	**<0.01**
>9 years	4,394 (50.8%)	3,780 (50.3%)	614 (54.6%)	
Health Outcomes				
10yo				
Asthma	530 (8.3%)	445 (8.5%)	85 (7.6%)	0.30
13yo				
Asthma	441 (9.6%)	355 (9.7%)	86 (9.1%)	0.53
Allergic rhinitis	557 (12.4%)	443 (12.4%)	114 (12.0%)	0.73
Atopic dermatitis	705 (15.4%)	573 (15.8%)	132 (13.9%)	0.16
Family history				
Allergy	906 (10.9%)	769 (10.6%)	137 (12.7%)	0.07

Data were presented as N (%). Bold values indicate a statistically significant difference. Yo: years old.
*Chi-square test.

Allergic sensitization affected 40.2% of screened children at the age of 10 years (N=452); specifically, 33.3% and 47.7% were girls and boys (p <0.01), respectively. Sensitized children at 10 years were more likely to be boys (59.1%), and to report asthma (14.4% at 10 years, 17.6% at

13 years) and rhinitis (21.6% at 13 years). No statistically significant differences were found for other baseline variables such as type of birth, socioeconomic factors, and history of allergy of the parents. In non-sensitized children, the prevalence of asthma reported at 10 years follow-up was 3.0%. Among IgE-sensitized children at age of 10 years, 83% were tested a second time with the ImmunoCAP™ ISAC array and considered in **study II,** completing a total of 1,049 participants when including non-sensitized children. Compared with remaining sensitized children (N=76), no statistically significant differences were found for demographic and clinical characteristics (Table 6). In **study I**, among sensitized participants at 10 years old, 186 had serum samples collected at 4 and 7 years that were analyzed using the ImmunoCAP™ ISAC test. Statistically significant differences were found for the characteristics of children with and without data available in the three follow-ups (Table 6). Children included in **Study I** presented a higher prevalence of allergic rhinitis at age 13 years.

Table 6 - Characteristics and differences of IgE-sensitized participants.

	ISAC testing (10yo)	**No ISAC testing (10yo)**	**ISAC testing (4, 7, 10yo)**	**No ISAC testing (4, 7, 10yo)**
N	**376 (83.2%)**	**76 (16.8%)**	**186 (41.2%)**	**266 (58.8%)**
Sex				
Boys	217 (57.7%)	50 (65.8%)	105 (56.5%)	104 (39.1%)
Type of birth				
Vaginal	181 (48.1%)	33 (43.4%)	90 (48.4%)	130 (48.9%)
Caesarean	136 (36.2%)	28 (36.8%)	67 (36.0%)	94 (35.3%)
Other	59 (15.7%)	15 (19.7%)	29 (15.6%)	42 (15.8%)
Neighbourhood socioeconomic deprivation (distribution by quintiles, Q)				
Q1 (least deprived)	53 (14.1%)	14 (18.4%)	25 (13.4%)	42 (15.8%)
Q2	70 (18.6%)	19 (25.0%)	37 (19.9%)	52 (19.5%)
Q3	81 (21.5%)	18 (23.7%)	34 (18.3%)	65 (24.4%)
Q4	101 (26.9%)	16 (21.1%)	55 (29.6%)	62 (23.3%)
Q5 (most deprived)	71 (18.9%)	9 (11.8%)	35 (18.8%)	45 (16.9%)
House occupation: number of household occupants/number of rooms				
<1.5	283 (82.0%)	85 (79.4%)	149 (81.4%)	219 (83.9%)
≥1.5	56 (16.2%)	20 (18.7%)	34 (18.6%)	42 (16.1%)
Maternal education				
≤9 years	174 (46.3%)	35 (46.1%)	87 (46.8%)	122 (45.9%)
>9 years	202 (53.7%)	41 (53.9%)	99 (53.2%)	144 (54.1%)
Health Outcomes				
Asthma, 10yo	54 (14.4%)	11 (14.5%)	32 (17.4%)	33 (12.4%)
Asthma, 13yo	55 (17.4%)	12 (18.8%)	30 (19.5%)	37 (16.4%)
Allergic rhinitis, 13yo	70 (22.2%)	12 (19.0%)	**42 (27.3%)**	**40 (17.5%)**
AD, 13yo	49 (15.5%)	10 (15.6%)	23 (14.9%)	36 (15.9%)
Family history				
Allergy	49 (13.7%)	15 (20.3%)	27 (15.2%)	37 (14.6%)

Data were presented as proportions, N (%). AD: atopic dermatitis; yo: years old.

Bold values indicate a statistically significant difference (P-value <0.05) and were calculated using chi-square test.

Among 376 children tested using a molecular approach (ImmunoCAP™ ISAC) at age 10 years, 6.9% were negative for all molecules tested (112 allergen components/molecules). Those who tested positive on the ImmunoCAP™ ISAC were sensitized to at least one aeroallergen and food sensitization reached 12.2%. Mite sensitization was the most common reaching 76.9% of sensitized children, with Der p 23 being the most prevalent allergen component (62.0%) (Annex 2). Grass pollen sensitization was the second most common, hitting 51.6% of sensitized children. Among these, 99% were sensitized to at least one component of timothy grass and nearly 60% were also sensitized to bermuda grass, a subtropical grass pollen. Phl p 1, a marker of "true sensitization" to grass pollen, was positive in 87% of sensitized children to grass pollen. Tree pollen sensitization affected 13.3% of children. As expected in Mediterranean countries, sensitization to olive tree pollen was the most common among the seven tree species evaluated in this multiplex assay. These results should be interpreted with cautious since IgE reactivity due to cross-reactive carbohydrate determinants (CCD), represented in this array by the MUXF3 molecule, was present in 28 children (7.5%). Weed pollen sensitization was positive in 45 children (12.0%). Sensitization to furry animals reached 27.7%; cat (23.7%) was the most predominant, followed by dog (10.4%), and horse (1.3%). The most relevant animal allergen component was Fel d 1 (22.6%). Allergic sensitization to fungi and cockroaches reached 8.5% and 3.5%, respectively. Sensitization against food components was found in 46 children (12.2%) and the most common were shrimp (5.3%), kiwi (4.8%), Herring worm (2.9%), soy bean (1.3%), and peach (1.3%). These descriptive results using ImmunoCAP™ ISAC are documented in Annex 2.

5.2. Trajectories of allergic sensitization (Study I)

In **study I**, LCA clustered participants into three classes, each one characterized by distinct weights attributed to allergen components considered in the analysis. The average probability of a participant belonging to class 1 was 99.3%, class 2 was 96.5%, and class 3 was 99.3%. Based on the most relevant allergen components comprising each class (considering probabilities ≥0.5), the first class was labelled as "few or no sensitizations", the second as "grass pollen", and the third as "HDM" (Table 7). The proportion of participants within each class at different ages is shown in Table 8 and Figure 8. At 4 years old, 64.5% of children was classified in class 1, 2.7% in class 2, and 32.8% in class 3. The proportion of children who moved from class 1 (at 4 years old) to class 3 and class 2 (at 7 years old) was 19.2% and 9.2%, respectively. At the same follow-up, the proportion of children who moved from class 3 to class 2 was 9.8%, and from class 3 to class 1 was 3.3%. At 7 years old, the proportion of children classified in class 1 who moved to class 3 and 2, at 10 years old, was 37.5% and 14.8%, respectively. The proportion of children who moved from class 3 to class 2 was 7.9% and from class 2 to class 3 was 4.5%. The proportion of children who moved from class 3 or class 2 to class 1 was 1.3% and 4.5%, respectively. Therefore, with increasing age, there was a higher proportion of children moving into class 3

("HDM") followed by class 2 ("grass pollen"). The proportion of rhinitis and children born by caesarean section was higher among children in the "grass pollen" class, and the proportion of children with asthma was higher among participants in the "HDM" class. Rhinitis at age 13 years was also increased among children in the "HDM" class. The prevalence of allergic multimorbidities at 13 years was higher among participants assigned to the "HDM" class at 4 and 7 years.

Table 7 – Probabilities assigned to allergen components in each cross-sectional latent class (only probabilities equal or greater than 0.200 were presented in this table).

Components	**Class 1**	**Components**	**Class 2**	**Components**	**Class 3**
Der p 23	0.2402	**Phl p 1**	**0.9522**	**Der f 2**	**0.8700**
		Cyn d 1	**0.8123**	**Der p 2**	**0.8635**
		Phl p 4	**0.6614**	**Der p 23**	**0.8110**
		Phl p 2	**0.6445**	**Der p 1**	**0.7558**
		Phl p 5	**0.5814**	**Der f 1**	**0.6873**
		Phl p 6	0.4849	Phl p 1	0.3316
		Der p 1	0.4418	Fel d 1	0.2540
		Der p 23	0.4372	Lep d 2	0.2381
		Der p 2	0.4049		
		Der f 2	0.4069		
		Der f 1	0.3180		
		Fel d 1	0.2836		
		Hev b 8	0.2628		
		Mer a 1	0.2473		
		Lep d 2	0.2168		
		Bet v 2	0.2164		
		Phl p 11	0.2164		
		Pla l 1	0.2113		
		Alt a 1	0.2011		
"Few or no sensitizations"		**"Grass pollen"**		**"HDM"**	

Values/components in bold indicate an assigned probability equal or greater than 0.500. HDM: house dust mites.

Table 8 – Distribution and characteristics of G21 participants assigned to sensitization classes defined by Latent Class Analysis at 4, 7, and 10 years old.

	4 years old				7 years old				10 years old			
	Class 1	**Class 2**	**Class 3**	**p**	**Class 1**	**Class 2**	**Class 3**	**p**	**Class 1**	**Class 2**	**Class 3**	**p**
N	120	5	61		88	22	76		44	39	103	
Sex, male	67 (55.8%)	4 (80.0%)	34 (55.7%)	0.56	49 (55.7%)	17 (77.3%)	39 (51.3%)	0.09	24 (54.5%)	25 (64.1%)	56 (54.4%)	0.56
Maternal education												
≤9 years	57 (47.5%)	1 (20.0%)	29 (47.5%)	0.48	43 (48.9%)	9 (40.9%)	35 (46.1%)	0.79	23 (52.3%)	18 (46.2%)	46 (44.7%)	0.70
>9 years	63 (52.5%)	4 (80.0%)	32 (52.5%)		45 (51.1%)	13 (59.1%)	41 (53.9%)		21 (47.7%)	21 (53.8%)	57 (55.3%)	
Family history of allergy	16 (13.9%)	1 (20.0%)	10 (17.2%)	0.81	9 (10.6%)	3 (14.3%)	15 (20.8%)	0.20	4 (9.1%)	4 (10.5%)	19 (19.8%)	0.17
Type of birth												
Vaginal	58 (48.3%)	0	32 (52.5%)	**0.02**	38 (43.2%)	3 (13.6%)	49 (64.5%)	**<0.01**	21 (47.7%)	12 (30.8%)	57 (55.3%)	**0.01**
Caesarean	39 (32.5%)	5 (100%)	23 (37.7%)		30 (34.1%)	18 (81.8%)	19 (25.0%)		16 (36.4%)	23 (59.0%)	28 (27.2%)	
Other	23 (19.2%)	0	6 (9.8%)		20 (22.7%)	1 (4.5%)	8 (10.5%)		7 (15.9%)	4 (10.3%)	18 (17.5%)	
Medical diagnosis of allergic conditions												
Asthma (4yo), N=180	4 (3.4%)	0	6 (10.5%)	0.13	4 (4.6%)	1 (4.8%)	5 (6.9%)	0.80	3 (6.8%)	0	7 (7.1%)	0.24
Allergic rhinitis (4yo), N=180	4 (3.4%)	1 (25.0%)	5 (8.5%)	0.09	4 (4.6%)	2 (10.0%)	4 (5.5%)	0.64	1 (2.3%)	6 (16.2%)	3 (3.0%)	**<0.01**
AD (4yo), N=185	9 (7.5%)	2 (40.0%)	8 (13.3%)	**0.04**	5 (5.7%)	3 (13.6%)	11 (14.7%)	0.15	1 (2.3%)	5 (12.8%)	13 (12.7%)	0.14
Asthma (7yo), N=183	7 (5.9%)	0	12 (20.0%)	**0.01**	5 (5.7%)	1 (4.5%)	13 (17.6%)	**0.03**	2 (4.5%)	3 (7.7%)	14 (14.0%)	0.19
Allergic rhinitis (7yo), N=185	10 (8.4%)	2 (40.0%)	14 (23.0%)	**0.01**	7 (8.0%)	4 (18.2%)	15 (19.7%)	0.08	4 (9.1%)	6 (15.4%)	16 (15.7%)	0.55
AD (7yo), N=185	15 (12.6%)	1 (20.0%)	8 (13.1%)	0.89	11 (12.6%)	4 (18.2%)	9 (11.8%)	0.73	4 (9.1%)	6 (15.8%)	14 (13.6%)	0.64
Asthma (10yo), N=184	13 (10.9%)	0	19 (31.7%)	**<0.01**	9 (10.5%)	1 (4.5%)	22 (28.9%)	**<0.01**	4 (9.1%)	4 (10.3%)	24 (23.8%)	**0.04**
Asthma (13yo), N=154	11 (11.0%)	0	19 (38.8%)	**<0.01**	6 (8.2%)	2 (10.5%)	22 (35.5%)	**<0.01**	2 (5.0%)	4 (11.4%)	24 (30.4%)	**<0.01**
Allergic rhinitis (13yo), N=154	19 (19.0%)	3 (60.0%)	20 (40.8%)	**<0.01**	12 (16.4%)	7 (36.8%)	23 (37.1%)	**0.02**	4 (10.0%)	13 (37.1%)	25 (31.6%)	**0.014**
AD (13yo), N=154	10 (9.9%)	1 (20.0%)	12 (25.0%)	0.051	6 (8.2%)	4 (21.1%)	13 (21.0%)	0.09	3 (7.5%)	6 (17.1%)	14 (17.7%)	0.31
Multimorbidities (13yo)												
Asthma + allergic rhinitis, N=153	4 (4.0%)	0	11 (22.4%)	**<0.01**	2 (2.7%)	2 (10.5%)	11 (18.0%)	**0.01**	0	4 (11.4%)	11 (14.1%)	**0.05**
Asthma + AD, N=153	3 (3.0%)	0	4 (8.3%)	0.31	2 (2.7%)	1 (5.3%)	4 (6.6%)	0.57	1 (2.5%)	2 (5.7%)	4 (5.1%)	0.76
AD + rhinitis, N=153	3 (3.0%)	0	9 (18.8%)	**<0.01**	1 (1.4%)	2 (10.5%)	9 (14.8%)	**0.01**	0	3 (8.6%)	9 (11.5%)	0.09
Asthma + allergic rhinitis + AD, N=152	2 (2.0%)	0	4 (8.3%)	0.16	1 (1.4%)	1 (5.3%)	4 (6.7%)	0.28	0	2 (5.7%)	4 (5.2%)	0.33
Allergic sensitization to ISAC components												
Nb of sensitizations*	0.48 (0.89)	7.60 (3.78)	5.02 (1.81)	**<0.01**	0.94 (1.09)	8.95 (3.64)	5.66 (2.06)	**<0.01**	1.86 (0.98)	10.82 (4.39)	6.36 (2.45)	**<0.01**

Data are presented as N (%) unless otherwise stated. *Data presented as mean (sd). Values in bold indicate statistically significant differences for the comparison of demographic and health outcomes between the 3 classes (a P-value ≤0.05 was considered statistically significant). Chi-square test was used to compare categorical variables; Kruskal-Wallis was used for continuous variables. AD: atopic dermatitis; HDM: house dust mites; nb: number; yo: years old. Class 1: "no or few sensitizations", Class 2: "grass pollen", Class 3: "HDM".

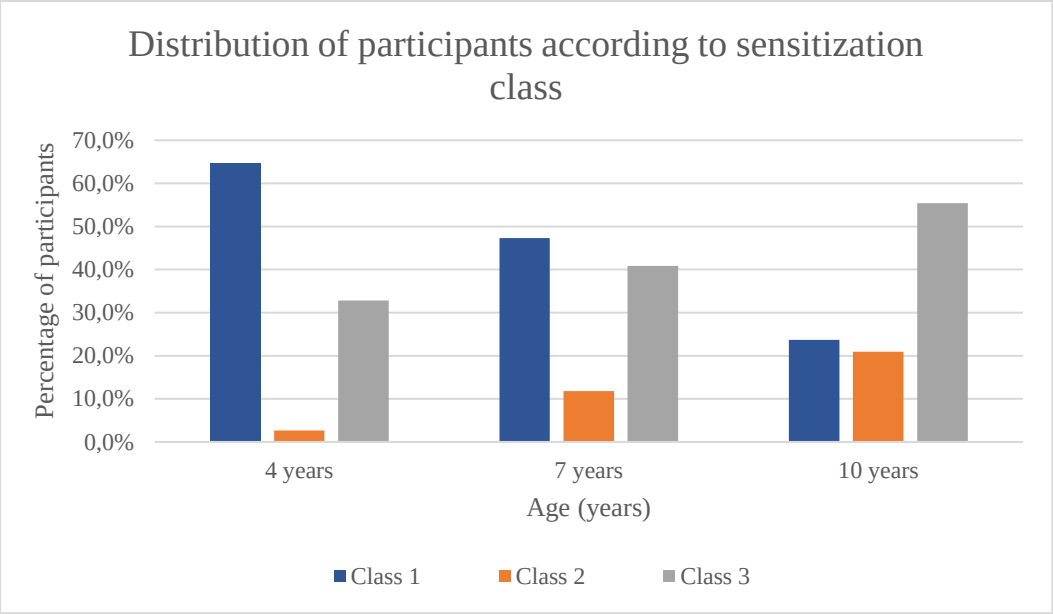

Figure 8 – Distribution of participants according to classes defined using LCA at 4, 7, and 10 years old. Class 1: "no or few sensitizations"; Class 2: "grass pollen"; Class 3: "HDM".

A solution of five sensitization trajectories was proposed based on the most prevalent transitions between classes. As previously mentioned, the most common transitions were participants who moved from class 1 ("nor or few sensitizations") to class 3 ("HDM"), followed by class 1 to class 2 ("grass pollen"), and class 3 to class 2. Furthermore, these transitions mainly occurred from age 7 to 10 years. Therefore, participants who were stable at class 1 in all follow-ups (N=41) or classified in this class at 10 years old (N=3) were grouped as "no or few sensitizations" trajectory. Participants who were stable at class 3 (N=50) or who started at 4 years and finished at 10 years in class 3 (N=2) were grouped as "early persistent HDM". Children who were stable at class 2 (N=5) or who moved from class 3 to class 2 at any time and remained in class 2 at 10 years old (N=10) were classified into "HDM and grass pollen" trajectory. Children who moved from class 1 to class 2 and remained in class 2 were classified as "late grass pollen" (N=24). Finally, children who moved from class 1 to class 3 and remained in class 3 were classified as "late HDM" (N=51). These data are summarized in Table 9.

The characterization of sensitization trajectories is presented in Table 10. Children in the "HDM and grass pollen" trajectory were characterized by the highest number of sensitizations and 50.0% reported allergic rhinitis at the age of 13 years, while asthma only affected 7.1% of these children. This trajectory was positively associated with allergic rhinitis in the adjusted model (OR, 95% CI: 12.1, 2.6 – 56.7) (Figure 9). The "early persistent HDM" trajectory was characterized by the highest proportion of children with asthma and was positively associated with asthma (OR, 95% CI: 14.4, 3.0 – 69.0) and allergic rhinitis (OR, 95% CI: 7.3, 2.1 – 25.4) (Figure 9). The "late HDM" and "late grass pollen" trajectories were characterized by 15.8% and 14.3% of children with asthma,

respectively. These trajectories were not associated with allergic diseases (asthma, allergic rhinitis, and atopic dermatitis).

Table 9 - Characterization of sensitization trajectories defined by stability of clusters identified in the LCA and by moving between clusters (most prevalent transitions).

Trajectories	N total	Sensitization class by age*			N
		4 years	7 years	10 years	
No or few sensitizations [stable in class 1 over time or in class 1 at 10 years of age]	**44**	1	1	1	41
		1	3	1	1
		3	1	1	1
		3	2	1	1
Early persistent HDM [stable in class 3 over time or starting at 4 years and finishing at 10 years in class 3]	**52**	3	3	3	50
		3	2	3	1
		3	1	3	1
HDM and grass pollen [stable in class 2 over time or moving from class 3 to class 2 at any time and remain in class 2]	**15**	1	3	2	3
		3	3	2	3
		3	2	2	4
		2	2	2	5
Late grass pollen [moving from class 1 to class 2, regardless of time, and remain in class 2]	**24**	1	2	2	11
		1	1	2	13
Late HDM [moving from class 1 to class 3, regardless of time, and remain in class 3]	**51**	1	3	3	19
		1	1	3	32

*The classes identified by LCA are represent numerically as following: 1 represents “no/few sensitizations”, 2 represents “grass pollen”, 3 represents “HDM”. HDM: house dust mites, LCA: Latent class analysis.

Table 10 - Distribution and characteristics of participants assigned to each sensitization trajectory.

	No/few sensitization	Early persistent HDM	HDM and grass pollen	Late grass pollen	Late HDM	p-value
N children	44	52	15	24	51	
Sex, males	24 (54.5%)	26 (50.0%)	12 (80.0%)	13 (54.2%)	30 (58.8%)	0.34
Maternal education						
≤9 years	23 (52.3%)	25 (48.1%)	8 (53.3%)	10 (41.7%)	21 (41.2%)	0.79
>9 years	21 (47.7%)	27 (51.9%)	7 (46.7%)	14 (58.3%)	30 (58.8%)	
Family history of allergy	4 (9.1%)	9 (18.4%)	2 (13.3%)	2 (8.7%)	10 (21.3%)	0.43
Type of birth						
Vaginal	21 (47.7%)	30 (57.7%)	4 (26.7%)	8 (33.3%)	27 (52.9%)	**0.02**
Caesarean	16 (36.4%)	16 (30.8%)	11 (73.3%)	12 (50.0%)	12 (23.5%)	
Other	7 (15.9%)	6 (11.5%)	0	4 (16.7%)	12 (23.5%)	
Medical diagnosis of allergic conditions						
Asthma (4yo), N= 180	3 (6.8%)	6 (12.5%)	0	0	1 (2.0%)	0.09
Allergic rhinitis (4yo), N= 180	1 (2.3%)	3 (6.0%)	3 (21.4%)	3 (13.0%)	0	**0.01**
AD (4yo), N= 185	1 (2.3%)	8 (15.7%)	2 (13.3%)	3 (12.5%)	5 (9.8%)	0.29
Asthma (7yo), N= 183	2 (4.5%)	12 (23.5%)	1 (6.7%)	2 (8.3%)	2 (4.1%)	**<0.01**
Allergic rhinitis (7yo), N= 185	4 (9.1%)	12 (23.1%)	4 (26.7%)	2 (8.3%)	4 (8.0%)	0.07
AD (7yo), N= 185	4 (9.1%)	7 (13.5%)	2 (13.3%)	4 (17.4%)	7 (13.7%)	0.91
Asthma (10yo), N=184	4 (9.1%)	17 (33.3%)	2 (13.3%)	2 (8.3%)	7 (14.0%)	**0.01**
Asthma (13yo), N= 154	2 (5.0%)	18 (43.9%)	1 (7.1%)	3 (14.3%)	6 (15.8%)	**<0.01**
Allergic rhinitis (13yo), N= 154	4 (10.0%)	17 (41.5%)	7 (50.0%)	6 (28.6%)	8 (21.1%)	**<0.01**
AD (13yo), N= 154	3 (7.5%)	10 (25.0%)	3 (21.4%)	3 (14.3%)	4 (10.3%)	0.19
Multimorbidities (13yo)						
Asthma + allergic rhinitis, N= 153	0	10 (24.4%)	1 (7.1%)	3 (14.3%)	1 (2.7%)	**<0.01**
Asthma + AD, N= 153	1 (2.5%)	4 (10.0%)	0	2 (9.5%)	0	0.15
AD + allergic rhinitis, N= 153	0	8 (20.0%)	1 (7.1%)	2 (9.5%)	1 (2.6%)	**0.01**
Asthma + allergic rhinitis + AD, N=152	0	4 (10.0%)	0	2 (9.5%)	0	0.05
Allergic sensitization to ISAC components						
Nb of sensitizations*, 4yo	0.34 (0.94)	4.81 (1.67)	5.87 (3.64)	1.00 (1.32)	0.49 (0.76)	**<0.01**
Nb of sensitizations*, 7yo	0.86 (1.21)	6.19 (2.77)	9.20 (2.88)	3.96 (3.21)	2.29 (2.13)	**<0.01**
Nb of sensitizations*, 10yo	1.86 (0.97)	7.19 (2.50)	12.40 (4.03)	9.83 (4.40)	5.51 (2.10)	**<0.01**

Data are presented as N (%) unless otherwise stated. *Data presented as mean (sd). Values in bold indicate statistically significant differences for the comparison of demographic and health outcomes variables between the 5 trajectories (P-value, represented as p, ≤0.05 was considered statistically significant). AD: atopic dermatitis; HDM: house dust mites; nb: number; yo: years old.

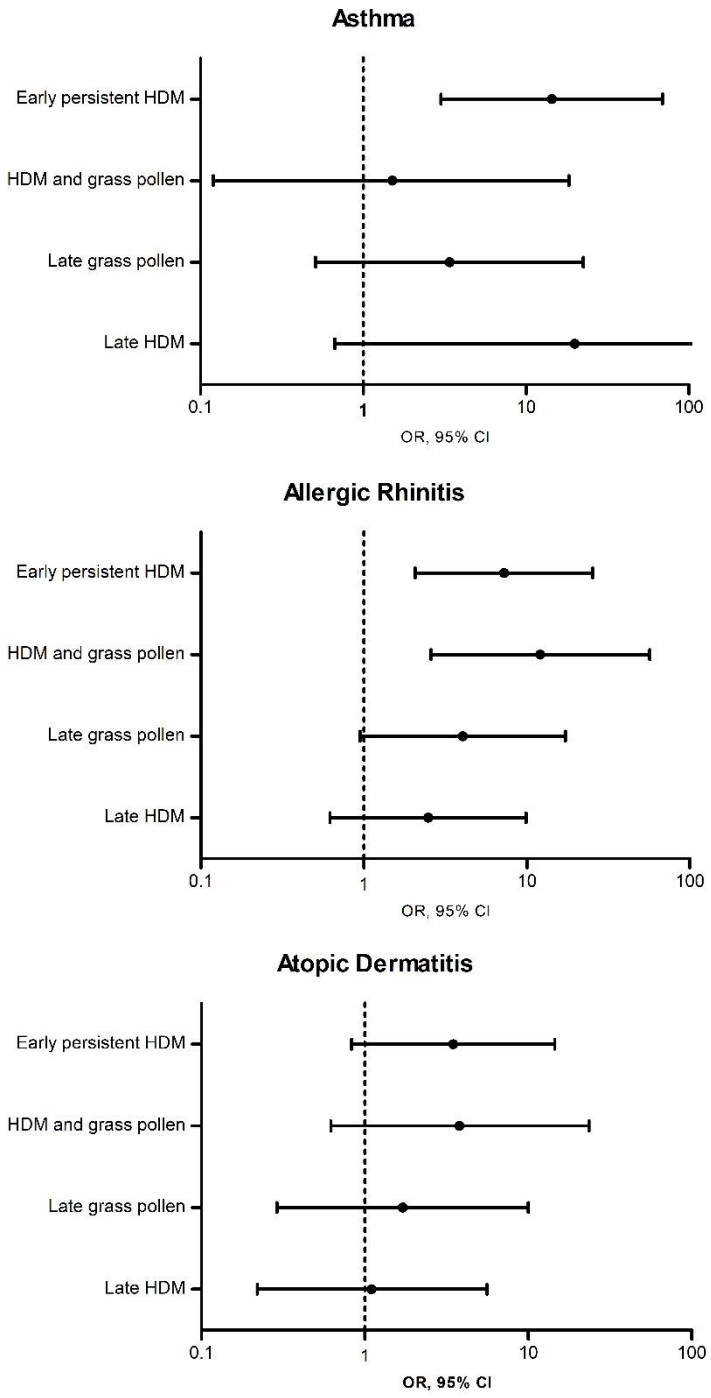

Figure 9 - Associations between the sensitization trajectories and allergic outcomes (asthma, allergic rhinitis and atopic dermatitis) assessed at 13 years old evaluation, adjusted for sex, parents' history of allergy, and maternal education. Results are expressed as odds ratios (OR) and 95% confidence intervals (CI). The reference category is the "no/few sensitizations" trajectory.

5.3. Asthma predictive tool (Study II)

Based on RMSEP and R^2 metrics in the PLS analysis, the optimal number of latent components (LC) was two for allergic sensitization data at 10 years old. These results were reinforced by the data available at 4 and 7 years old. The weights of the most relevant predictors in the first latent component (LC1), presented in Table 11, were the most constant over time and positively related (Y loadings) with asthma, followed by rhinitis and atopic dermatitis. However, at 4 years old some positive weights from grass pollen molecules seemed relevant to maximize the score of LC1 and its association with asthma did not remain, but was relevant for rhinitis. The highest weights in LC1 were mainly HDM molecules (Der p 1, Der p 2, Der p 23, Der f 1, Der f 2), cat (Fel d 1) and dog dander (Can f 1, Can f 2). The second latent component (LC2), mainly composed of negative weights, was inversely associated with asthma and suggested that children sensitized to most relevant grass and tree pollen and some food molecules were likely to present rhinitis. Grass pollen-related molecules such as Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6 and Cyn d 1 were characterized by negative weights, especially at early assessments (at 4 and 7 years) and negative Y loadings were related with rhinitis followed by atopic dermatitis, but not asthma. Food allergens such as cashew, peanut, hazelnut, walnut, sesame, egg, soy and peach presented high negative weights at 7 and 10 years (Ana o 2, Ara h 1, Ara h 9, Cor a 9, Jug r 3, Ses i 1, Gal d 1, Gal d 5, Gly m 5, Gly m 6, Pru p 3) in LC2. At 4 years old, LC2 was related with asthma but not rhinitis; at this age, some HDM molecules seemed relevant to balance the effects of the negative weights of pollen-related molecules. The full list of predictors (allergen components) and corresponding weights in each latent component (LC1 and LC2) is presented in Annex 3.

The previous PLS results (LC1 and LC2) were combined with other relevant variables assessed in previous asthma risk tools. The associations were stronger when evaluating predictors at 10 years and outcomes at 13 years and were supported by the classification metrics (Table 12). Family history of allergy and history of early wheezing symptoms (previous to 4 years old) were relevant predictors to improve the accuracy to 0.82 when allergic sensitization was assessed at 10 years of age. This result was robust when evaluating allergic sensitization at 4 and 7 years (Table 12). Sensitivity and specificity reached 75% and 81%, while positive predictive value (PPV) and negative predictive value (NPV) were 28% and 97%.

Table 11 – Partial Least Squares weight scores of the most relevant predictors (i.e., allergenic molecules with weights ≤-0.100 or ≥0.100) by latent component (LC1, LC2) and time of assessment (4, 7, 10 years). Y loadings and % of variance are presented in the end of the table by latent component and according to allergic diseases (asthma, rhinitis, atopic dermatitis) diagnosed at 13 years old.

	4 years		7 years		10 years	
Molecules	**LC1***	**LC2**	**LC1***	**LC2**	**LC1**	**LC2**
Der f 1	0.359	0.137	0.352		0.322	
Der f 2	0.411	0.105	0.345		0.346	0.119
Der p 1	0.395	0.111	0.339		0.306	
Der p 2	0.407	0.105	0.336		0.345	0.120
Der p 10					0.143	0.137
Der p 23	0.346	0.138	0.269		0.235	0.149
Blo t 5			0.189		0.144	0.111
Lep d 2					0.177	0.116
Fel d 1	0.194		0.230		0.236	
Can f 1			0.155		0.215	
Can f 2	0.151	0.110	0.312		0.144	
Phl p 1	0.211	-0.135		-0.213		-0.127
Phl p 2	0.150	-0.315		-0.247		
Phl p 4	0.100	-0.174		-0.254		-0.161
Phl p 5		-0.215		-0.165		
Phl p 6	0.161	-0.312		-0.221		-0.100
Phl p 12	0.161	-0.312		-0.184		
Cyn d 1	0.142	-0.242		-0.245		-0.147
Ole e 1	0.127	-0.238	0.142	-0.101	0.114	
Ole e 7	0.127	-0.238				
Pla a 3					0.137	-0.255
Bet v 2	0.161	-0.312	0.104	-0.160		
Mer a 1	0.161	-0.312		-0.144		
Art v 3					0.137	-0.255
Pla l 1	0.128	-0.271		-0.171		
Ana o 2				-0.328		-0.287
Ara h 1						-0.287
Ara h 9	0.127	-0.238			0.137	-0.255
Cor a 9				-0.328		-0.287
Jug r 3					0.137	-0.255
Ses i 1				-0.328		-0.233
Gal d 1				-0.328		-0.287
Gal d 5				-0.270		-0.159
Gly m 5				-0.328		-0.188
Gly m 6				-0.328	0.132	-0.192
Pru p 3					0.135	-0.339
Colour legend						
>0.25	0.200 to 0.250		0.150 to 0.200		0.100 to 0.150	
<-0.25	-0.200 to -0.250		-0.150 to -0.200		-0.100 to -0.150	
% Variance explained						
Asthma	7.770	11.610	18.650	20.320	10.130	18.640
Allergic rhinitis	7.660	9.190	8.310	15.680	20.670	22.280
AD	3.660	6.550	3.550	7.030	2.530	2.550
Y Loadings						
Asthma	0.144	0.093	0.223	0.066	0.176	0.143
Allergic rhinitis	0.100	-0.040	0.104	-0.100	0.176	-0.040
AD	0.073	-0.060	0.066	-0.070	0.060	0

*The values from this latent component were inverted (negatives to positives, positives to negatives) to allow a better interpretation of results, including Y loadings. AD: atopic dermatitis; LC: latent component.

Table 12 - Logistic regression results including additional predictors to identify asthma at 13 years old by time of allergic sensitization assessment (4, 7, and 10 years) and corresponding classification metrics.

	IgE 10 years old	IgE 7 years old	IgE 4 years old
N	793	649	649
Model parameters; OR (95% CI), p-value			
LC1	**1.86 (1.56, 2.19), <0.01**	**2.27 (1.81, 2.87), <0.01**	1.34 (0.77, 1.97), 0.21
LC2	**1.36 (1.15, 1.79), <0.01**	1.18 (0.90, 1.86), 0.35	**3.10 (1.47, 9.74), 0.02**
Family history of allergy	**2.84 (1.26, 6.02), <0.01**	1.90 (0.66, 4.74), 0.20	1.78 (0.63, 4.41), 0.24
Early wheezing	**2.52 (1.41, 4.70), <0.01**	1.90 (0.95, 3.99), 0.08	1.78 (0.89, 3.75), 0.11
Classification metrics			
AUC	0.82	0.77	0.77
Sensitivity	75%	62%	57%
Specificity	81%	89%	91%
PPV	28%	30%	33%
NPV	97%	97%	96%
Cut-off	0.085	0.073	0.084

AUC: Area under the curve, CI: confidence interval, LC: latent component (from the PLS analysis), NPV: negative predictive value, OR: Odds ratio, PPV: positive predictive value. Values in bold indicate statistically significant associations.

5.4. Allergen immunotherapy effects on the prevention of asthma onset (Study III)

We included 24 studies in the qualitative analysis and 18 were suitable for the quantitative analysis. Regarding study design, 12 RCTs[226-237] and 12 NRSI[156, 238-248] were included. Six NRSI used secondary data from healthcare databases; namely, one analyzed real-world data from a population-based administrative healthcare database[240], and five used real-world data from a health insurance prescription database[156, 238, 239, 246, 247]. The population in each study varied, ranging from 28 up to 118,754 subjects. Most studies were performed in a population with allergic rhinitis (n=12), followed by rhinoconjunctivitis (n=9), atopic dermatitis (n=2), and healthy individuals (n=1). Eleven studies also included participants with allergic asthma; nine presented the data for the subpopulation without asthma at baseline. Asthma onset was the primary outcome/endpoint only in seven publications. SLIT was evaluated in thirteen studies, SCIT in eight, and both in three studies of which two studies presented data separately according to the administration route. The allergens used for AIT were grass pollen (n=7), HDM (n=5), birch pollen (n=2), and *Parietaria judaica* (n=1). The characteristics and main findings of each study were presented in the Original Article.

The risk-of-bias results are summarized in Figures 10 and 11 for RCTs and NRSI studies, respectively. Five RCTs were considered to be at high risk of bias mainly due to the use of a control group instead of a placebo group, which raised concerns due to unblinding of participants and assessors when measuring the outcome; and due to missing data of participants that were lost to the follow-up without specifying the reasons and if the dropouts were balanced or unbalanced

between the groups. As expected, the risk of bias was higher in NRSI mainly due to indication, selection, and detection bias reflecting the real-world nature of these studies, and consequently, confounding effects. We considered only one non-randomized study of moderate quality because of the appropriate measures taken to minimize bias and confounding (matching and multivariate modelling) and the proper definition and assessment of the outcome.

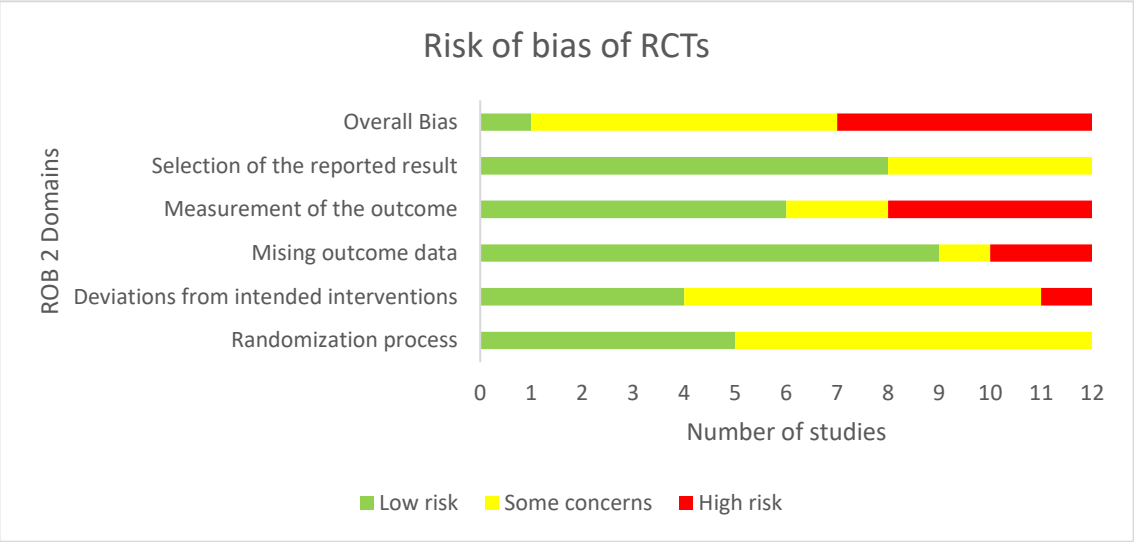

Figure 10 - Risk of Bias Assessment of randomized clinical trials (RCTs) using the ROB 2 Tool.

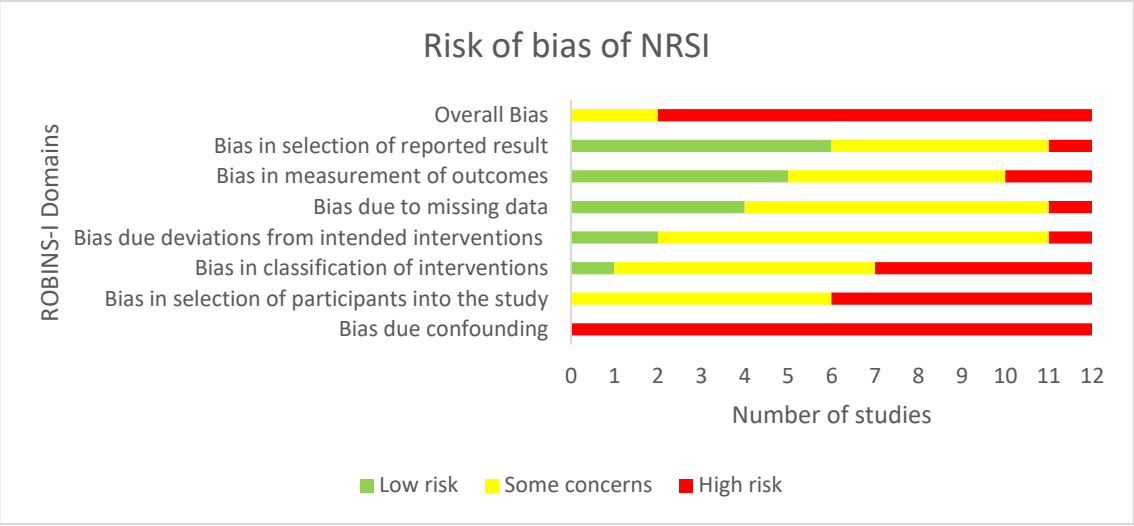

Figure 11 - Risk of Bias Assessment of non-randomized studies of intervention (NRSI) using ROBINS-I Tool.

5.4.1. Random effects meta-analysis

The pooled risk ratio (RR) was 0.75 (95% CI: 0.64 - 0.88; N=17), demonstrating a significant decrease in the risk of developing asthma using AIT by 25% (Figure 12). One study was considered an outlier and not included is this estimate. The three indicators of between-study heterogeneity showed moderate-to-high heterogeneity between the pooled studies (Figure 12). The broad prediction interval, which surpassed the value one, did not allow to overly confidence in the preventive effect of the intervention, meaning that AIT may not be protective in every context. In addition, in the sensitivity analysis the result did not remain significant (RR, 95% CI: 0.76, 0.45-1.27; N=7). When including the study considered an outlier (*Fritzsching et al, 2021*[247]) and when excluding influential studies from the analysis (*Fritzsching et al, 2021*[247] and *Wahn et al, 2018*[246]), the results were similar (Supporting information of the Original Article - see section "Original Publications").

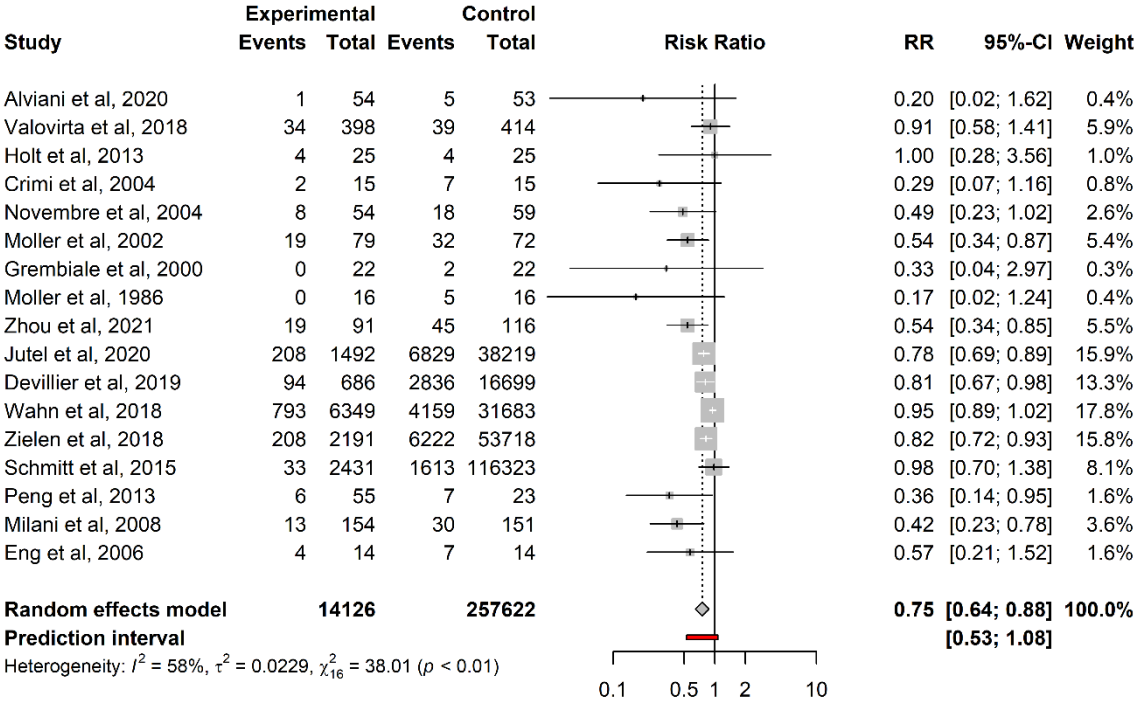

Figure 12 - Random-effects meta-analysis of effectiveness of AIT in the prevention of asthma (N of studies=17). Diamond: pooled effect; Grey square: represents study weight; black horizontal line: study confidence interval.

The funnel plot (Figure 13) shows substantial asymmetry and the Egger's test was statistically significant (p<0.01), denoting publication bias. When performing the trim-and-fill analysis to impute data from missing studies, the result remained marginally significant (RR, 95% CI: 0.82, 0.68-0.98) and the heterogeneity remained moderate-to-high (I^2= 59.7%).

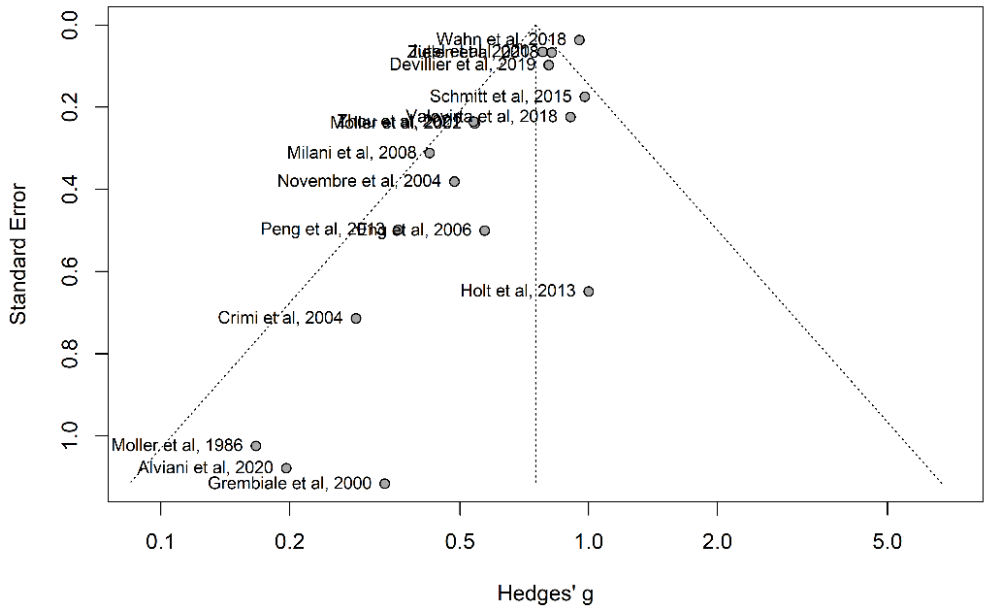

Figure 13 - Publication bias assessed by funnel plot.

5.4.2. Subgroup analysis

The analysis by study design highlighted the protective effect of AIT in preventing asthma onset when evaluating evidence from RCTs (RR, 95% CI: 0.59, 0.40-0.88; I^2=17%), while in NRSI studies the heterogeneity was higher and the effect was not significant (RR, 95% CI: 0.82, 0.66-1.01; I^2: 86%) (Table 13). The administration route and type of allergen did not influence the results since these were significant for both SCIT and SLIT, and HDM and grass pollen, respectively. Concerning SLIT administration, both drops and tablets were statistically significant, with a larger effect observed when administering SLIT drops. Other results from this analysis (Table 13) showed that AIT was beneficial in specific circumstances: studies conducted only in children (RR 95% CI: 0.71, 0.53-0.96; N= 8); mono-sensitized patients (RR 95% CI: 0.49, 0.39-0.61; N= 6); AIT administration for at least three years (RR 95% CI: 0.64, 0.47-0.88; N= 8); and, follow-up after AIT discontinuation for up to three years (RR 95% CI: 0.74, 0.62-0.88; N= 14). When asthma was defined by mixed criteria (symptoms, medication, and lung function) or only by medication, the results did not remain significant.

Table 13 – Subgroup analysis when combining all studies and by AIT administration route (SLIT or SCIT). For each output we report the corresponding RR (95% CI), the heterogeneity parameters, and the number of studies included.

	All studies	**SCIT**	**SLIT**
Study Design			
RCT, RR (95% CI)	**0.59 (0.40; 0.88)**	**0.50 (0.27; 0.93)**	0.63 (0.29; 1.35)
Heterogeneity: I2; Q	**17%; 8.44;**	**0%; 0.87**	33%; 5.93
N	**8**	**3**	5
NRSI, RR (95% CI)	0.82 (0.66; 1.02)	0.78 (0.59; 1.04)	**0.78 (0.66; 0.93)**
Heterogeneity: I2; Q	86%; 65.41;	73%; 18.41	**15%; 4.71**
N	10	6	**5**
Outcome			
Primary analysis, RR (95% CI)	0.84 (0.67; 1.06)	0.83 (0.59; 1.17)	0.76 (0.57; 1.03)
Heterogeneity: I2; Q	64%; 13.89	78%; 13.75	0%; 2.35
N	6	4	4
Secondary analysis, RR (95% CI)	**0.67 (0.50; 0.89)**	**0.48 (0.36; 0.65)**	0.73 (0.54; 1.00)
Heterogeneity: I2; Q	**83%; 63.74**	**0%; 1.34**	40%; 8.38
N	**12**	**5**	6
Population			
Children, RR (95% CI)	**0.71 (0.53; 0.96)**	0.71 (0.37; 1.38)	0.63 (0.29; 1.35)
Heterogeneity: I2; Q	**30%; 10.04**	48%; 3.81	33%; 5.93
N	**8**	3	5
Adults, RR (95% CI)	0.75 (0.56; 1.01)	0.54 (0.21; 1.35)	0.78 (0.58; 1.05)
Heterogeneity: I2; Q	45%; 10.89	43%; 5.24	30%; 4.28
N	7	4	4
Therapy duration			
<3 years, RR (95% CI)	0.85 (0.67; 1.07)	0.87 (0.59; 1.36)	**0.76 (0.61; 0.94)**
Heterogeneity: I2; Q	85%; 60.37	79%; 9.69	**32%; 8.77**
N	10	3	**7**
≥3 years, OR (95% CI)	**0.64 (0.47; 0.88)**	**0.60 (0.42; 0.85)**	0.78 (0.35; 1.74)
Heterogeneity: I2; Q	**42%; 12.01**	**22%; 6.43**	2%; 2.04
N	**8**	**6**	3
Follow-up after AIT discontinuation			
Short-term (≤3 years), RR (95% CI)	**0.74 (0.62; 0.88)**	**0.70 (0.50; 0.97)**	**0.77 (0.65; 0.91)**
Heterogeneity: I2; Q	**63%; 34.92**	**77%; 25.55**	**23%; 9.12**
N	**14**	**7**	**8**
Long-term (>3 years), RR (95% CI)	0.88 (0.51; 1.49)	0.76 (0.42; 1.37)	
Heterogeneity: I2; Q	67%; 11.93	0%; 1.62	Not enough data
N	5	3	
Allergen			
Grass pollen, RR (95% CI)	**0.79 (0.68; 0.92)**	**0.55 (0.42; 0.72)**	**0.81 (0.71; 0.94)**
Heterogeneity: I2; Q	**5%; 5.28**	**0%; 0.01**	**0%; 2.09**
N	**6**	**2**	**4**
House dust mite, RR (95% CI)	**0.60 (0.38; 0.96)**	0.63 (0.38; 1.05)	
Heterogeneity: I2; Q	**40%; 6.63**	41%; 5.10	Not enough data
N	**5**	4	
Sensitization			
Only mono-sensitized, RR (95% CI)	**0.49 (0.39; 0.61)**	**0.49 (0.36; 0.66)**	
Heterogeneity: I2; Q	**0%; 1.36**	**0%; 1.36**	Not enough data
N	**6**	**5**	
Mono/poly-sensitized, RR (95% CI)	0.85 (0.70; 1.03)	0.83 (0.58; 1.17)	**0.79 (0.69; 0.90)**

Heterogeneity: I2; Q	84%; 63.43	79%; 14.04	**15%;7.53**
N	11	4	**8**
SLIT type			
Drops, RR (95% CI)			**0.65 (0.48; 0.89)**
Heterogeneity: I2; Q	-	-	**5%; 5.28**
N			**6**
Tablets, RR (95% CI)			**0.82 (0.70; 0.96)**
Heterogeneity: I2; Q	-	-	**0%; 2.65**
N			**4**
Asthma definition			
*Mixed criteria, RR (95% CI)	0.77 (0.34; 1.73)		0.85 (0.36; 2.03)
Heterogeneity: I2; Q	46%; 5.57	Not enough data	0%; 1.95
N	4		3
Symptoms/medication, RR (95% CI)	**0.52 (0.45; 0.60)**		**0.50 (0.40;0.61)**
Heterogeneity: I2; Q	**0%; 0.07**	Not enough data	**0%; 0**
N	**3**		**2**
Medication, RR (95% CI)	0.91 (0.72; 1.14)		**0.80 (0.71; 0.91)**
Heterogeneity: I2; Q	92%; 47.42	Not enough data	**0%; 0.65**
N	5		**3**
Symptoms, RR (95% CI)	**0.45 (0.29; 0.72)**	0.44 (0.16; 1.16)	0.47 (0.10; 2.24)
Heterogeneity: I2; Q	**0%; 3.24**	0%; 0.73	20%; 2.51
N	**6**	3	3

*Mixed criteria were defined as a combination of symptoms, medication and lung function assessments to diagnose asthma; thus, in these studies, asthma diagnosis was more accurate because it followed a more complete and strict criteria. One of these studies reported an asthma diagnosis based on the GINA guidelines.

Abbreviations. CI: confidence interval; I2: Higgins & Thompson's I^2 Statistic; N= number of studies; NRSI: non-randomized studies of interventions; Q: Cochran's Q; RCT: randomized controlled trial; RR: risk ratio; SCIT: subcutaneous immunotherapy; SLIT: sublingual immunotherapy.

5.5. Cost-effectiveness of allergen immunotherapy in children with allergic HDM-driven asthma or grass pollen rhinitis (Study IV and V)

The cost-effectiveness analysis of SCIT and SLIT tablets compared to the SOC strategy, in children with allergic HDM-driven asthma (**study IV**), estimated an ICER of 1,281€ and 7,717€, per QALY gained, respectively. The base case results are presented in Table 14. The major contributors to these results were AIT effects on health outcomes by reducing moderate and severe asthma exacerbations and asthma controller medication. For the SCIT strategy and in the time horizon considered in the analysis, we expected a reduction of 1,898 moderate exacerbations and 45 severe exacerbations, corresponding to cost differences of 242,304€ and 125,542€, respectively. We anticipated a less optimistic scenario for the SLIT tablets strategy; namely, a reduction of 764 moderate exacerbations and 19 severe exacerbations. The reduction of asthma medication and scheduled medical appointments led to savings of 439,199€ and 154,521€ in SCIT and SLIT tablets strategies, respectively. Regarding the simulation of adherence rates, 570

children were expected to complete three years of SCIT as opposed to 227 children in the sublingual strategy.

In children with grass pollen-induced allergic rhinitis (**study V**), the analysis yielded an ICER of 6,318€ and 12,605€, per QALY gained, for SCIT and SLIT, respectively, compared with SOC strategy (Table 15). SCIT demonstrated to be less costly than SLIT mainly due to savings in asthma costs and AIT acquisition price. Over the 10-year time horizon, the number of patients experiencing allergic asthma were 193, 181, and 172 in SOC, SLIT, and SCIT strategies, respectively. According to the model, 339 and 470 patients completed three years of immunotherapy (SLIT and SCIT, respectively).

Table 14 - Base case results of costs and health gains for SOC, SLIT, and SCIT strategies at the end of the model calculation (10-year horizon) in patients with HDM-driven allergic asthma.

Base Case Scenario	**SOC**	**SCIT**	**SLIT**
Asthma medication + scheduled visits	2,915,378€	2,476,179€	2,760,857€
Intervention costs (SLIT or SCIT + administration)	0€	1,176,098€	1,327,165€
Moderate exacerbations costs	506,673€	264,369€	409,105€
Number of moderate exacerbations	3,969	2,071	3,205
Severe exacerbations costs	243,756€	118,214€	189,383€
Number of severe exacerbations	88	43	69
Total cost	3,665,807€	4,034,859€	4,686,510€
Total cost (discount rate)	3,209,552€	3,647,624€	4,231,070€
Utilities	17,785	17,949	17,842
Disutilities	480	250	387
QALYs	17,305	17,699	17,455
QALYs (discount rate)	15,096	15,438	15,228
Cost difference	Reference	438€	1,021€
Effect difference	Reference	0.342	0.132
ICER	Reference	1,281€	7,717€

ICER: incremental cost-effectiveness ratio, QALYs: quality-adjusted life-years, SCIT: subcutaneous immunotherapy, SLIT: sublingual immunotherapy, SOC: standard-of-care.

Table 15 - Base case results of costs and health gains for SOC, SLIT, and SCIT strategies at the end of the model calculation (10-year horizon) in patients with grass pollen-induced allergic rhinitis.

Base Case Scenario	**SOC**	**SCIT**	**SLIT**
AR costs	288,495€	248,095€	257,814€
AIT costs	0€	1,144,970€	1,571,120€
AA costs	495,501€	282,076€	334,882€
Healthcare resource costs	1,500,002€	1,531,000€	1,531,000€
Total cost	2,283,999€	3,206,141€	3,694,815€
Total cost (discount rate)	1,988,279€	2,921,615€	3,396,562€
QALYs	6,702	6,868	6,827
QALYs (discount rate)	5,889	6,037	6,001
Cost difference	Reference	933€	1,408€
Effect difference	Reference	0.148	0.112
ICER	Reference	6,318€	12,605€

AA: allergic asthma, AIT: allergen immunotherapy, ICER: incremental cost-effectiveness ratio, QALY: quality-adjusted life-years, Ref: reference, SCIT: subcutaneous immunotherapy, SLIT: sublingual immunotherapy, SOC: standard-of-care.

5.5.1. Sensitivity analysis

In both studies (**study IV and V**), the robustness of the model was assessed through deterministic and probabilistic sensitivity analysis. In the model developed for children with asthma (**study IV**), the variation of each parameter uncertainty according to lower and higher boundaries led to an ICER ranging between 138€ and 6,628€ for SCIT, and 3,273€ and 46,710€ for SLIT tablets. The Tornado plots are presented in the Original Article (see section "Original Publications"). In the PSA analysis, Figure 14, the ICER after 1,000 simulations was 1,387€ for SCIT and 8,498€ for SLIT. For the SLIT tablet strategy, some simulations were plotted in a non-cost-effectiveness region (upper left quadrant of the graph), and costs were markedly higher for most simulations when compared to SCIT. The results were used to compute cost-effectiveness acceptability curves that showed a probability of cost-effectiveness around 75% and 99% for SCIT and SLIT, respectively, for a WTP threshold of 20,000€ (section "Original Publications"). When evaluating other possible scenarios, none of the ICER values reached the CE threshold. In a perfect scenario of full adherence to AIT or considering a 50% discount in AIT acquisition prices, a marked reduction of the ICER was observed. When AR medication costs were included, the ICER decreased, particularly in the SCIT strategy because adherence rates to SCIT are higher and more children benefit from a reduction in AR drugs use. If the effects of AIT in asthma remission were considered the same as in the SOC arm, the ICER increased. If we do not take into account the effects of AIT on moderate and severe exacerbations for the entire time horizon, as expected, the ICER values increased. Still, SCIT results remained optimistic (3,539€ and 17,437€ for SCIT and SLIT, respectively).

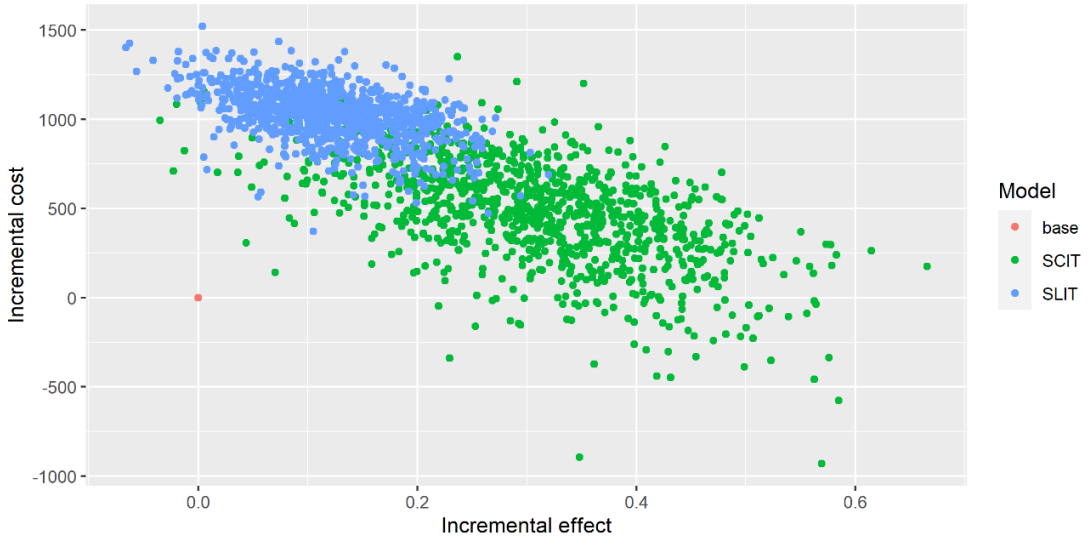

Figure 14 - Results of the probabilistic sensitivity analysis (PSA) represented on a cost-effectiveness plan for the simulation with children with HDM-driven allergic asthma (Study IV). Incremental cost axis is presented in euros (€) and Incremental effect axis in QALYs. Green: subcutaneous immunotherapy, Blue: sublingual immunotherapy, Orange: standard-of-care (reference). SCIT: subcutaneous immunotherapy, SLIT: sublingual immunotherapy.

In **study V**, the DSA led to variations in ICER ranging between 2,093€ and 8,417€ for SCIT and 4,185€ and 20,290€ for SLIT. For both AIT strategies, the natural probability of asthma development (assumed in the SOC arm) over the years was the main driver for change in costs, followed by annual discount rate, treatment discontinuation, and AIT costs (particularly, in SLIT arm). In the PSA, Figure 15, the ICER average was 6,249€ and 12,599€ and the probability of being cost-effective under a threshold of 20,000€ was 98% and 60% for SCIT and SLIT, respectively. SCIT presented higher values for QALYs and lower costs which translated to a lower ICER value. When evaluating other possible scenarios, full-adherence to AIT and a 50% discount on AIT acquisition costs had a significant effect on ICER as the probability of SLIT and SCIT being cost-effective increased. The ICER remained similar to the base case when asthma and allergic rhinitis symptomatic treatment costs varied. The results from other scenarios analysis are fully described and presented in the original articles of **studies IV and V** (see section "Original Publications").

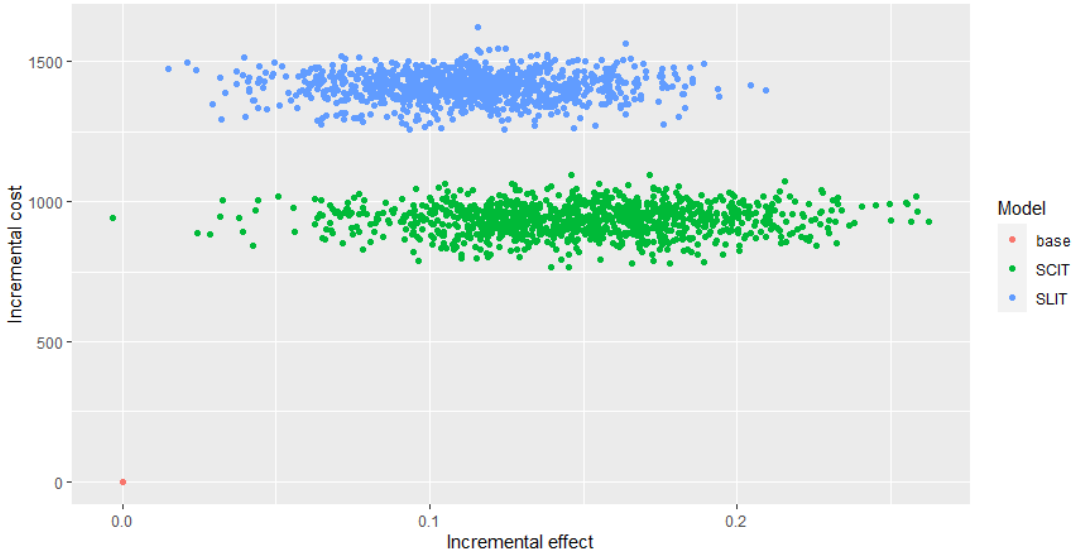

Figure 15 - Results of the probabilistic sensitivity analysis (PSA) represented on a cost-effectiveness plan for the simulation with children with grass pollen-induced allergic rhinitis (Study IV). Incremental cost axis is presented in euro (€) and Incremental effect axis in QALYs. Green: subcutaneous immunotherapy, Blue: sublingual immunotherapy, Orange: standard-of-care (reference). SCIT: subcutaneous immunotherapy, SLIT: sublingual immunotherapy.

6. Discussion

6.1. Summary of findings

This thesis was mainly focused on the prevention of allergic diseases. Primary prevention has the ultimate goal of preventing disease in healthy people before it develops by implementing effective primary prevention plans for which it is necessary to know biological, clinical, and epidemiological data of the disease and the population in question.[249] Secondary prevention aims to identify people at high risk for the development of a disease in order to detect it and intervene early, that is, to detect the disease earlier than it would have been detected with usual care.[249] **Studies I and II** focused on exploring childhood allergic sensitization trajectories, evaluating their relation with allergic outcomes, and translating the applicability of CRD tests in a Portuguese population. Allergic mechanisms contribute to approximately 70% of asthma cases in children[250, 251]; therefore, understanding the particularities of different populations in relation to allergic sensitization profiles is essential to increase our knowledge not only in terms of mechanisms, but also to investigate how primary and secondary prevention can be more effective in identifying children at high risk of disease. In **study III**, the emphasis was on secondary prevention by evaluating evidence of the effectiveness of allergen immunotherapy in preventing asthma onset. In tertiary prevention, the disease has already been diagnosed and the goal is to provide adequate treatment to prevent its progression and mitigate complications.[249] Therefore, evaluating the treatment effects of allergen immunotherapy in terms of costs, clinical benefits, and quality of life has become crucial for comparing treatment approaches. Cost-effectiveness studies, **studies IV and V**, allow simulating the effects of a specific treatment in a hypothetical cohort of patients and comparing strategies. Ultimately, this type of studies aims to inform about the most effective approaches, both for patients and health systems, being a unique tool to improve and guide health decisions.

In **studies I and II**, a sample of participants from the G21 birth cohort was randomly selected for a screening test to assess allergic sensitization. About 40% of children were IgE sensitized by age of 10 years. Most children were sensitized to HDM, specifically to the Der p 23 component, followed by grass pollen, animal, and tree and weed pollen. This study reinforced mites as the leading cause of allergic sensitization in Portugal while in other European countries, birch and grass pollens are the major contributors.[6] Dust mites grow mostly in regions with temperate climates and damp homes.[6, 252] Houses and schools in Portugal are typically characterized by moisture, cold problems and poor ventilation, especially in winter, which may partially explain these findings.[253, 254] On the contrary, northern European regions are characterized by dry and heated interior spaces that limit the development of mites.[252] Additionally, Der p 23 was the most prevalent allergen component among sensitized children, highlighting the importance of this component in southern European regions. Der p 23 was a major sensitizer that was found in

moderate to severe patients with asthma and in household dust samples in Spain.[255, 256] Finally, differences between regions within the same country are also expected. In some central country side regions of Portugal, grasses were reported to have the highest prevalence of sensitization compared to HDM that affected only 33% of allergic patients.[257] In the same study, olive tree sensitization affected 28% of children, while in our study it was present in 6% of IgE-sensitized children. Olive trees are intensively cultivated in the interior and southern regions, and the pollen counts of grasses and trees are generally higher in these locations, which may explain some of the differences across the country.[257, 258]

In **study I**, a subsample of IgE-sensitized children was evaluated during childhood using CRD tests. Three sensitization classes were identified through LCA and defined as "no/few sensitizations", "HDM", and "grass pollen" which were grouped into five trajectories. The trajectories revealed different associations with allergic diseases, supporting clinically relevant sensitization phenotypes and their impact on the risk of developing allergic diseases. The trajectory "early persistent HDM" was positively associated with asthma and allergic rhinitis, and "HDM and grass pollen", a broader trajectory characterized by multiple sensitizations to individual components, was associated with allergic rhinitis. In **study II**, molecular characterization of allergic sensitization at 10 years was beneficial to predict asthma at 13 years old. The area under the curve was acceptable and specificity and NPV were good, but sensitivity and PPV were low. Similar to other asthma risk scores, the addition of clinically relevant risk factors, such as early wheezing and family history of allergy, improved the accuracy of the prediction model and the sensitivity reached 75%, a superior performance compared to other risk scores. These results were reinforced by a subsample of data available at 4 and 7 years of age and are in line with the results of **study I**. Both studies provide important evidence to improve health prevention plans and identify children at high risk of developing allergic outcomes earlier.

Secondary prevention of asthma with AIT was evaluated in **study III**. This study suggested a preventive effect of AIT on the development of asthma. The preventive effect of AIT was remarkable in children, mono-sensitized patients, and when administered for at least three years, regardless of allergen (grass pollen or HDM) and administration route (SCIT, SLIT tablets, or SLIT drops), indicating an overall effect of this therapy on the outcome of interest. The results were robust for the short-term (three years of follow-up). It can be argued that secondary prevention of asthma using AIT has its best chance of stopping the progression of allergic multimorbidities when prescribed early in life, particularly, at the onset of sensitization and if other allergic diseases are present. Furthermore, the identification of high-risk children (**studies I and II**) might be relevant to improve the prescription and effectiveness of secondary prevention using AIT.

In **studies IV and V**, the cost-effectiveness of AIT in the treatment of HDM-driven allergic asthma and grass pollen-induced allergic rhinitis, respectively, was evaluated by developing a

Markov model adapted to the Portuguese context. AIT, both for subcutaneous and sublingual administration, appears to be cost-effective in children with moderate HDM-driven allergic asthma, considering a CE-threshold in Portugal of 18,482.80€ (ICER of 1,281€ for SCIT, and 7,717€ for SLIT, per QALY gained). The core parameters that contributed to these ICER values were the effects of AIT in reducing asthma-related exacerbations and asthma symptomatic treatment. Considering the same CE-threshold, SCIT and SLIT seem to be cost-effective in children with grass pollen-induced allergic rhinitis (ICER of 6,318€ and 12,605€, per QALY gained, respectively). The key factors were the reduction of asthma-related costs due to the prevention of asthma cases and the lower acquisition price of SCIT. In both studies, sensitivity analysis demonstrated the robustness of the results, particularly in patients with HDM-driven allergic asthma.

6.2. Impact on allergic diseases of childhood allergic sensitization characterized by component-resolved diagnosis (studies I and II)

The characteristics of allergic sensitization are complex and vary by regions[6, 259, 260]; therefore, characterizing sensitization trajectories in different populations is essential to identify differences and evaluate the impact on the development of allergic outcomes. In **study I**, we evaluated for the first time the trajectories of allergen-specific IgE sensitization in a southern European country using CRD. Some of the results were in agreement with other European studies, particularly, Germany, UK, and France.[67, 79, 81, 83] First, as the age of assessment increases, the proportion of allergic sensitized children increases, as does the number of allergen components a person is sensitized to, which is remarkably represented in children of the trajectories "HDM and grass pollen" and "late grass pollen". However, only the first trajectory was associated with allergic rhinitis reported at the 13 years follow-up, which may indicate the relevance of co-sensitization to HDM and grass pollen. Two previous studies defined a similar trajectory named "severe atopy" characterized by sensitization to a variety of perennial and seasonal allergens and found positive associations with rhinitis.[79, 81] Second, the "early persistent HDM" trajectory was positively associated with rhinitis and the only trajectory with a positive association with asthma. Sensitization trajectories in the MAAS study revealed similar findings[7, 8, 67]; specifically, a profile towards full sensitization to HDM components showed the strongest association with asthma[67]. In **study I**, at 4 years of age, a considerable proportion of children was already sensitized to multiple components of HDM, which seems to impose a higher risk of developing asthma later in life. *Posa et al*. characterized 722 participants in the German MAS birth cohort using 12 molecules of *Dermatophagoides pteronyssinus*.[83] Sensitization started with Der p 1, Der p 2, and Der p 23, as in our study, but later expanded to other molecules not represented in the ISAC array, such as Der p 5, Der p 7, Der p 4, Der p 21 and others. Early onset of sensitization was associated with a broader polymolecular profile, and participants in this trajectory were at higher risk for

rhinitis and asthma, as demonstrated in our study despite the underrepresentation of HDM molecules to confirm this reasoning. Finally, in Singapore, a non-European country, "early food and mite" sensitization was associated with an increased risk of wheezing at 3, 5 and 8 years of age and eczema in the first 8 years.[87]

The results of **study I** also highlight some differences, specifically, with northern European countries. In Finland, a "HDM"-related class was not found and "birch pollen", "animals", and "severe" classes were associated with wheeze, allergic rhinitis and eczema.[81] The "severe" class in this group comprised seasonal (timothy and birch pollen) and perennial (cat and dog) allergens. In Estonian children from the same study, a latent class characterized by HDM and cat allergens was associated with wheeze and allergic rhinitis, and the "severe" class, characterized by specific IgE to cat, dog, HDM, birch and grass allergens, was associated with wheeze, allergic rhinitis and eczema.[81] In **study I**, children co-sensitized to grass pollen and mite molecules ("HDM and grass pollen" trajectory) were not at a higher risk of developing asthma. In other study conducted in Danish and Swedish children with asthmatic mothers, sensitization rates against grass and birch pollen were higher and associations with both asthma and rhinitis were found. However, in **study I**, the trajectory "HDM and grass pollen" was associated only with allergic rhinitis, while "late grass pollen" was not associated to allergic diseases.[80]

These differences in allergic sensitization trajectories by regions and their associations with allergic outcomes should be explored during adolescence and adulthood to assess their impact across the lifespan. Previously, the evolution of total IgE was measured and characterized by an increase until the age of 10 years, a plateau until the age of 13 and a slight decrease until the age of 20 years.[261] The same evolution was demonstrated for Der p 23 sIgE with a peak in late childhood followed by a decrease.[262] A French high-risk cohort evaluated sensitization profiles in childhood (mean age [min-max]: 11 [4.5– 16] years) and 12 years later; specifically, they showed a similar molecular composition and few transitions occurred between the four profiles during the evaluation time.[84] In both cross-sectional periods, the trajectories classes "most prevalent HDM" and "many allergens" were associated with asthma and allergic rhinitis, while "pollen/animal allergens" were associated only with rhinitis, indicating possible stability of sensitization profiles and their association with allergic diseases in late adolescence/early adulthood.[84]

The information provided by CRD tests is complex and requires technical knowledge for the interpretation of results. **Study II** aimed to expand the clinical applicability of molecular allergen assays in clinical practice by combining molecular sensitization and clinical data to predict asthma in early adolescence. Different allergen components presented distinct weights for the risk of developing allergic diseases. Molecules related to HDM presented the highest weights when defining LC1 score and were associated with asthma, rhinitis and atopic dermatitis at 13 years old. Allergic sensitization to cat and dog allergens was also relevant for LC1, particularly Fel d 1. Pollens and some food components (LC2) seemed to have an additional role when uncovering

the risk of allergic rhinitis. At 4 years old, LC1 was only associated with allergic rhinitis since higher scores corresponded to allergic sensitization to both HDM and grass/tree pollen components. On the contrary, LC2 at 4 years was associated with asthma and higher scores represented sensitization to HDM components but not to grass pollen, which would reduce the score due to the negative weights, showing the importance of pollen molecules when assessing the risk of developing allergic diseases. Atopic dermatitis was less explained by latent components. These findings are corroborated by the results of **study I** and previous literature.[67, 84, 86] Specifically, *Custovic et al.*[67] found that complete sensitization to several HDM molecules posed the highest risk for the development of asthma and allergic multimorbidity. Early onset of grass sensitization was associated with asthma, rhinitis and eczema while late onset was predictive of rhinitis only. *Gabet et al.*[86] also found that HDM sensitization was the strongest risk factor for asthma and rhinitis in the PARIS cohort. A recent study conducted in Spain demonstrated that in patients with grass pollen allergy, the main predictor for asthma development was sensitization to *D. pteronyssinus*.[263] *Siroux et al.*[84] found an association between rhinitis and serum sIgE of grass pollen and animal allergens; and between asthma, with or without rhinitis, and sensitization to HDM alone or a mixture of allergens (HDM, pollens, animals, latex).

History of early atopic dermatitis was not relevant to improving asthma prediction (**study II**), but including history of early wheezing and family history of allergy improved accuracy of the prediction model by outperforming previous predictive models. The Asthma Predictive Index (API) was the first to be published and it is the most well recognized.[94] This Index focused on wheezing characteristics, history of eczema, parental history of asthma, allergic rhinitis, and eosinophilia as predictors. For the loose index, sensitivity, PPV, specificity and NPV reached 39%, 32%, 82%, and 87%, respectively, for prediction of asthma at 13 years old, indicating a high specificity but low sensitivity.[94] Different studies applying variations of API (such as mAPI and ucAPI) have been published after that, but sensitivity did not improve substantially despite an increase in the specificity (94 to 99%).[95, 96] Recently, *Myers et al.*[107] published the Paediatric Asthma Risk Score (PARS) as an improvement of the API by adding some relevant predictors; namely, sensitization to two or more food and/or aeroallergens and race. Sensitivity increased to 68% while specificity was reduced to 77% compared to API and the results were externally validated. In **study II**, by adding information on the molecular sensitization characteristics of children, we demonstrated that the accuracy of asthma prediction models can be improved. Specifically, considering data on allergen components, early wheezing, and parental history of allergy, both sensitivity and specificity improved. However, the PPV remained low, meaning that despite a positive test result, the probability of having asthma at 13 years old is low. Contrarily, a high NPV suggests that most children with a predicted negative result are not likely to develop asthma by age 13 years.

6.3. Prevention and management of allergic outcomes

6.3.1. The role of allergen immunotherapy (study III)

The systematic review and meta-analysis (**study III**) suggested a preventive effect of allergen immunotherapy on the development of asthma in high-risk individuals with other allergic diseases (rhinitis and rhinoconjunctivitis). The preventive effect of AIT was notable in children, mono-sensitized, and if administered for at least three years, independently of the allergen (grass pollen or HDM) and administration route (SCIT, SLIT tablets, or SLIT drops). The results were robust for short-term follow-up (three years). This is the first meta-analysis that combines all evidence, regardless of study design, since RCTs and NRSIs were reviewed, analyzed, and included in the analysis whenever possible. Thus, it was possible to gather data from eighteen studies, reaching a relevant number of participants with a moderate heterogeneity between included studies.

Evidence from RCTs studies was more robust when compared to NRSI studies due to the randomization process that makes results less prone to bias. Furthermore, adherence to therapy and duration of treatment according to recommendations tended to be better achieved and evaluated in RCTs, which may have contributed to the better asthma prevention effects observed in these studies. However, some of the RCTs included in this review raised some concerns due to the unblinding of participants and outcome assessors, contributing to a lower quality of evidence.[230-232, 234, 243, 245] Some authors justified the lack of use of controls due to ethical concerns about using placebo in children for a long time and difficulties in justifying its use to parents when asthma is a possible outcome.[234, 245] The recent RCT by *Valovirta et al.*[227] was performed in children using a large placebo group and was considered to be at low risk of bias. The study was primarily designed to detect differences in the onset of asthma that was defined by different criteria. When considering asthma defined by symptoms and medication use, a 33% reduction in the risk of developing asthma was reported.[227]

Evidence from NRSI is better suited to assessing the effectiveness of AIT in the real-world, but raises serious concerns regarding the selection of participants and confounding. Individuals prescribed with AIT were generally from a higher socioeconomic status and have better access to healthcare specialists, which may had overestimated the effect. However, better access to the health system reduces the probability of undiagnosed asthma in the intervention group, which may be the opposite in patients with less access or in the control group, causing a possible underestimation of the results. Another limitation is the incompleteness or absence of data on relevant prognostic variables, particularly in retrospective studies, such as multiple sensitization status, severity and duration of allergic disease for which AIT is being prescribed, and previous history of wheezing. The previous characteristics can affect and underestimate the results because patients prescribed with AIT tend to have a longer and more severe disease, previous personal or

family history of asthma, and might be sensitized to multiple allergens. The retrospective study by *Schmitt et al.*[240] was considered of moderate quality due to efforts in minimizing bias; namely, the criteria for selecting controls and adjusting the effect measure for *a priori*-defined confounders (age, sex, healthcare use, antihistamines use), as well as the criteria for outcome definition. Asthma was defined based on the registration of the ICD-10 code in the administrative database at least twice and according to the prescription of asthma medications. At baseline, patients in the AIT group were more likely to present more antihistamines prescriptions and healthcare use, being more likely to be diagnosed with asthma, which may underestimated the effect.[240] Some studies may have reported overestimated results since control patients had more severe disease (higher number of allergic rhinitis prescriptions).[238, 239]

The study by *Schmitt et al.*[240] reported a 40% decrease in asthma onset, but the result calculated in this random effect meta-analysis was based on raw event counts without any adjustments for covariates. Adjusted results highlighted a greater preventive effect for SCIT, independently of allergen (seasonal or perennial) and duration of intervention.[240] We cannot rule out indication bias, which is inherent in these studies, as well as reporting bias since patients adhering to AIT are accompanied more carefully by physicians over time and asthma diagnosis can be reported more frequently. Finally, the study considered as an outlier showed different effects on asthma prevention.[247] The authors explained that the results were due to reporting bias since patients in the intervention group visit specialists more frequently and the definition of asthma required a medical diagnosis coded in the database. This reasoning was supported by the major conclusions of the study that pointed a preventive effect of AIT in asthma progression from mild to severe.[247]

AIT can not only treat allergic diseases, but also slow down or prevent the progression of disease severity and allergic multimorbidities. This reinforces the allergic multimorbidity framework in which a single allergic disease has no priority and that allergic diseases reflect different manifestations of the same systemic inflammatory disease. Treatment of allergic multimorbidities can prevent the onset of asthma by inducing allergen-specific immune tolerance that involves innate and adaptive immune responses, impacting the underlying mechanisms of asthma development.[131, 148] Furthermore, these effects are prolonged after the stopping AIT and are explained by the increase in allergen-neutralizing antibodies such as IgG1 and IgG4.[140] IgG1 and IgG4 compete with IgE for allergen binding, avoiding the link between allergen-IgE complexes and mast cells and basophils and suppressing histamine release.[148] This neutralizing effect was correlated with clinical response in patients with allergic rhinitis.[138] Additionally, IgE-driven Th2 responses are diminished as the presentation of IgE-allergen complexes to B and T cells is reduced.[148] The effect suggested in this study was more evident for SCIT, which may be partially explained by differences in their mechanisms of action. *Shamji et al.*[144] reported a higher induction of IgA1 and IgA2 in nasal and serum samples from patients under SLIT compared to

SCIT and placebo, which were correlated with clinical symptoms.[144] The same study demonstrated increased levels of IgG and IgG4 in nasal and serum samples from patients in the SCIT group. Both antibody responses measured at baseline and one year after treatment enabled to identify non-responders to AIT. Different mechanisms of action may be involved in SCIT and SLIT and, hence, distinct biomarkers for the prognosis of treatment should be investigated and considered.[144, 264]

6.3.2. The importance of cost-effectiveness studies to improve the management of healthcare resources and allergic diseases (studies IV and V)

To the best of our knowledge, **studies IV and V** were the first studies to estimate the effects of SCIT and SLIT tablets on health and costs in children with allergic asthma and allergic rhinitis in Portugal, addressing the limitations of previous economic analyses carried out in other countries. **Study IV** was adapted from a recent work conducted by *Parra-Padilla et al.*[189] in children with asthma, but some new considerations were added to better reflect the real world. The model was restructured to allow the flow of patients between health-states representing different medication requirements as defined in GINA guidelines; therefore, medication step-up was allowed instead of medication step-down only as in the previous study. AIT compliance was included in the model as it is a major concern for clinicians, patients, and policymakers and this can have a major impact on the outcomes. AIT adherence rates significantly impacted results, highlighting the value of implementing strategies to promote adherence rates once the treatment is prescribed. An alternative scenario of full adherence to the therapy would generate an ICER of 917€ and 4,032€, per QALY gained, for SCIT and SLIT, respectively.

The results of the base case analysis in **study IV** are in agreement with those obtained for SCIT in the aforementioned study[19]; the small differences may be due to the inclusion of compliance data and medication step-up in our model, as well as the differences in the input data according to each country specifications. Another study evaluated the cost-effectiveness of SLIT in adults with HDM-driven allergic asthma in three European countries.[265] Our ICER estimate was slightly lower when compared to Czech Republic, Poland, and Slovakia (7,455€, 7,492€, and 8,814€, respectively). However, differences in the model assumptions, WTP thresholds, lack of compliance data and population's age might have led to these differences. Our results agreed with previous ones and highlighted the higher likelihood of cost-effectiveness for the subcutaneous route due to lower acquisition prices and greater improvements in health and quality of life that were amplified by better adherence rates than SLIT.

Regarding **study V**, *Vogelberg et al.*[223] performed a similar analysis in children for the sublingual administration route, in Germany. Our results were similar in terms of QALYs gained per patient and higher in terms of costs, resulting in a relatively higher ICER estimate. The differences in costs can be explained by different assumptions made in each study; that is, the

authors considered only mild cases of asthma and an additional health-state to account for the improvement in severity of rhinitis ("mild rhinitis").[223] Furthermore, our study evaluated for the first-time grass pollen SCIT in a paediatric cohort, which was shown to be more cost-effective than SLIT, particularly when evaluating the results of the sensitivity analysis and alternative scenarios. The main reasons for this effect were the lower acquisition costs of SCIT and the greater effect on asthma prevention and, consequently, on asthma-related costs, which were higher than rhinitis-related costs. This study assumes a higher relevance because it simulates a paediatric cohort of patients in which preventive effects could be more prominent; when evaluating studies conducted in adult patients, the ICER was usually higher for both strategies.[207, 266] The estimation of QALYs for children may also partly explain this difference, as the values differed from adults, highlighting the scarcity of studies conducted in children.[198]

Despite the variations in model assumptions, we sought to assess which strategy is more cost-effective in a paediatric population. Different sensitivity and scenario analyses demonstrated a favourable result towards SCIT mainly due to the lower acquisition costs, lower discontinuation rates, and greater effects on asthma prevention (**study V**) and asthma-related costs (**studies IV and V**). However, SCIT is not always the preferred route of administration in children due to frequent hospital visits, and discomfort intrinsically related to the administration of injections. New routes of administration are being developed and evaluated, such as epicutaneous, intradermal, and intralymphatic[130], but there is limited evidence of effectiveness, especially in young children in whom AIT is more likely to prevent new sensitizations and asthma.

6.4. Limitations and Strengths

The children selected and screened for **studies I and II** were from a birth-cohort and differences in socioeconomic status were observed due to losses in follow-up; children of lower socioeconomic status may be underrepresented, but significant differences in sIgE sensitization rates were not found for the upper and lower socioeconomic strata. In **study I**, the number of children with complete molecular sensitization data for the LCA analysis was limited by the serum samples and tests available for the three consecutive follow-ups. A sample of at least 300 participants is recommended to perform LCA.[267] In **study I**, data of participants from the three follow-ups (N=558) was used to identify the clusters using LCA. However, other clusters may exist if a larger population is considered. Furthermore, children included in this analysis were participants from a cohort retained through several follow-ups and presented some differences regarding the diagnosis of allergic rhinitis at 13 years old compared to children without allergic sensitization data, compromising the generalizability of the findings. Therefore, the proportion of children reporting allergic rhinitis, especially in classes characterized by higher proportions such as "HDM and grass pollen", might be overestimated. The results of **studies I and II** have not been validated in an external population with similar characteristics. In fact, the characteristics of

allergic sensitization, such as the type of allergen involved, vary by region and country as demonstrated by the Global Allergy and Asthma European Network (GA^{2}LEN) study.[6] Consequently, the results may be limited to the northern region of Portugal; even though the main allergen sensitizer in the Portuguese population is HDM, as reported in this work and previously[6, 258], differences by regions may occur, as highlighted by a study conducted in an central country side region where the main sensitization of participants was grass pollen.[257]

Another limitation of **studies I and II** is related to the method used to characterize the sensitization profiles of children. The ImmunoCAP™ ISAC multiplex array is limited to 112 components and other molecules could be relevant for establishing sensitization profiles; specifically, other HDM molecules, such as Der p 4, Der p 5, Der p 7, and Der p 21.[83] It should be noted that allergen-specific IgE measurements with ImmunoCAP™ ISAC may underestimate the levels of sIgE due to competition of allergen-specific IgG4 for allergen binding within the assay, as allergen concentrations in the assay are low.[194, 268] The clinical relevance of positive allergen sIgE responses was not evaluated and it may vary according to the allergens in question and geographic location.[6] This differential distribution of the clinical relevance by allergen was suggested to be partially explained by differences in their allergenicity.[269, 270] Lastly, allergic sensitization data assessed earlier in life (before 4 years old) were not available and results demonstrated that the time of onset seems relevant for the development of allergic diseases.[67]

It should be recognized that the number of classes identified in **study I** using LCA did not vary in time, while participants were allowed to moving between classes. In LCA, individuals were attributed to classes based on probabilities calculated according to the scores they have on variables (allergen components) and the probability of participants being classified in other classes was not considered; still, the average probability of classification in the three classes considered in this study was higher than 96%. In addition, LCA handles with categorical variables and the quantitative specific-IgE data might also have a role when defining trajectories.[67, 267] An advantage of this statistical approach is the "person-oriented analysis" that allows grouping individuals based on their characteristics rather than a "variable-centric approach" that prioritizes the relationship between variables.[267] In **study II**, PLS was chosen over principal component analysis (PCA) to maximize the variance of dependent variables that are explained by a set of independent variables.[271] However, the percentage of variance explained by the latent components was low, which may affected the final estimates, since some allergen components could be underrepresented in the chosen latent classes. Nevertheless, an internal validation of the PLS analysis was performed to confirm the optimal number of LCs. Using two different approaches, an unsupervised in **study I** and supervised in **study II**, demonstrated the consistency of the findings, since results and interpretability pointed in the same direction.

In **studies I and II**, allergic outcomes were self-reported by the child's legal guardians using questionnaires based on the ISAAC study and other definitions of disease or severity were not

considered; therefore, over- or under-reporting cannot be ruled out. *Silva et al*.[272] demonstrated that asthma defined solely on the basis of a reported medical diagnosis is not completely reliable for estimating current asthma as a composite score including medical diagnosis, symptoms, and lung function. Other confounding variables not considered or unmeasured may have affected the estimates presented in **study I**. Family history of allergy only included data from one of the parents who completed the questionnaire at baseline and possibly is underreported. Additional immunological and clinical parameters not considered in **study II** may increase the accuracy of this predictive model, such as exhaled VOCs and gene expression.[102] However, the assessment of several parameters in clinical practice may compromise their applicability. Despite some improvement in sensitivity compared to similar predictive models, PPV remained low[94, 95, 107]; in other words, the proportion of children who will develop asthma and who will return a positive result increased, but a high number of these children will not develop asthma because the probability of developing asthma given a positive result remained low (around 30%).

Previous studies carried out in Portugal to assess allergic sensitization were performed in patients with a history of allergic diseases or allergic complaints.[257, 258, 273, 274] **Studies I and II** were performed on a sample of Portuguese children from a well-characterized population-based birth cohort. Children were first screened using an extract-based approach to measure sIgE levels, and then, among children who tested positive, a subsample was selected to analyse allergen-specific IgE components using CRD. The discrepancies between the two methods, one based on extracts and the other on allergen molecular components, were expected because the composition of the molecular array is limited to 112 components and some may not be represented as in molecular extracts.[10] Extract-based tests can also be positive by recognizing minor components and/or highly cross-reactive molecules rather than major and genuine molecules; for example, pan-allergens and cross-reactive carbohydrate determinants (CCD).[10] Therefore, measuring of allergen-specific IgE components using a CRD approach allowed to identify cross-reactive, minor and major allergen components, and provided a deeper understanding of individual sIgE responses. The selection method for including participants based on sensitization data at 10 years of age may have excluded children with transient sensitization at an earlier age. Transient sensitization to food allergens is common, but less studied for inhalant allergens.[275, 276] Nevertheless, early-transient sensitization has not been associated with allergic diseases in previous studies.[277, 278]

The trajectories of allergen-specific IgE responses in Portuguese children were presented for the first time in **study I**. The same participants were followed and evaluated at three time points in childhood, and allergic outcomes were reported from childhood to early adolescence. The characterization of sensitization classes and differences for health outcomes were similar at 4, 7, and 10 years old. Finally, the associations were determined within a subpopulation of IgE sensitized children screened at 10 years of age and did not include children that were negative for

the screening test, which in turn could have increased the associations found if they were included as a reference class.

Study II introduced an approach to incorporate molecular assays into clinical practice, although this method is not currently used as a routine test in the country due not only to high costs, but also the high level of expertise required to interpret the results. The composition of latent components and subsequent interpretation were supported by the literature and the results of **study I**. Common allergic diseases other than asthma were considered in the PLS analysis to improve weights assigned to allergens in each latent component and the interpretability of the PLS scores within a multimorbidity framework. Finally, the model was tested using allergic sensitization data at different follow-up times, and the results were similar.

The main limitation of the meta-analysis (**study III**) was the low number of high-quality studies included, as only one was considered to be at low risk-of-bias, which was highlighted in the sensitivity analysis. However, the biological plausibility of the effects of AIT in allergic diseases and the absence of studies pointing to an increased risk of AIT use corroborated the results. The small sample size of most RCTs contributed to the imprecision (wider confidence intervals) of these studies. The measure of effect generated in the NRSI was based on the raw results presented in each study. Four NRSI presented an adjusted relative risk ratio that was systematically lower than the relative risk obtained in this meta-analysis; thus, the results provided by these studies in the pooled meta-analysis may be underestimated. Still, the results of NRSI should be analyzed with caution, keeping in mind the risk of bias and confounders. Another limitation is the fact that in few studies the onset of asthma was a primary or a secondary endpoint, highlighting the need for studies specifically designed to assess the effects of AIT on asthma prevention. The definition of asthma varied between studies; specifically, when using a more complete definition of asthma (including symptoms, medication, and lung function) the results in asthma prevention were not significant. Although the results suggested publication bias, the evaluation using the trim-and-fill method to calculate and estimate the impact of small studies missing from the analysis did not change the estimate. Lastly, the subgroup analysis according to the route of administration of SLIT (drops or tablets) was hampered by the small number of studies.

Nevertheless, the strengths of **study III** must be recognized. The search strategy and eligibility criteria were comprehensive as NRSI evidence was included, which provided real-world evidence (RWE) of AIT considering a larger number of participants. These studies were important in contrasting the evidence from the RCTs because they reflect the complexity of clinical settings.[279] The results of AIT in clinical practice depend on the characteristics of the population, such as patient preferences, comorbidities, lifestyle, personal expectations and priorities, and the accessibility to a correct diagnosis and treatment. The overall heterogeneity between studies was moderate and may be explained by differences in study design, duration of

therapy, time of follow-up, and population considered in each study. The detailed subgroup analysis allowed the scrutiny of data, unravelling important clinical implications and supporting the consistency of the main results. For the first time, a meta-analysis pooled separate effect estimates for SLIT tablets and SLIT drops. Finally, it should be highlighted that AIT is currently not recommended in healthy individuals or in those with atopic dermatitis.[280] Still, **study III** included evidence from studies performed in the aforementioned population to ensure a comprehensive review of the literature.

Regarding the limitations of **studies IV and V**, both models were applied in the Portuguese context, but these can be adapted to other settings (hospital, region or country, for example) that need to estimate the ICER of immunotherapy in patients with asthma (**study IV**) or rhinitis (**study V**). In practice, this can be achieved by changing the values of the model parameters according to the specific data available for that setting, such as adherence rate and drug costs, for example. Additionally, these results provided relevant insights for clinicians and policymakers regardless of the country. As a direct limitation of the Markov models applied in **studies IV and V**, we should be aware that we calculated the expected costs and health benefits by simulating the progression of the disease based on the literature findings and we are not following each patient, as the model is memoryless.[163] Markov models are highly dependent on the underlying assumptions and data retrieved from the literature; this was demonstrated by the DSA analysis because some parameters led to considerable variations in cost and effect differences and, consequently, in the ICER. Specifically, in **study IV**, transition probabilities were calculated based on studies carried out in children under HDM SLIT tablets or SCIT except for two probabilities due to the lack of published data; first, the transition probability from the step 4 of GINA to step 3 was the same in all strategies because we did not find specific data for AIT, second, the transition probability from step 2 of GINA to step 3 in the SLIT strategy was assumed to be the same as in the SCIT strategy. In the DSA analysis, the variation of those specific parameters did not affect the ICER estimates significantly. Furthermore, the definition of QALYs using generic or disease-specific preference instruments to defined utilities may impact the results as QALYs may be different.[173] **Studies IV and V** highlighted the need for high-quality studies evaluating the long-term effects of AIT in children with asthma and rhinitis, particularly to assess the effects on symptomatic medication and asthma progression. For example, we lacked high-quality evidence on the effects of AIT on moderate and severe exacerbations of asthma in the short- and long-term, as these are the main drivers of costs. In **study V**, the efficacy of AIT on allergic rhinitis symptoms and medication use was assumed to be the same for both strategies based on a study performed for SLIT alone.[281] A meta-analysis revealed no differences between SCIT and SLIT in standardized mean differences for rhinitis medication scores in children[282] and there are no head-to-head comparisons.

Another concern in **studies IV and V** is related to the definition of the CE threshold. We proposed a CE threshold based on the lower limit suggested by the WHO. However, this limit should be interpreted with caution since the Portuguese authorities may consider another willingness to pay value for the healthcare system.[224] Another possible threshold relies on the estimates of the individuals' willingness to pay to improve their health; this approach defines the value of health consumption from a social perspective rather than that defined for the health system.[24] We did not find this estimate for Portugal, but for Spain it ranged from 10,119€ up to 28,187€.[283] Nevertheless, the extensive presentation of the results allows the interpretation using different WTP thresholds. It was not possible to estimate the cost-effectiveness of SLIT drops due to the lack of data on health effects and discontinuation rates. Nonmedical costs such as transportation to the hospital for SCIT administration, productivity losses, and management of adverse events due to AIT were not incorporated, which may increase the ICER to some extent. We believe that the impact of this parameter would be low because the differences found between AIT and placebo in RCTs were not statistically significant.[132, 281] The risk of death due to SCIT administration was not considered, but the risk exists despite being very low.[284] Finally, the analyses were limited to a time horizon of 10 years and we cannot predict the long-term effect of asthma development in adulthood, as well as the progression of rhinitis and asthma severity.

Studies IV and V provided valuable information on costs and health effects of different therapies in a given setting, allowing their comparison. Therefore, a number of strengths need to be recognized. In both studies, discontinuation rates were considered based on real-world studies to surpass the gaps from previous cost-effectiveness studies. Whenever possible, input data were retrieved from real-world studies conducted in children with HDM-driven asthma (**study IV**) and grass pollen allergic rhinitis (**study V**). Sensitivity analyses were extensive to test the robustness of the base case model, while alternative scenario analyses were relevant to identify core parameters that can contribute to improve cost-effectiveness in the real world and guide policy decisions.

In **study IV,** as emphasized by *Parra-Padilla et al*[189], the definition of health-states according to the steps of the GINA allows their use in different contexts and captures both the complexity of the disease and the effects of interventions. The length of each Markov cycle was chosen to represent the clinical response of patients and changes in medication needs according to the average number of scheduled medical appointments for asthma in Portugal. The base case model followed important conservative assumptions to avoid over or underestimation of the results. First, moving between health-states was gradual. Second, the probability of asthma remission was the same for all strategies and increased only for patients who completed three years of immunotherapy. Third, after discontinuation of AIT, patients followed the transition probabilities and health-states represented in the SOC strategy; thus, it was assumed that AIT had no effect in the subsequent years. Finally, an alternative scenario was considered that includes

costs due to allergic rhinitis, as this is a common comorbidity in children with asthma and an indication for AIT.[24] The results reinforced the cost-effectiveness of AIT, especially for SCIT, and were in agreement with the findings of the literature.[189]

In the same way, in **study V**, the parameters and assumptions of the base case model were conservative. First, the effect of AIT on asthma prevention was assumed only for the years of treatment, but if we assumed this effect in an alternative scenario for the remaining time-horizon, in patients who completed three years of treatment, the results did not differ significantly. Second, the effects of AIT on asthma prevention were recovered from the meta-analysis of **study III**, synthesizing multiple data sources to improve the precision of pooled estimates and resulting in a less optimistic estimate when compared to previous cost-effectiveness studies, avoiding overestimation of the results.[223, 285] To conclude, it was assumed that patients who discontinue AIT earlier the recommended duration would not receive any benefit from AIT (medication, QALYs, asthma prevention) thereafter.

6.5. Implications for practice and future research

The burden of allergic diseases is increasing for patients and society, with corresponding rising costs. Disease prevention is the ultimate goal of epidemiology and is important for the development and evaluation of public health policies. Although primary prevention for the whole population would be ideal, it is not feasible due to cost constraints and difficult implementation. Therefore, who needs preventive measures? The key is to identify the population that is at higher risk and eligible for secondary prevention, especially if we accurately identify those at risk earlier. Longitudinal characterization of childhood allergen-specific IgE profiles can be relevant to identify children at high risk of developing allergic diseases, improve their management at a clinical level, and provide adequate prevention health plans according to the characteristics of the populations.

Dust mites are the most frequent aeroallergen in the northern Portugal, followed by grass, furry animals, tree and weed pollen, fungi and cockroach. These data may be useful to clinicians for managing risk factors and to spread the knowledge and advantages of molecular-based diagnostic tests. At the research level, this extensive characterization can be important to design future studies and to improve our knowledge of the development of allergic diseases having in mind the specificities of allergic sensitization profiles in the country, which differs from other European regions. For pharmaceutical companies and patients, CRD may contribute to developing more precise allergen immunotherapy treatments. This study highlighted Der p 23 as an essential component in IgE-sensitized children which may contribute to improving specific immunotherapy to HDM allergy in southern European regions.

Despite similarities with studies performed in the UK and Germany, sensitization trajectories in Portugal, and possibly in other southern European countries, may differ from other European

countries, as well as their association with allergic outcomes. Longer follow-up periods and evaluations sharing similar methods for assessing allergen specific-IgE and allergic outcomes, using different populations, would provide more information to understand the impact of late-onset sensitization on the risk of allergic diseases in adolescence and adulthood. Specific molecular information of childhood allergic sensitization can improve the prediction of asthma and identify high risk children in a timely manner for secondary prevention approaches. When considering other predictive models, the sensitivity of this approach using CRD increased, but the PPV can still be improved in future works. Similar to other models, a negative test result has a high probability of ruling out the development of asthma. The need for a better characterization of who can be targeted for secondary prevention must be continuously investigated within a multimorbidity framework and considering different known and, most importantly, unknown or underexplored risk factors.

Pharmacological approaches to secondary prevention have long been explored. AIT is a promising therapy for this purpose, but the combination of evidence from different sources to support this effect was lacking. Therefore, our findings are important in terms of clinical practice because they may contribute to the further improvement of guidelines by extending the efficacy results of AIT in treating allergic diseases to its secondary preventive role in asthma. Moreover, it might be an additional reason for patients to initiate AIT and to be more compliant, although future expectations must be managed carefully as it is not fully understood how to select patients that will benefit the most from the preventive effect of AIT. Concerning research implications, our study reveals the lack of high-quality well-powered studies, regardless of study design, especially for the long-term effects after AIT discontinuation. Evidence for implementation is also lacking. RWE provided by health-related nationwide and representative databases may overcome such limitations; however, researchers must ensure a suitable and comparable control group of patients and minimize confounding effects whenever possible by employing appropriate methodologies. This study also provided data and highlighted the importance of including asthma prevention outcomes in cost-effectiveness models.

The demand for cost-effectiveness studies is increasing as health resources are limited. Health economics is important for health policy and management, as it shows which policies have the best cost-opportunity in generating health at an affordable cost, addressing health inequalities. The CEA studies in this thesis filled a gap in the literature in different ways. Firstly, in our model we included adherence rates to represent what happens in the real world. Secondly, it was simulated in a paediatric population for different administrations routes of AIT. Thirdly, we applied the model considering a Portuguese context. Both strategies were cost-effective. However, SCIT showed more favourable results and seemed to have a greater impact on clinical and quality of life outcomes. This evidence may drive future policy decisions and AIT prescribing habits. To perform reliable and accurate cost-effectiveness studies, AIT's long-term effects should

be addressed in high-quality studies. It was also demonstrated that AIT adherence rates significantly impact results, highlighting the value of implementing strategies to promote higher adherence rates.

7. Conclusions

Allergic multimorbidities need to be studied in light of the "One Health" concept when evaluating causes, prevention, and treatment strategies. Allergic sensitization profiles are different by country and regions, as well as their role as a risk factor for the development of allergic diseases. Furthermore, the demand for evidence to support secondary prevention of allergic multimorbidities and public health policies in non-communicable diseases is urgent. Based on the results presented in this thesis, the following conclusions can be drawn:

(1) Distinct allergic sensitization trajectories pose different risks in the development of allergic diseases and differed from those found in other countries, specifically, northern European countries. "Early persistent HDM" trajectory was the only positively associated with asthma at 13 years old. "HDM and grass pollen" and "early persistent HDM" trajectories were associated with rhinitis.

(2) The information provided by CRD tests is useful to improve the prediction of asthma and identify children at high risk in a timely manner. The sensitivity of the predictive model increased compared to previous ones, but the PPV can still be improved.

(3) The meta-analysis suggested a possible preventive role of AIT in the short-term and highlighted a more pronounced effect in children, mono-sensitized, and for the patients completing three years of therapy, independently of allergen used. However, we should be aware that this effect was observed when considering the diagnosis of asthma based on symptoms or medication, which may indicate that AIT can prevent the onset of asthma-like symptoms and the use of asthma medication.

(4) Considering the Portuguese health system, AIT was cost-effective in children with HDM-driven allergic asthma, especially when given by the subcutaneous route. In children with grass pollen-induced rhinitis, the probability of cost-effectiveness was lower, but still below the CE-threshold, especially for SCIT. AIT adherence rates greatly influenced CEA results, highlighting the value of implementing strategies to promote adherence rates.

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Annex 1

Table 16 – Description of the ImmunoCAP™ ISAC multiplex array – 112 allergenic components grouped by allergen source and group.

Allergenic component	Biological function	Allergen source (Species)	Allergen group
Der p 1	Cysteine protease	House dust mites (*Dermatophagoides pteronyssinus)*	Mites
Der p 2	NPC2 protein family		
Der p 10	Tropomyosin		
Der p 23	Peritrophin-like protein		
Der f 1	Cysteine protease	House dust mites (*Dermatophagoides farinae)*	
Der f 2	NPC2 protein family		
Lep d 2	NPC2 protein family	Storage mites *(Lepidoglyphus destructor)*	
Blo t 5	Unknown	Storage mites (*Blomia tropicalis)*	
Phl p 1	Beta-expansin	Timothy grass (*Phleum pratense*)	Grass pollen
Phl p 2	Grass group II/III		
Phl p 4	Berberine bridge enzyme		
Phl p 5	Unknown		
Phl p 6	Unknown		
Phl p 7	Polcalcin		
Phl p 11	Ole e 1-related protein		
Phl p 12	Profilin		
Cyn d 1	Beta-expansin	Bermuda grass (*Cynodon dactylon*)	
Fel d 1	Uteroglobin	Cat (*Felis domesticus*)	Furry animals
Fel d 2	Serum albumin		
Fel d 4	Lipocalin		
Can f 1	Lipocalin	Dog (*Canis familiaris*)	
Can f 2	Lipocalin		
Can f 3	Serum albumin		
Can f 4	Lipocalin		
Can f 5	Kallikrein		
Can f 6	Lipocalin		
Equ c 1	Lipocalin	Horse (*Equus caballus*)	
Equ c 3	Serum albumin		
Mus m 1	Lipocalin/Urinary prealbumin	Mouse (*Mus musculus*)	
Ole e 1	Common olive group 1	Olive (*Olea europaea*)	Tree pollen
Ole e 7	Putative non-specific lipid transfer protein		
Ole e 9	1,3-beta glucanase		
Bet v 1	PR-10, Bet v 1 family member	Birch (*Betula verrucosa*)	
Bet v 2	Profilin		
Bet v 4	Polcalcin		
Cup a 1	Pectate lyase	Cypress (*Cupressus arizonica*)	

Cry j 1	Pectate lyase	Sugi or japanese cedar (*Cryptomeria japonica*)	
Aln g 1	PR-10, Bet v 1 family member	Alder (*Alnus glutinosa*)	
Cor a 1 0101	PR-10, Bet v 1 family member	Hazel (*Corylus avellana*)	
Pla a 1	Putative invertase inhibitor	Plane tree (*Platanus acerifolia*)	
Pla a 3	Non-specific lipid transfer protein 1		
Pla l 1	Ole e 1-like protein	English plantain (*Plantago lanceolata*)	Weed pollen
Mer a 1	Profilin	Annual mercury (*Mercurialis annua*)	
Che a 1	Ole e 1-like protein	Goosefoot (*Chenopodium album*)	
Par j 2	Non-specific lipid transfer protein	Pellitory (*Parietaria judaica*)	
Sal k 1	Pectin methylesterase family	Russian thistle (*Salsola kali*)	
Amb a 1	Pectate lyase	Ragweed (*Ambrosia artemisiifolia*)	
Art v 1	Defensin-like protein	Mugwort (*Artemisia vulgaris*)	
Art v 3	Non-specific lipid transfer protein		
Alt a 1	Unknown	*Alternaria alternata*	Fungi
Alt a 6	Enolase		
Asp f 1	Ribotoxin	*Aspergillus fumigatus*	
Asp f 3	Peroxisomal protein		
Asp f 6	MnSOD		
Cla h 8	Mannitol dehydrogenase	*Cladosporium herbarum*	
Bla g 1	Midgut microvilli protein homolog	*Blattella germanica*	Kockroach
Bla g 2	Unusual aspartic protease		
Bla g 5	Glutathione S-transferase		
Bla g 7	Tropomyosin		
Bos d 4	Alpha-lactalbumin	Cow's milk (Bos domesticus)	Food
Bos d 5	Beta-lactoglobulin		
Bos d 6	Serum albumin		
Bos d 8	Casein		
Bos d lactoferrin	Transferrin		
Alpha-Gal	Galactose-α-1,3-galactose (sugar)	Red meat (Alpha-Gal)	
Gal d 1	Ovomucoid	Egg (*Gallus domesticus*)	
Gal d 2	Ovalbumin		
Gal d 3	Ovotransferrin		
Gal d 5	Serum albumin		
Fag e 2	2S albumin	Common buckwheat (*Fagopyrum esculentum*)	
Tri a 14	Non-specific lipid transfer protein 1	Wheat (*Triticum aestivum*)	
Tri a 19 0101	Omega-5 gliadin		
Tri a aA_TI	Alpha-Amylase / Trypsin Inhibitor		
Ana o 2	11S globulin	Cashew (*Anacardium occidentale*)	
Ana o 3	2S albumin		
Ara h 1	Cupin, 7S globulin	Peanut (*Arachis hypogaea*)	

Ara h 2	Conglutin, 2S albumin		
Ara h 3	Cupin, 11S globulin		
Ara h 6	Conglutin, 2S albumin		
Ara h 8	PR-10, Bet v 1 family member		
Ara h 9	Nonspecific lipid-transfer protein type 1		
Ber e 1	2S albumin	Brazil nut (*Bertholletia excelsa*)	
Cor a 1 0401	PR-10, Bet v 1 family member	Hazelnut (*Corylus avellana*)	
Cor a 8	Non-specific lipid transfer protein type 1		
Cor a 9	11S globulin		
Cor a 14	2S albumin		
Jug r 1	2S albumin	Walnut (*Juglans regia*)	
Jug r 3	Non-specific lipid transfer protein type 1 (nsLTP1)		
Ses i 1	2S albumin	Sesame (*Sesamum indicum*)	
Pen m 1	Tropomyosin	Shrimp (*Penaeus monodon*)	
Pen m 2	Arginine kinase		
Pen m 4	Sarcoplasmic calcium binding protein		
Gad c 1	Parvalbumin	Codfish (*Gadus callarias*)	
Ani s 1	Serine protease inhibitor	Herring worm (*Anisakis simplex*)	
Ani s 3	Tropomyosin		
Gly m 4	PR-10, Bet v 1 family member	Soy bean (*Glycine max*)	
Gly m 5	Beta-conglycinin, 7S globulin		
Gly m 6	Glycinin, 11S globulin		
Api g 1	PR-10, Bet v 1 family member	Celery (*Apium graveolens*)	
Act d 1	Cysteine protease (actinidin)	Kiwi fruit (*Actinidia deliciosa*)	
Act d 2	Thaumatin-like protein		
Act d 5	Kiwellin		
Act d 8	PR-10, Bet v 1 family member		
Mal d 1	PR-10, Bet v 1 family member	Apple (*Malus domestica*)	
Pru p 1	PR-10, Bet v 1 family member	Peach (*Prunus persica*)	
Pru p 3	Non-specific lipid transfer protein 1		
Hev b 1	Rubber elongation factor	Rubber tree (*Hevea brasiliensis*)	Latex
Hev b 3	Small rubber particle protein (SRPP)		
Hev b 5	Acidic protein, unknown function		
Hev b 6	Hevein precursor		
Hev b 8	Profilin		

Annex 2

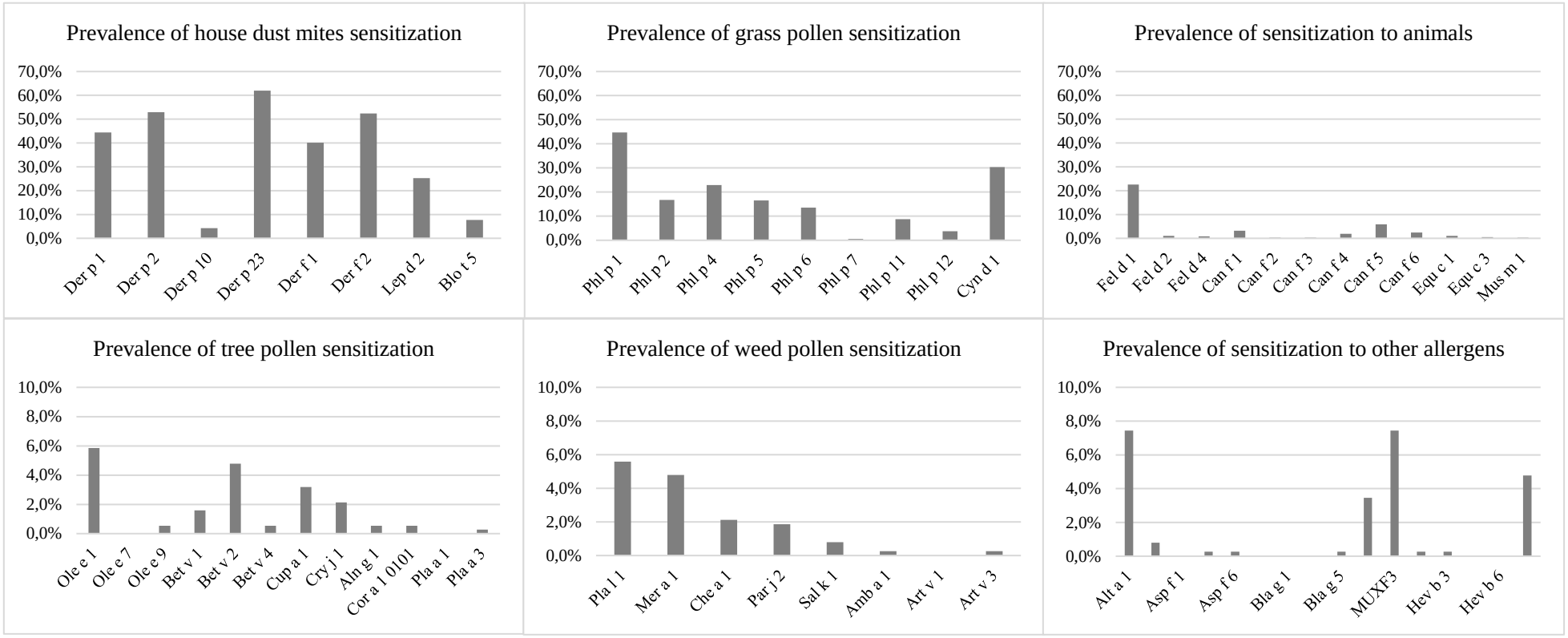

Figure 16 - Prevalence of IgE sensitization by allergenic component grouped by main sources (HDM, grass pollen, animals, tree pollen, weed pollen, and others) among IgE-sensitized children from Generation XXI tested with ImmunoCAP™ ISAC (N=376). X-axis: allergen components; y-axis: percentage of sensitized children.

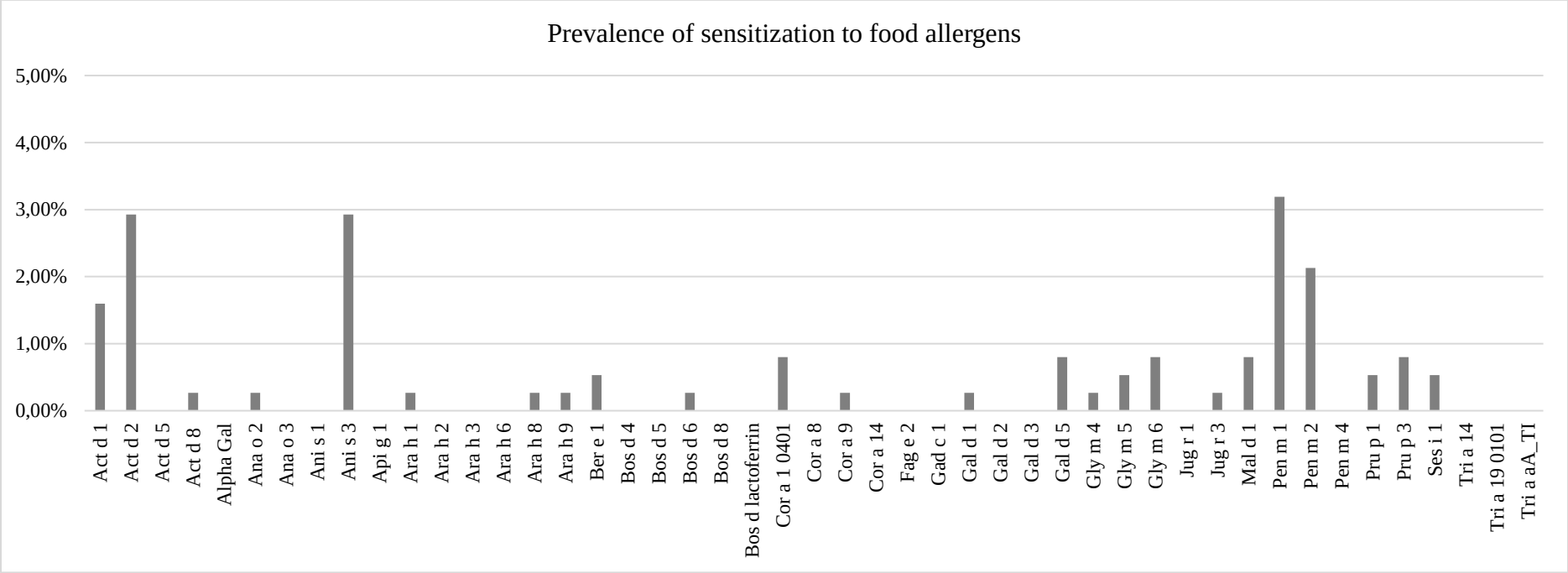

Figure 17 - Prevalence of IgE sensitization by food allergen (allergenic components) among IgE-sensitized children from Generation XXI tested with ImmunoCAP™ ISAC (N=376). X-axis: allergen components; y-axis: percentage of sensitized children.

Annex 3

Table 17 - PLS weight scores of allergenic components (molecules with weights ≤-0.100 or ≥0.100 are in a colour scale) by latent component (LC1, LC2) and time of assessment (4, 7, 10 years). Y loadings and % of variance explained results are presented in the end of the table by latent component and according to allergic diseases (asthma, rhinitis, eczema) at 13 years old.

Molecules	4 years		7 years		10 years	
	LC1*	LC2	LC1*	LC2	LC1	LC2
Act d 1	0.016	-0.094	0.015	-0.048	0.087	-0.116
Act d 2	-0.033	-0.014	0.059	-0.078	0.141	-0.135
Act d 8					-0.114	0.048
Aln g 1	0.074	0.012	-0.105	0.045	-0.110	0.077
Alt a 1			0.012	-0.102	0.074	-0.060
Alt a 6					-0.003	0.030
Amb a 1					0.023	-0.006
Ana o 2			-0.008	-0.328	0.093	-0.287
Ani s 3	-0.058	-0.002	0.052	0.005	0.121	0.132
Api g 1			-0.105	0.045		
Ara h 1					0.093	-0.287
Ara h 8					-0.114	0.048
Ara h 9	0.127	-0.238			0.137	-0.255
Art v1			-0.057	-0.020		
Art v 3					0.137	-0.255
Asp f 3					-0.042	-0.006
Asp f 6	0.003	0.007			0.048	-0.063
Ber e 1					-0.031	-0.020
Bet v 1			-0.105	0.045	-0.048	0.081
Bet v 2	0.161	-0.312	0.104	-0.160	-0.085	-0.064
Bet v 4					-0.064	-0.024
Bla g 5					0.085	0.060
Bla g 7	-0.058	-0.002	0.091	-0.012	0.139	0.141
Blo t 5	0.090	0.044	0.189	0.055	0.144	0.111
Bos d Lacto			0.221	0.103		
Bos d 4	0.019	0.062				
Bos d 6					-0.008	-0.043
Can f 1	0.085	-0.006	0.155	0.002	0.215	-0.039
Can f 2	0.151	0.110	0.312	0.073	0.144	0.077
Can f 3			0.043	0.042	-0.008	-0.043
Can f 4	0.166	0.117	0.193	0.008	0.072	0.015
Can f 5			0.107	0.042	0.125	0.048
Can f 6	0.041	-0.023	0.241	0.066	0.145	-0.002

	4 years		7 years		10 years	
	LC1*	LC2	LC1*	LC2	LC1	LC2
Che a 1			-0.032	-0.083	-0.038	0.001
Cor a 1 0101			-0.105	0.045	-0.110	0.077
Cor a 1 0401			-0.105	0.045	-0.085	0.093
Cor a 9	0.003	-0.061	-0.008	-0.328	0.093	-0.287
Cry j 1			0.023	-0.118	0.127	-0.069
Cup a 1	0.127	-0.238	0.015	-0.098	0.111	0.033
Cyn d 1	0.142	-0.242	0.085	-0.245	0.089	-0.147
Der f 1	0.359	0.137	0.352	-0.012	0.322	0.090
Der f 2	0.411	0.105	0.345	0.009	0.346	0.119
Der p 1	0.395	0.111	0.339	-0.033	0.306	0.082
Der p 10	-0.058	-0.002	0.081	0.015	0.143	0.137
Der p 2	0.407	0.105	0.336	0.009	0.345	0.120
Der p 23	0.346	0.138	0.269	0.005	0.235	0.149
Equ c 1	-0.009	-0.028	0.187	0.103	0.053	0.029
Equ c 3					-0.014	-0.031
Fel d 1	0.194	0.036	0.230	-0.037	0.236	0.033
Fel d 2			0.043	0.042	-0.076	0.000
Fel d 4	-0.009	-0.028	0.064	0.014	0.061	-0.044
Gad c 1	0.032	-0.020	0.038	-0.090		
Gal d 1	0.003	-0.061	-0.008	-0.328	0.093	-0.287
Gal d 2	0.080	-0.001	0.051	-0.053		
Gal d 5			-0.018	-0.270	-0.025	-0.159
Gly m 4			-0.105	0.045	-0.114	0.048
Gly m 5			-0.008	-0.328	0.086	-0.188
Gly m 6	0.003	-0.061	-0.008	-0.328	0.132	-0.192
Hev b 1	0.053	0.012	0.052	-0.045	0.108	-0.028
Hev b 3					0.108	-0.028
Hev b 8	0.161	-0.312	0.071	-0.144	-0.088	-0.069
Jug r 3					0.137	-0.255
Lep d 2	-0.012	0.009	0.039	0.045	0.177	0.116
Mal d 1					-0.085	0.093
Mer a 1	0.161	-0.312	0.071	-0.144	-0.085	-0.064
Mus m 1	0.096	-0.036	0.052	-0.138	0.001	0.012

	4 years		7 years		10 years	
	LC1	LC2	LC1*	LC2	LC1*	LC2
MUXF3	0.032	-0.020	0.026	-0.187	-0.005	-0.090
Ole e 1	0.127	-0.238	0.142	-0.101	0.114	0.003
Ole e 7	0.127	-0.238				
Ole e 9					0.047	-0.002
Par j 2			-0.029	-0.012	-0.024	0.010
Pen m 1	-0.058	-0.002	0.074	0.019	0.138	0.136
Pen m 2					0.009	0.047
Phl p 1	0.211	-0.135	0.045	-0.213	0.064	-0.127
Phl p 11			-0.056	-0.087	-0.033	-0.041
Phl p 12	0.161	-0.312	-0.007	-0.184	-0.063	-0.069
Phl p 2	0.150	-0.315	0.013	-0.247	0.004	-0.087
Phl p 4	0.100	-0.174	0.018	-0.254	0.037	-0.161
Phl p 5	0.044	-0.215	0.045	-0.165	-0.007	-0.098
Phl p 6	0.161	-0.312	0.006	-0.221	-0.021	-0.100
Phl p 7					-0.046	-0.037
Pla a 3					0.137	-0.255
Pla l 1	0.128	-0.271	-0.009	-0.171	-0.048	-0.056
Pru p 1					-0.110	0.077
Pru p 3	0.061	0.053	-0.032	0.018	0.135	-0.339
Sal k 1					-0.019	-0.021
Ses i 1			-0.008	-0.328	0.060	-0.233
Tri a 14	0.127	-0.238				
Colour scale						
>0.250	0.200 to 0.250		0.150 to 0.200		0.100 to 0.150	
<-0.250	-0.200 to 0.250		-0.150 to 0.200		-0.100 to 0.150	
% variance explained						
Asthma	10.13	18.64	18.65	20.32	7.77	11.61
Rhinitis	20.67	22.28	8.31	15.68	7.66	9.19
Eczema	2.53	2.55	3.55	7.03	3.66	6.55
Y Loadings						
Asthma	0.18	0.14	0.22	0.07	0.14	0.09
Rhinitis	0.18	-0.04	0.10	-0.10	0.10	-0.04
Eczema	0.06	0	0.07	-0.07	0.07	-0.06

*The values from this latent component were inverted (negatives to positives, positives to negatives) to allow a better interpretation of results, including Y loadings.

Original Publications (I, III – V) and Copyrights

Scientific Publication I

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ORIGINAL ARTICLE

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Sensitization trajectories to multiple allergen components in a population-based birth-cohort

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Abstract

Background: The characteristics of allergic sensitization profiles can differ between populations and geographic regions, contributing differently to the association with allergic diseases. Consequently, the sensitization trajectories found in previous studies conducted in Northern Europe may not apply in Southern European countries.

Objective: To identify trajectories of allergic sensitization profiles during childhood and evaluate the association with allergic outcomes, using data from a Portuguese birth cohort.

Methods: A random sample from Generation XXI was screened for allergic sensitization at 10 years of age. Among 452 allergic sensitized children, 186 were tested with ImmunoCAP™ ISAC multiplex array that detects 112 molecular components, at three follow-ups (4, 7, and 10 years old). Information on allergic outcomes (asthma, rhinitis, atopic dermatitis) was obtained at the 13-year-old follow-up. Latent class analysis (LCA) was used to identify clusters of participants with similar sensitization profiles. Then, sensitization trajectories were defined based on the most prevalent transitions between clusters over time. Logistic regression was applied to estimate the association between sensitization trajectories and allergic diseases.

Results: Five trajectories were proposed: "no/few sensitizations," "early persistent house dust mites (HDM)," "early HDM and persistent/late grass pollen," "late grass pollen," and "late HDM." The "early HDM and persistent/late grass pollen" trajectory was associated with rhinitis and "early persistent HDM" with asthma and rhinitis.

Conclusion: Distinct sensitization trajectories pose different risks in the development of allergic diseases. These trajectories present some differences from those in Northern European countries and are important for planning adequate prevention health plans.

KEYWORDS
allergen, allergic diseases, asthma, atopic dermatitis, birth-cohort, molecular sensitization, rhinitis, sensitization trajectories

1 | INTRODUCTION

Atopy is a strong risk factor for the development of allergic diseases and multimorbidities, and both have risen in recent decades.[1–3] The cause for this increase has been attributed to changes in environmental exposures and lifestyle.[4–6] However, the effects of environmental exposures have generated inconsistent results; for example, the effect of green spaces and air pollution on developing allergic sensitization and rhinitis.[7–9] These disparities can be explained by differences in geographic location, time or method of exposure/outcome assessment, study participants, outcome definition, or even diet, which may modulate the effects of air pollution on asthma, but the definition and diagnostic accuracy of atopy also play a role in this phenomenon.[10–13]

The development of allergic sensitization and diseases usually occurs during childhood and can persist throughout life, causing a significant burden on the daily lives of patients.[14] *Simpson* et al.[12] demonstrated that allergic sensitization assessed as a dichotomous variable may over aggregate the underlying complexity of sensitization to multiple allergen components. Later, *Hatzler* et al.[15] showed that early IgE sensitization to timothy grass pollen can start before the first symptoms of seasonal allergic rhinitis, predicting the future onset of disease. Recent work on the trajectories of allergen component-specific IgE demonstrated that different trajectories associated with specific allergic outcomes.[16–18]

To better understand the development and rising of allergic diseases, we should further explore the multiple atopic vulnerabilities using allergen-specific IgE profiles,[12] considering different populations because sensitization rates and allergens involved in allergic sensitization of individuals are different and vary by geographic region.[10] Thus, we hypothesize that sensitization profiles and trajectories found in previous studies conducted in Northern Europe may not apply in Southern European countries. Therefore, our aim was to identify the sensitization trajectories of allergen component-specific IgE profiles in childhood and evaluate the association with allergic outcomes, using a Portuguese population-based birth cohort.

Key Message

Allergic sensitization trajectories in southern European countries may differ from those in the north, as well as the impact on the development of allergic diseases, providing relevant information for the elaboration of adequate health prevention plans.

2 | METHODS

2.1 | Study participants

The study used data collected as part of the evaluations of Generation XXI (G21) birth cohort, which includes 8495 mothers and 8647 newborns delivered in the Porto Metropolitan Area in Portugal. The recruitment took place between April 2005 and September 2006 at all five available public maternity units, where 95% of the region's births occurred. All participants were invited to be re-evaluated at 4, 7, 10, and 13 years of age corresponding to a participation of 86%, 80%, 74% and 54%, respectively. The assessment at age 13 was interrupted in March 2020 due to the COVID-19 pandemic, explaining the low participation in this follow-up. More details regarding the cohort have been previously published.[19,20] Ethical approval for the study was obtained from the Ethics Committee of the University Hospital Center of São João (CES-01/2017) and signed informed consent was obtained from all participant's caregivers. All phases of the study followed the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki.

Among 6397 children evaluated at the 10-year follow-up visit, 4047 collected blood samples, and 1125 were randomly selected by a computer-generated randomization sequence and screened for measurement of sIgE levels using Phadiatop Infant. From this subsample, 452 children (40%) were positive for sIgE and 186 of these had serum samples available at 4- and 7-years old evaluation; thus, these children were further characterized using the ImmunoCAP® ISAC to identify sIgE antibodies against 112 components of food and airborne allergens at the three follow-ups. The flowchart of participants is presented in Figure 1. The description of allergic

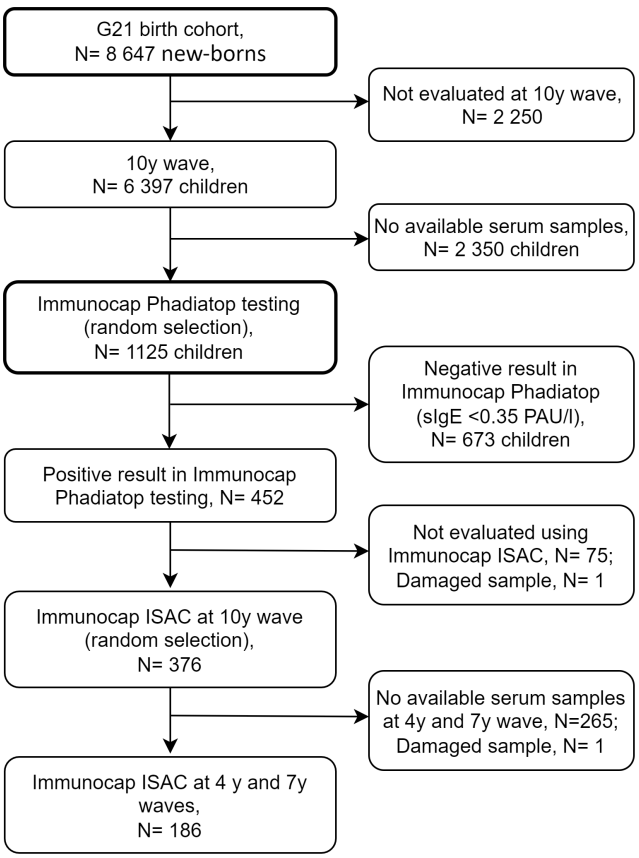

FIGURE 1 Flow diagram of participants included in the study.

sensitization assessment using Phadiatop Infant and ImmunoCAP® ISAC tests is in the Supplemental Material.

2.2 | Statistical analysis

The data analysis was performed using the RStudio Software version 4.1.3 (R Foundation for Statistical Computing, Vienna, Austria) with the poLCA package. The characteristics of participants were compared using Chi-square test and a *p*-value ≤.05 was considered statistically significant. Quantitative results from the multiplex array ImmunoCAP™ ISAC were categorized according to the threshold suggested by the manufacturer instructions (≥0.35 PAU/l [Pharmacia Arbitrary Units per liter]) and are presented in Appendix S1: Figure S1 and S2.

Latent class analysis (LCA) was used to identify clusters of individuals based on component-specific IgE to different allergens using all the measurements of the three follow-ups (186 measurements at each follow-up). Among the 112 allergenic components, 30 were never positive in any child at any time. The optimal number of clusters or latent classes was defined based on Bayesian information criteria (BIC), starting from one class and increasing until the number of classes did not lead to a decrease in BIC. The rank of probabilities attributed to each allergen component was used to interpret and label the clusters (based on probabilities higher than 0.500). Children were then assigned to each cluster according to the highest probability of class membership. At each follow-up, demographic and clinical characteristics were compared between clusters using Chi-square and Kruskal–Wallis tests for categorical and continuous variables, respectively.

After the classification of the individuals in clusters at each follow-up, the number and proportion of transitions between clusters over time were obtained. Sensitization trajectories were determined based on the stabilization of individuals classified in the same cluster throughout the follow-up and according to the most prevalent transitions between clusters, regardless of the time at which they occurred. The trajectories were characterized and compared using Chi-square and Kruskal–Wallis tests, as appropriate. Associations between sensitization trajectories and allergic outcomes (asthma, allergic rhinitis, atopic dermatitis) at 13 years old were estimated by crude and adjusted odds ratios (OR) and respective 95% confidence interval (95% CI) using logistic regression models. Sex, parents' history of allergy, and maternal education were considered in the adjusted models.

3 | RESULTS

The baseline characteristics of participants selected for the screening test (*N* = 1125) and the remaining cohort revealed that children screened at 10 years old were from a higher socioeconomic status (Appendix S1: Table S1). The baseline characteristics between IgE sensitized children with complete molecular sensitization data included in this study (*N* = 186) and those excluded due to lack of molecular sensitization testing (*N* = 266), presented in Table 1, showed that the latter were more likely to report allergic rhinitis at the age 13 years.

LCA clustered participants into three classes, each one represented by distinct weights attributed to allergen components. Based on allergen components comprising each class, we labeled the first class as "few or no sensitizations," the second as "grass pollen," and the third as "house dust mite (HDM)" (Figure 2 and Appendix S1: Table S2). Food allergens affected few participants (Appendix S1: Figure S2) and were mostly represented in the second class followed by third class, but their weights were very low (<0.100) (Appendix S1: Table S2). The proportion of participants within each latent class at different ages is shown in Table 2, Appendix S1: Figure S3 and Figure S4. At 4 years old, 64.5% of children were classified in class 1, 2.7% in class 2, and 32.8% in class 3. The proportion of children who moved from class 1 (at 4 years old) to class 3 and class 2 (at 7 years old) was 19.2% and 9.2%, respectively. At the same follow-up, the proportion of children who moved from class 3 to class 2 was 9.8%, and from class 3 to class 1 was 3.3%. At 7 years old, the proportion of children classified in class 1 who moved to class 3 and 2, at 10 years old, was 37.5% and 14.8%, respectively. The proportion of children who moved from class 3 to class 2 was 7.9% and from class 2 to class 3 was 4.5%. The proportion of children who moved from class 3 or class 2 to class 1 was 1.3% and 4.5%, respectively. Participants' flow is represented in Figure S4.

3.1 | Longitudinal sensitization profiles

Based on the most prevalent transitions between clusters we proposed a solution of five sensitization trajectories (Figure 3). The most common transitions were participants who moved from class 1 to class 3, followed by class 1 to class 2, and class 3 to class 2 (Figure 3, Appendix S1: Figure S4). Furthermore, these transitions mainly occurred between ages 7 and 10 years. Therefore, participants who were stable at class 1 (*N* = 41) or terminated classified in this class at 10 years old (*N* = 3) were grouped as "no or few sensitizations" trajectory. Participants who were stable at class 3 (*N* = 50) or who started at 4 years and finished at 10 years in class 3 (*N* = 2) were grouped as "early persistent HDM." Children who were stable at class 2 (*N* = 5) or who moved from class 3 to class 2 at any time and remained in class 2 at 10 years old (*N* = 10) were classified into "HDM and grass pollen" trajectory. Children who moved from class 1 to class 2 and remained in class 2 were classified as "late grass pollen" (*N* = 24). Children who moved from class 1 to class 3 and remained in class 3 were classified as "late HDM" (*N* = 51). These data are summarized in Figure 3, Appendix S1: Table S3 and Figure S4.

The characterization of sensitization trajectories is presented in Table 3. Children in the "HDM and grass pollen" trajectory were had the highest number of sensitizations and 50.0% reported rhinitis at the age of 13 years, while asthma only affected 7.1%. This trajectory was positively associated with rhinitis in the adjusted model

TABLE 1 Characteristics of allergic sensitized participants at 10 years old, with (*N* = 186) and without (*N* = 266) serum samples analyzed for allergen component-specific IgE (molecular assessment) using the ImmunoCAP™ ISAC test.

	IgE sensitized children	Children with molecular sensitization data (included)	Children without molecular sensitization data (excluded)	*p*-value*
N	452	186	266	
Sex				
Girls	185 (40.9%)	81 (43.5%)	104 (39.1%)	.33
Boys	267 (59.1%)	105 (56.5%)	162 (60.9%)	
Privation index (distribution by quintiles, Q)				
Q1	67 (14.8%)	25 (13.4%)	42 (15.8%)	.38
Q2	89 (19.7%)	37 (19.9%)	52 (19.5%)	
Q3	99 (21.9%)	34 (18.3%)	65 (24.4%)	
Q4	117 (25.9%)	55 (29.6%)	62 (23.3%)	
Q5	80 (17.7%)	35 (18.8%)	45 (16.9%)	
House occupation: number of household occupants/number of rooms				
<1,5	368 (81.4%)	149 (81.4%)	219 (83.9%)	.49
>=1,5	76 (16.8%)	34 (18.6%)	42 (16.1%)	
Maternal education				
≤9 years	209 (46.2%)	87 (46.8%)	122 (45.9%)	.85
≥9 years	243 (53.8%)	99 (53.2%)	144 (54.1%)	
Type of birth delivery				
Vaginal	220 (48.7%)	90 (48.4%)	130 (48.9%)	.98
Caesarean	161 (35.6%)	67 (36.0%)	94 (35.3%)	
Other	71 (15.7%)	29 (15.6%)	42 (15.8%)	
Health outcomes				
10 years old				
Asthma, *N* = 450	65 (14.4%)	32 (17.4%)	33 (12.4%)	.14
13 years old				
Asthma, *N* = 380	67 (17.6%)	30 (19.5%)	37 (16.4%)	.44
Rhinitis, *N* = 382	82 (21.5%)	42 (27.3%)	40 (17.5%)	**.03**
Atopic dermatitis, *N* = 380	59 (15.5%)	23 (14.9%)	36 (15.9%)	.79
Information of parents				
History of allergy, *N* = 432	64 (14.8%)	27 (15.2%)	37 (14.6%)	.86

Note: Children with available data (molecular sensitization) were included in this study. Data were presented as frequencies, *N* (%).

*Comparisons of baseline characteristics and health outcomes between participants included and not included in the analysis of sensitization trajectories were assessed using chi-square tests (*p*-value ≤ .05 was considered statistically significant).

(OR, 95% CI: 12.1, 2.6–56.7, Appendix S1: Figure S5). The "early persistent HDM" trajectory was characterized by the highest proportion of children with asthma (43.9%) and was positively associated with asthma (OR, 95% CI: 14.4, 3.0–69.0) and rhinitis (OR, 95% CI: 7.3, 2.1–25.4, Appendix S1: Figure S5). The "late HDM" and "late grass pollen" trajectories were characterized by 15.8% and 14.3% of children with asthma, respectively.

4 | DISCUSSION

In this study, we present the evolution of sIgE sensitization during childhood to a comprehensive panel of 112 allergenic components in a Portuguese birth cohort. Participants were clustered in three classes using LCA that we defined as "no/few sensitizations," "HDM," and "grass pollen" and then were grouped into five trajectories. The trajectories revealed different associations with allergic diseases, supporting that distinct atopic vulnerabilities have a specific impact on the risk of developing allergic diseases. Specifically, only "early persistent HDM" trajectory was positively associated with asthma, and both trajectories of "early persistent HDM" and "HDM and grass pollen," a broader trajectory characterized by a high number of sensitizations, were associated with rhinitis.

One of the limitations of this study might be related with the characteristics of children with complete molecular sensitization data for the LCA. First, a sample of at least 300 participants is recommended to perform LCA.[21] In this analysis, the data of the 186

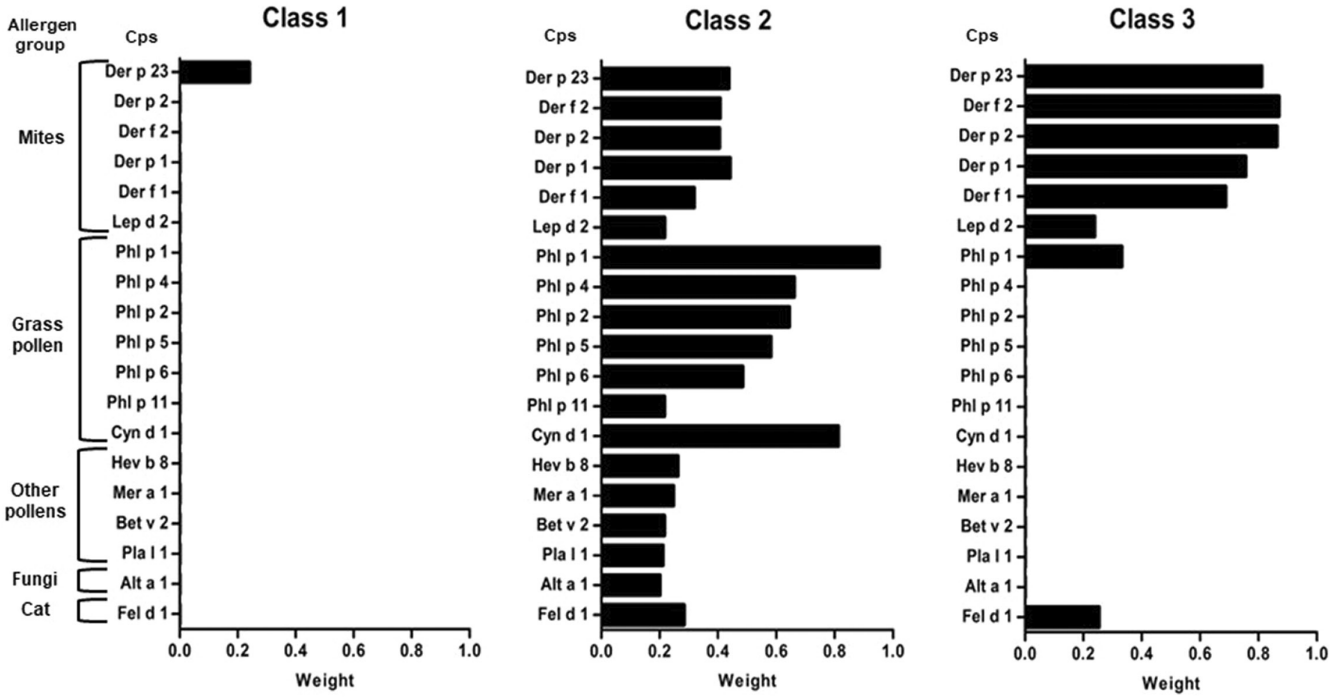

FIGURE 2 Weights of the most relevant allergenic components assigned to each latent class with probabilities equal or greater than 0.200. The data of all participants ($N = 186$) from the three follow-ups were used to identify the classes (i.e., $186 \times 3 = 558$ observations). Most relevant components were considered as those with an assigned probability equal or greater than .500 and the clusters were labeled according to them. HDM: house dust mites. Allergen nomenclature was based on scientific name of plant or animal species (in latin) and composed by three letters (genus), followed by one letter (species) and one number (usually indicates order of discovery). Alt a: *Alternaria alternata* (fungi); Bet v: *Betula verrucosa* (birch); Cyn d: *Cynodon dactylon* (Bermuda grass); Der f: *Dermatophagoides farinae* (HDM); Der p: *Dermatophagoides pteronyssinus* (HDM); Fel d: *Felis domesticus* (cat); Hev b: *Hevea brasiliensis* (rubber tree or latex); Lep d: *Lepidoglyphus destructor* (storage mites); Mer a: *Mercurialis annua* (plant pollen); Phl p: *Phleum pratense* (timothy grass); Pla l: *Platanus acerifolia* (plane tree).

participants from the three follow-ups were used to identify the clusters (completing 558 observations). However, we recognize that other clusters may exist if a larger population was considered since many allergen components were positive in few children, which may have hindered their relevance. Additionally, a different data-driven approach, such as latent transition analysis, could be able to identify relevant transitions between classes over time, but a higher sample size would be needed since it requires one LCA model at each time point. Second, the children considered for this analysis were participants from a cohort retained through several follow-ups and presented some differences regarding the diagnosis of allergic rhinitis at 13 years old compared with children with incomplete sensitization data, compromising the generalizability of the findings. Therefore, the proportion of children reporting allergic rhinitis, especially in classes characterized by higher proportions, might be overestimated. Third, we did not validate sensitization trajectories in an external population with similar characteristics. Allergic sensitization profiles vary by region and country as demonstrated by the Global Allergy and Asthma European Network (GA^{2}LEN) study.[10] Consequently, the results of this study may be limited to the Northern region of Portugal; even though the main allergen sensitizer of the Portuguese population is HDM, as reported in this work and previously,[10,22] differences by regions may occur as highlighted by a study conducted in an interior central region where the main sensitization was to grass pollen.[23] Finally, the ISAC multiplex array is limited to 112 components and other molecules might be relevant for establishing sensitization profiles.[24] In fact, a small proportion of participants positive to the screening test (extract-based) did not show any positive response to the molecular array and few individuals showed sensitization to few allergen components; therefore, were grouped in class 1 (no/few sensitizations).

We must also recognize strengths. Previous studies were performed on patients with a history of allergic diseases or complaints.[22] This study presents for the first time allergic sensitization trajectories of a population-based sample of children from a well-characterized birth cohort. The evaluation of allergen-specific IgE using a CRD approach allowed us to identify cross-reactive, minor, and major allergen components, providing a deeper understanding of individual sensitization profiles. The same participants were followed and evaluated at three-time points throughout childhood and allergic outcomes were reported from childhood to early adolescence. The characterization of latent classes and differences regarding health outcomes were similar at 4-, 7-, and 10-years old follow-ups. Finally, the associations were determined within a subpopulation of allergen sensitized children screened at 10 years of age and did not include children that were negative for the screening test, which in turn could have increased the associations found if they were included as a reference class.

TABLE 2 Distribution and characteristics of participants ($N = 186$) assigned to latent classes at 4, 7, and 10 years old according to the highest probability of class membership using LCA.

	4 years				7 years				10 years			
	Class 1	Class 2	Class 3	*p*	Class 1	Class 2	Class 3	*p*	Class 1	Class 2	Class 3	*p*
N	120	5	61		88	22	76		44	39	103	
Sex, male	67 (55.8%)	4 (80.0%)	34 (55.7%)	.56	49 (55.7%)	17 (77.3%)	39 (51.3%)	.09	24 (54.5%)	25 (64.1%)	56 (54.4%)	.56
Maternal education												
≤9 years	57 (47.5%)	1 (20.0%)	29 (47.5%)	.48	43 (48.9%)	9 (40.9%)	35 (46.1%)	.79	23 (52.3%)	18 (46.2%)	46 (44.7%)	.70
>9 years	63 (52.5%)	4 (80.0%)	32 (52.5%)		45 (51.1%)	13 (59.1%)	41 (53.9%)		21 (47.7%)	21 (53.8%)	57 (55.3%)	
Family history of allergy	16 (13.9%)	1 (20.0%)	10 (17.2%)	.81	9 (10.6%)	3 (14.3%)	15 (20.8%)	.20	4 (9.1%)	4 (10.5%)	19 (19.8%)	.17
Type of birth delivery												
Vaginal	58 (48.3%)	0	32 (52.5%)	**.02**	38 (43.2%)	3 (13.6%)	49 (64.5%)	**<.01**	21 (47.7%)	12 (30.8%)	57 (55.3%)	**.01**
Caesarean	39 (32.5%)	5 (100%)	23 (37.7%)		30 (34.1%)	18 (81.8%)	19 (25.0%)		16 (36.4%)	23 (59.0%)	28 (27.2%)	
Other	23 (19.2%)	0	6 (9.8%)		20 (22.7%)	1 (4.5%)	8 (10.5%)		7 (15.9%)	4 (10.3%)	18 (17.5%)	
Medical diagnosis of allergic conditions												
Asthma (4 y) [missings: 6]	4 (3.4%)	0	6 (10.5%)	.13	4 (4.6%)	1 (4.8%)	5 (6.9%)	.80	3 (6.8%)	0	7 (7.1%)	.24
Rhinitis (4 y) [missings: 6]	4 (3.4%)	1 (25.0%)	5 (8.5%)	.09	4 (4.6%)	2 (10.0%)	4 (5.5%)	.64	1 (2.3%)	6 (16.2%)	3 (3.0%)	**<.01**
AD (4 y) [missings: 1]	9 (7.5%)	2 (40.0%)	8 (13.3%)	**.04**	5 (5.7%)	3 (13.6%)	11 (14.7%)	.15	1 (2.3%)	5 (12.8%)	13 (12.7%)	.14
Asthma (7 y) [missings: 3]	7 (5.9%)	0	12 (20.0%)	**.01**	5 (5.7%)	1 (4.5%)	13 (17.6%)	**.03**	2 (4.5%)	3 (7.7%)	14 (14.0%)	.19
Rhinitis (7 y) [missings: 1]	10 (8.4%)	2 (40.0%)	14 (23.0%)	**.01**	7 (8.0%)	4 (18.2%)	15 (19.7%)	.08	4 (9.1%)	6 (15.4%)	16 (15.7%)	.55
AD (7 y) [missings: 1]	15 (12.6%)	1 (20.0%)	8 (13.1%)	.89	11 (12.6%)	4 (18.2%)	9 (11.8%)	.73	4 (9.1%)	6 (15.8%)	14 (13.6%)	.64
Asthma (10 y) [missings: 2]	13 (10.9%)	0	19 (31.7%)	**<.01**	9 (10.5%)	1 (4.5%)	22 (28.9%)	**<.01**	4 (9.1%)	4 (10.3%)	24 (23.8%)	**.04**
Asthma (13 y) [missings: 32]	11 (11.0%)	0	19 (38.8%)	**<.01**	6 (8.2%)	2 (10.5%)	22 (35.5%)	**<.01**	2 (5.0%)	4 (11.4%)	24 (30.4%)	**<.01**
Rhinitis (13 y) [missings: 32]	19 (19.0%)	3 (60.0%)	20 (40.8%)	**<.01**	12 (16.4%)	7 (36.8%)	23 (37.1%)	**.02**	4 (10.0%)	13 (37.1%)	25 (31.6%)	**.014**
AD (13 y) [missings: 32]	10 (9.9%)	1 (20.0%)	12 (25.0%)	.05	6 (8.2%)	4 (21.1%)	13 (21.0%)	.09	3 (7.5%)	6 (17.1%)	14 (17.7%)	.31
Multimorbidities (13 y)												
Asthma + rhinitis [missings: 33]	4 (4.0%)	0	11 (22.4%)	**<.01**	2 (2.7%)	2 (10.5%)	11 (18.0%)	**.01**	0	4 (11.4%)	11 (14.1%)	**.05**
Asthma + AD, [missings: 33]	3 (3.0%)	0	4 (8.3%)	.31	2 (2.7%)	1 (5.3%)	4 (6.6%)	.57	1 (2.5%)	2 (5.7%)	4 (5.1%)	.76
AD + rhinitis [missings: 33]	3 (3.0%)	0	9 (18.8%)	**<.01**	1 (1.4%)	2 (10.5%)	9 (14.8%)	**.01**	0	3 (8.6%)	9 (11.5%)	.09
Asthma + rhinitis + AD, [missings: 33]	2 (2.0%)	0	4 (8.3%)	.16	1 (1.4%)	1 (5.3%)	4 (6.7%)	.28	0	2 (5.7%)	4 (5.2%)	.33
Allergic sensitization to ISAC components												
Nb of sensitizations[a]	0.48 (0.89)	7.60 (3.78)	5.02 (1.81)	**<.01**	0.94 (1.09)	8.95 (3.64)	5.66 (2.06)	**<.01**	1.86 (0.98)	10.82 (4.39)	6.36 (2.45)	**<.01**

Note: Data are presented as *N* (%) unless otherwise stated. Values in bold indicate statistically significant differences for the comparison of demographic and health outcomes variables between the three latent classes (*p*-value, represented as *p*, ≤.05 was considered statistically significant).

Abbreviations: AD, atopic dermatitis; HDM, house dust mites; nb, number; y, years; Class 1, "no or few sensitizations," Class 2, "grass pollen," Class 3, "HDM."

[a]Data presented as mean (SD).

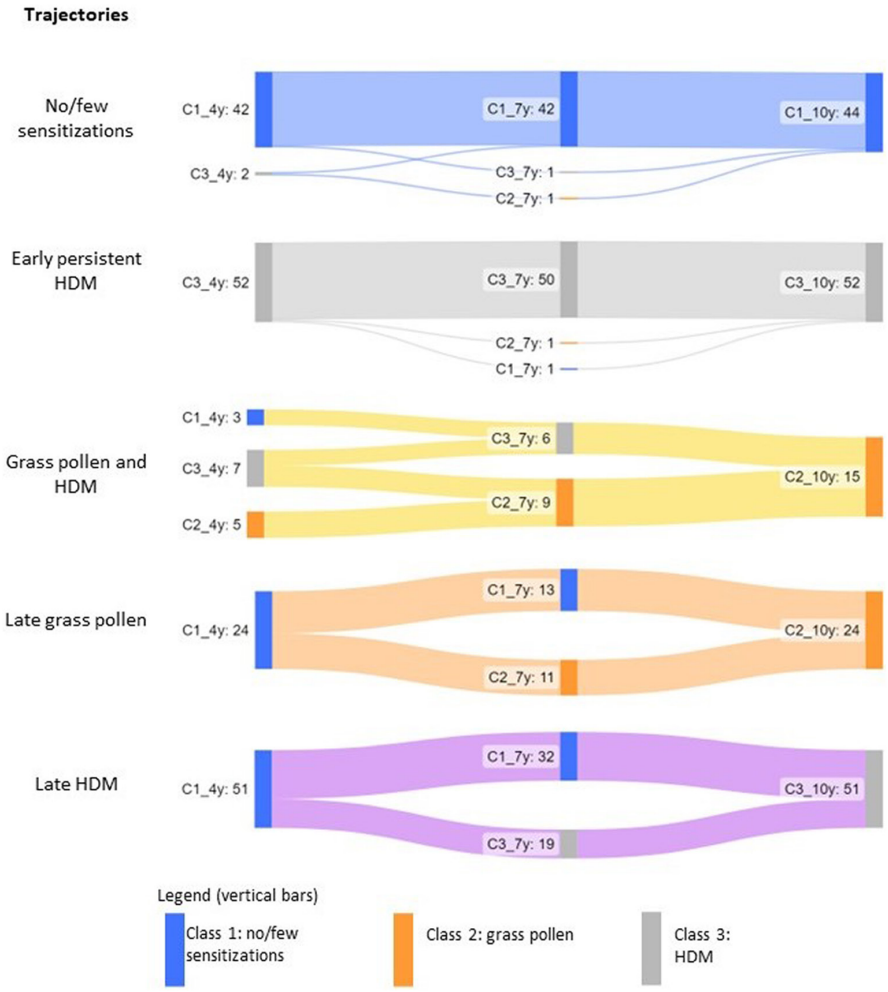

FIGURE 3 Sankey plot presenting the transitions of participants over time and how were grouped into five distinct trajectories: "no/few sensitizations" (light blue), "early persistent HDM" (light gray), "HDM and grass pollen" (yellow), "late grass pollen" (light orange), "late HDM" (violet). The definition of trajectories is also presented in Appendix S1: Table S3. The values after each class name (defined as C1_4y, e.g., class 1 at 4 years) represents the number of children within that class, at a specific follow-up. Blue vertical bars represents class 1. Orange vertical bars represents class 2. Gray vertical bars represents class 3. C1: Class 1 (no/few sensitization); C2: Class 2 (grass pollen); C3: Class 3 (HDM); 4 y: 4 years; 7 y: 7 years; 10 y: 10 years; HDM: house dust mites.

Our results are in agreement with some European studies.[13,17,18,24] The "HDM and grass pollen" trajectory was associated with rhinitis reported at 13 years, which may indicate the relevance of co-sensitization of HDM and grass pollen. Two previous studies using LCA defined a similar trajectory named "severe atopy" characterized by sensitization to a variety of perennial and seasonal allergens and found significant associations with rhinitis.[13,18] The trajectory "early persistent HDM" was positively associated with rhinitis and the only trajectory with a positive association with asthma. Sensitization trajectories in different studies conducted in the Manchester Asthma and Allergy Study (MAAS) revealed similar findings[12,16,17]; specifically, a profile towards full sensitization to HDM components showed the strongest association with asthma using a two-stage LCA approach.[17] In our study, at 4 years of age, a considerable proportion of children was already sensitized to multiple components of HDM, which seems to impose a higher risk of developing asthma later in life. *Posa* et al. characterized 722 participants in a German birth cohort using 12 molecules of *Dermatophagoides pteronyssinus*.[24] Sensitization started with Der p 1, Der p 2, and Der p 23, as in our study, but later expanded to other molecules not represented in the ISAC array. Early onset of sensitization was associated with a broader polymolecular profile and participants in this trajectory presented the highest risk for rhinitis and asthma, as demonstrated in our study despite the underrepresentation of HDM molecules to confirm this reasoning.

In this study, we assessed for the first time the trajectories of allergen-specific IgE profiles in a Southern European country using CRD. In Finland, an "HDM" latent class was not found, and "birch pollen," "animals," and "severe" classes were associated with wheeze, allergic rhinitis, and eczema.[13] In Estonian children from the same study, a class characterized by HDM and cat allergens was associated with wheeze and allergic rhinitis, and the "severe" class, characterized by IgE specific to cat, dog, HDM, birch, and grass allergens, was associated with wheeze, allergic rhinitis, and eczema.[13] In our study, children co-sensitized to grass pollen and mite molecules ("HDM and grass pollen") were not at a higher risk of developing asthma. In other study conducted in Danish and Swedish children with asthmatic mothers, sensitization rates against grass and birch pollen were higher and associations with both asthma and rhinitis were found. However, in our study, the trajectory "HDM and grass pollen" was associated only with rhinitis, while "late grass pollen" was not associated with allergic diseases.[25] Previous studies also found a trajectory mainly comprising food allergens, which was not the case in our study.[13,18] Food allergens were present in few participants and

TABLE 3 Distribution and characteristics of participants ($N = 186$) assigned to each sensitization trajectory.

	No/few sensitizations	Early persistent HDM	HDM and grass pollen	Late grass pollen	Late HDM	*p*-value
N children	44	52	15	24	51	
Sex, males	24 (54.5%)	26 (50.0%)	12 (80.0%)	13 (54.2%)	30 (58.8%)	.34
Maternal education						
≤9 years	23 (52.3%)	25 (48.1%)	8 (53.3%)	10 (41.7%)	21 (41.2%)	.79
>9 years	21 (47.7%)	27 (51.9%)	7 (46.7%)	14 (58.3%)	30 (58.8%)	
Parents history of allergy	4 (9.1%)	9 (18.4%)	2 (13.3%)	2 (8.7%)	10 (21.3%)	.43
Type of birth delivery						
Vaginal	21 (47.7%)	30 (57.7%)	4 (26.7%)	8 (33.3%)	27 (52.9%)	**.02**
Caesarean	16 (36.4%)	16 (30.8%)	11 (73.3%)	12 (50.0%)	12 (23.5%)	
Other	7 (15.9%)	6 (11.5%)	0	4 (16.7%)	12 (23.5%)	
Medical diagnosis of allergic conditions						
Asthma (4 y) [missings: 6]	3 (6.8%)	6 (12.5%)	0	0	1 (2.0%)	.09
Rhinitis (4 y) [missings: 6]	1 (2.3%)	3 (6.0%)	3 (21.4%)	3 (13.0%)	0	**.01**
AD (4 y) [missings: 1]	1 (2.3%)	8 (15.7%)	2 (13.3%)	3 (12.5%)	5 (9.8%)	.29
Asthma (7 y) [missings: 3]	2 (4.5%)	12 (23.5%)	1 (6.7%)	2 (8.3%)	2 (4.1%)	**<.01**
Rhinitis (7 y) [missings: 1]	4 (9.1%)	12 (23.1%)	4 (26.7%)	2 (8.3%)	4 (8.0%)	.07
AD (7 y) [missings: 1]	4 (9.1%)	7 (13.5%)	2 (13.3%)	4 (17.4%)	7 (13.7%)	.91
Asthma (10 y) [missings: 2]	4 (9.1%)	17 (33.3%)	2 (13.3%)	2 (8.3%)	7 (14.0%)	**.01**
Asthma (13 y) [missings: 32]	2 (5.0%)	18 (43.9%)	1 (7.1%)	3 (14.3%)	6 (15.8%)	**<.01**
Rhinitis (13 y) [missings: 32]	4 (10.0%)	17 (41.5%)	7 (50.0%)	6 (28.6%)	8 (21.1%)	**<.01**
AD (13 y) [missings: 32]	3 (7.5%)	10 (25.0%)	3 (21.4%)	3 (14.3%)	4 (10.3%)	.19
Multimorbidities (13 y)						
Asthma + rhinitis [missings: 33]	0	10 (24.4%)	1 (7.1%)	3 (14.3%)	1 (2.7%)	**<.01**
Asthma + AD, [missings: 33]	1 (2.5%)	4 (10.0%)	0	2 (9.5%)	0	.15
AD + rhinitis [missings: 33]	0	8 (20.0%)	1 (7.1%)	2 (9.5%)	1 (2.6%)	**.01**
Asthma + rhinitis + AD, [missings: 33]	0	4 (10.0%)	0	2 (9.5%)	0	.05
Allergic sensitization to ISAC components						
Nb of sensitizations[a], 4 y	0.34 (0.94)	4.81 (1.67)	5.87 (3.64)	1.00 (1.32)	0.49 (0.76)	**<.01**
Nb of sensitizations[a], 7 y	0.86 (1.21)	6.19 (2.77)	9.20 (2.88)	3.96 (3.21)	2.29 (2.13)	**<.01**
Nb of sensitizations[a], 10 y	1.86 (0.97)	7.19 (2.50)	12.40 (4.03)	9.83 (4.40)	5.51 (2.10)	**<.01**

Note: Data are presented as *N* (%) unless otherwise stated. Values in bold indicate statistically significant differences for the comparison of demographic and health outcomes variables between the five trajectories (*p*-value, represented as *p*, ≤.05 was considered statistically significant).

Abbreviations: AD, atopic dermatitis; HDM, house dust mites; nb, number; y, years.

[a]Data presented as mean (SD).

were mainly grouped in class 2, but their weight within this class was very low (<0.100). The sample size may explain this finding as previous studies included a higher number of participants; however, an extract-based test was used to assess sensitization in those studies.

In conclusion, distinct trajectories of allergen-specific IgE pose different risks in the development of allergic diseases in children. Despite similarities with studies conducted in the United Kingdom and Germany, sensitization trajectories might differ if compared with other Northern European countries, as well as the association with allergic outcomes. Longer follow-up times and evaluations sharing similar methods to assess specific-IgE and outcomes, using different populations, would be useful to provide further information and understand the impact of late-onset sensitization on the risk of allergic diseases in late adolescence and adulthood.

AUTHOR CONTRIBUTIONS

Mariana Farraia: Writing – original draft; data curation; formal analysis; investigation; visualization. **Francisca Castro Mendes:** Investigation; writing – review and editing; data curation. **Oksana Sokhatska:** Investigation; writing – review and editing; data curation. **Milton Severo:** Investigation; writing – review and editing; visualization; formal analysis; software; methodology. **João Cavaleiro Rufo:**

Investigation; writing – review and editing; supervision. **Henrique Barros:** Investigation; funding acquisition; writing – review and editing; supervision; methodology; conceptualization; validation; project administration. **André Moreira:** Supervision; writing – original draft; funding acquisition; investigation; conceptualization; methodology; validation; project administration.

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CONFLICT OF INTEREST STATEMENT

None of the authors report any conflict of interest in relation to this work.

PEER REVIEW

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Supplemental Material

Methods

Allergic sensitization

Serum samples collected from children at 10 years of age were screened for sIgE antibodies, using the Phadiatop Infant ImmunoCAP™ (Thermo Fisher Scientific, Uppsala, Sweden), including hen's egg, cow's milk, peanut, shrimp, cat epithelium and dander, dog dander, house dust mite, common silver birch, timothy, ragweed, and wall pellitory (*Parietaria Judaica*). ImmunoCAP™ fx1 nut mix (peanut, hazelnut, Brazil nut, almond and coconut) and fx22 nut mix (pecan nut, cashew nut, pistachio and walnut) were also tested. Sensitization was defined as a sIgE level of 0.35 (PAU/l) (Pharmacia Arbitrary Units per litre) or greater according to the manufacturer's instructions and was coded as positive in the dichotomous analyses.[1]

The levels of 112 allergenic components from 48 allergen sources were assessed using the multiplex array ImmunoCAP™ ISAC sIgE 112 (Thermo Fisher, Uppsala, Sweden) in the serum samples of children collected at ages 4, 7, and 10 years that tested positive in the screening test. The levels of each allergen component were reported in ISAC standardized units (ISU) whenever the threshold was achieved (≥0.30 ISU).[2, 3]

Health outcomes

At each follow-up, past medical diagnosis of allergic conditions were reported by the participants' legal guardians based on the International Study on Allergy and Asthma Meeting (ISAAC) appropriate questions.[4] Asthma prevalence was defined as a positive answer to the question "*Has your child ever been diagnosed with asthma by a physician?*". Rhinitis and eczema were defined as a positive answer to the questions "*Has your child ever been diagnosed with allergic rhinitis by a physician?*" and "*Has your child ever been diagnosed with eczema by a physician?*". The diagnosis of asthma was assessed at 4, 7, 10 and 13 years of age, and rhinitis and eczema were evaluated at 4, 7 and 13 years.

Results

Table S1 – Sociodemographic characteristics and differences of the included participants (randomly selected for allergic sensitization screening at 10 years old) and the remaining cohort.

	Birth cohort	Not included	Included	p value
N	**8,647**	**7,522**	**1,125**	
Sex				
Girls	4,237 (49.0%)	3,681 (48.9%)	556 (49.4%)	0.761
Boys	4,410 (51.0%)	3,841 (51.1%)	569 (50.6%)	
Privation index (distribution by quintiles, Q)				
Q1	1,105 (12.8%)	957 (12.8%)	148 (13.2%)	0.177
Q2	1,681 (19.5%)	1,455 (19.4%)	226 (20.1%)	
Q3	2,138 (24.8%)	1,858 (24.8%)	280 (24.9%)	
Q4	1,924 (22.3%)	1,654 (22.1%)	270 (24.0%)	
Q5	1,766 (20.5%)	1,565 (20.9%)	201 (17.9%)	
House occupation: number of household occupants/number of rooms				
<1,5	6,868 (79.4%)	5,942 (79.0%)	926 (82.3%)	**0.035**
≥1,5	1,496 (17.3%)	1,327 (17.6%)	169 (15.0%)	
Maternal education				
≤9 years	4,253 (49.2%)	3,742 (49.7%)	511 (45.4%)	**0.007**
>9 years	4,394 (50.8%)	3,780 (50.3%)	614 (54.6%)	
Health Outcomes				
10 years old				
Asthma, N=6,356	530 (8.3%)	445 (8.5%)	85 (7.6%)	0.304
13 years old				
Asthma, N=4,598	441 (9.6%)	355 (9.7%)	86 (9.1%)	0.527
Rhinitis, N=4,509	557 (12.4%)	443 (12.4%)	114 (12.0%)	0.730
Atopic dermatitis, N=4,580	705 (15.4%)	573 (15.8%)	132 (13.9%)	0.155

Data were presented as proportions, N (%).

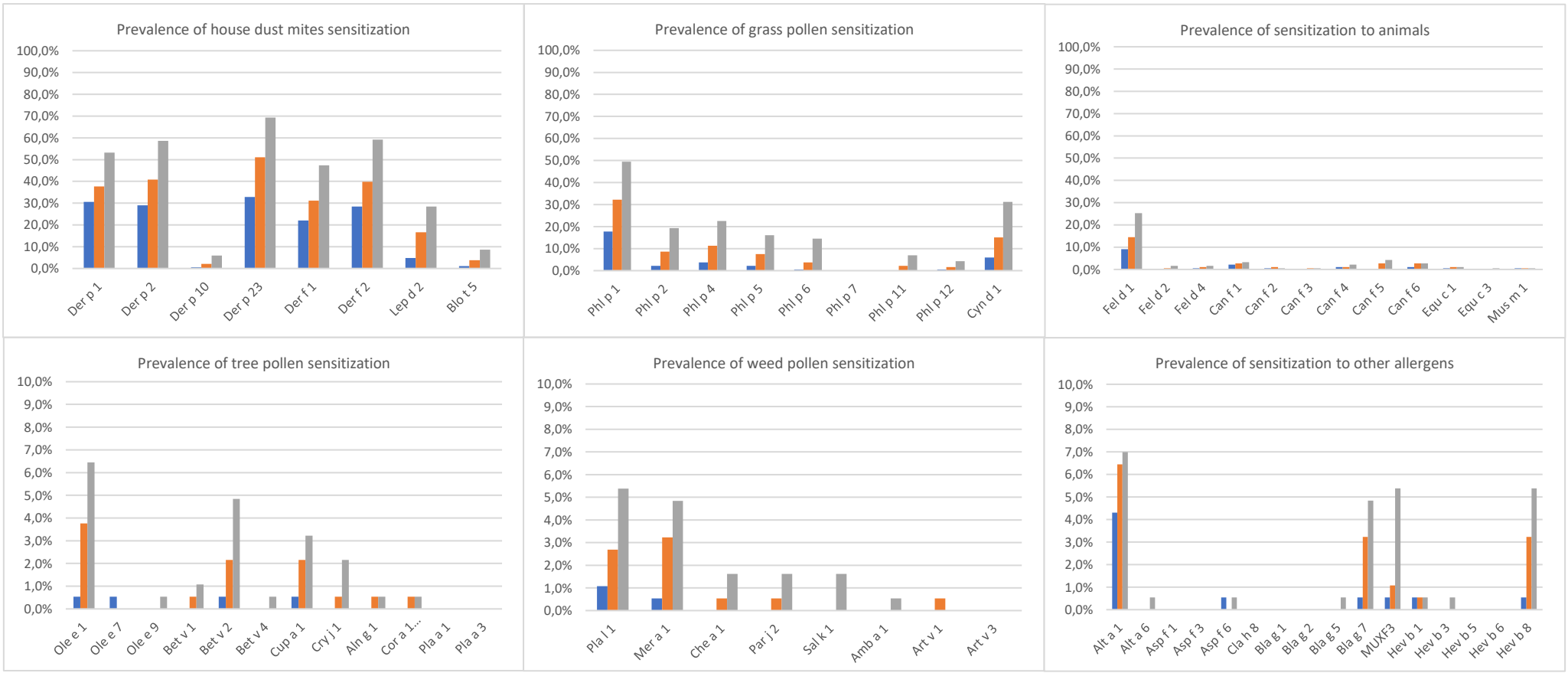

Figure S1 – Prevalence of IgE sensitization by allergenic component grouped by main sources (HDM, grass pollen, animals, tree pollen, weed pollen, and others). Prevalence is presented over time at 4- (blue), 7- (orange), and 10-years old (grey). Total number of participants considered in each follow-up: 186.

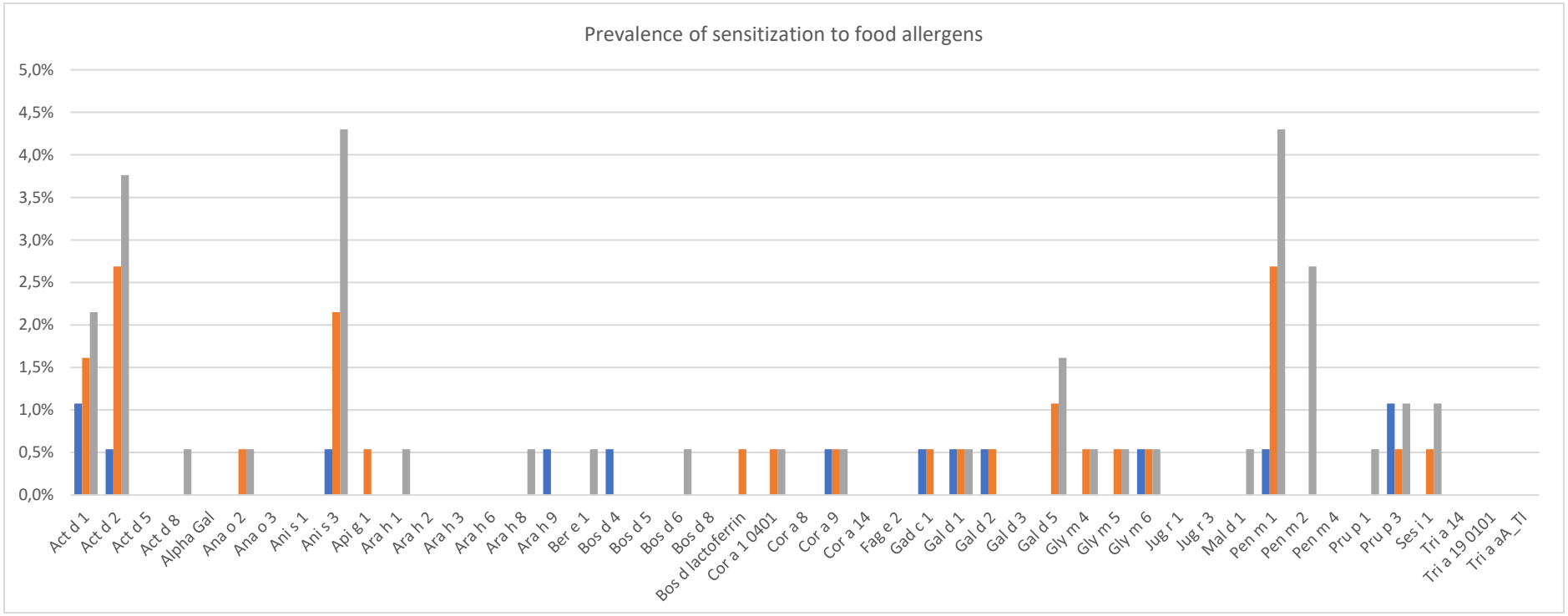

Figure S2 - Prevalence of IgE sensitization to food components. Prevalence is presented over time at 4- (blue), 7- (orange), and 10-years old (grey). Total number of participants considered in each follow-up: 186.

Table S2 – Probabilities assigned to each component at each cross-sectional latent class.

	Probabilities						
Components	**Class 1**	**Class 2**	**Class 3**	**Components**	**Class 1**	**Class 2**	**Class 3**
Act d 1	0.0000	0.0927	0.0124	Equ c 1	0.0000	0.0000	0.0206
Act d 2	0.0079	0.1367	0.0090	Equ c 3	0.0000	0.0000	0.0041
Act d 8	0.0000	0.0155	0.0000	Fel d 1	0.0444	**0.2836**	**0.2540**
Aln g 1	0.0000	0.0309	0.0000	Fel d 2	0.0000	0.0309	0.0083
Alt a 1	0.0355	**0.2011**	0.0416	Fel d 4	0.0000	0.0000	0.0248
Alt a 6	0.004	0.0000	0.0000	Gad c 1	0.0000	0.0167	0.0038
Amb a 1	0.0000	0.0155	0.0000	Gal d 1	0.0000	0.0464	0.0000
Ana o 2	0.0000	0.0309	0.0000	Gal d 2	0.0000	0.0000	0.0083
Ani s 3	0.0000	0.0169	0.0492	Gal d 5	0.0000	0.0773	0.0000
Api g 1	0.0000	0.0155	0.0000	Gly m 4	0.0000	0.0309	0.0000
Ara h 1	0.0000	0.0155	0.0000	Gly m 5	0.0000	0.0309	0.0000
Ara h 8	0.0000	0.0155	0.0000	Gly m 6	0.0000	0.0464	0.0000
Ara h 9	0.0000	0.0155	0.0000	Hev b 1	0.0000	0.0000	0.0124
Art v1	0.0000	0.0155	0.0000	Hev b 3	0.0000	0.0000	0.0041
Art v 3	0.0000	0.0000	0.0000	Hev b 8	0.0000	**0.2628**	0.0000
Asp f 3	0.0000	0.0000	0.0000	Jug r 3	0.0000	0.0000	0.0000
Asp f 6	0.0040	0.0155	0.0000	Lep d 2	0.0849	**0.2168**	**0.2381**
Ber e 1	0.0000	0.0155	0.0000	Mal d 1	0.0000	0.0155	0.0000
Bet v 1	0.0000	0.0464	0.0000	Mer a 1	0.0000	**0.2473**	0.0000
Bet v 2	0.0000	**0.2164**	0.0000	Mus m 1	0.0000	0.0154	0.0083
Bet v 4	0.0000	0.0155	0.0000	MUXF3	0.0000	0.1867	0.0038
Bla g 5	0.0000	0.0155	0.0000	Ole e 1	0.0077	**0.1998**	0.0213
Bla g 7	0.0000	0.0169	0.0616	Ole e 7	0.0000	0.0155	0.0000
Blo t 5	0.0030	0.0161	0.0959	Ole e 9	0.0000	0.0000	0.0041
Bos d Lacto	0.0000	0.0000	0.0041	Par j 2	0.0040	0.0309	0.0041
Bos d 4	0.004	0.0000	0.0000	Pen m 1	0.0000	0.0155	0.0537
Bos d 6	0.0000	0.0000	0.0041	Pen m 2	0.0000	0.0000	0.0206
Can f 1	0.0000	0.0630	0.0451	Phl p 1	0.1717	**0.9522**	**0.3316**
Can f 2	0.0000	0.0155	0.0124	Phl p 11	0.0080	**0.2164**	0.0041
Can f 3	0.0000	0.0000	0.0083	Phl p 12	0.0000	0.1855	0.0000
Can f 4	0.0000	0.0319	0.0245	Phl p 2	0.0314	**0.6445**	0.0265
Can f 5	0.0155	0.0477	0.0249	Phl p 4	0.0526	**0.6614**	0.0578
Can f 6	0.0079	0.0155	0.0372	Phl p 5	0.0236	**0.5814**	0.0184
Che a 1	0.0000	0.0618	0.0000	Phl p 6	0.0046	**0.4849**	0.0102
Cor a 1 0101	0.0000	0.0309	0.0000	Phl p 7	0.0000	0.0000	0.0000
Cor a 1 0401	0.0000	0.0309	0.0000	Pla a 3	0.0000	0.0000	0.0000
Cor a 9	0.0000	0.0464	0.0000	Pla l 1	0.0039	**0.2113**	0.0097
Cry j 1	0.0000	0.0621	0.0041	Pru p 1	0.0000	0.0155	0.0000
Cup a 1	0.0000	0.1085	0.0164	Pru p 3	0.0080	0.0309	0.0041
Cyn d 1	0.0462	**0.8123**	0.1356	Sal k 1	0.0000	0.0464	0.0000
Der f 1	0.0000	**0.3180**	**0.6873**	Ses i 1	0.0000	0.0309	0.0041
Der f 2	0.0000	**0.4069**	**0.8700**	Tri a 14	0.0000	0.0155	0.0000
Der p 1	0.0573	**0.4418**	**0.7558**				
Der p 10	0.0000	0.0309	0.0578				
Der p 2	0.0147	**0.4049**	**0.8635**				
Der p 23	0.2402	**0.4372**	**0.8110**				

Values in bold indicate a probability equal or higher than 0.200.

Allergen nomenclature is based on scientific name of plant or animal species (in latin) and composed by three letters (genus), followed by one letter (species) and one number (usually indicates order of discovery). Act d: *Actinidia deliciosa* (kiwi); Aln g: *Alnus glutinosa* (alder); Alt a: *Alternaria alternata* (fungi); Amb a: *Ambrosia artemisiifolia* (ragweed); Ana o: *Anacardium occidentale* (cashew); Ani s:

Anisakis simplex; Api g: *Apium graveolens* (celery); Ara h: *Arachis hypogaea* (peanut); Art v: *Artemisia vulgaris* (mugwort); Asp f: *Aspergillus fumigatus* (fungi); Ber e: *Bertholletia excelsa* (Brazil nut); Bet v: *Betula verrucosa* (birch); Bla g: *Blattella germanica* (kockroach); Blo t: *Blomia tropicalis* (storage mites); Bos d: *Bos domesticus* (cow's milk); Can f: *Canis familiaris* (dog); Che a: *Chenopodium album* (goosefoot, plant); Cor a: *Corylus avellana* (hazel); Cry j: *Cryptomeria japonica* (japanese cedar); Cup a: *Cupressus arizonica* (cypress); Cyn d: *Cynodon dactylon* (Bermuda grass); Der f: *Dermatophagoides farina*e (HDM); Der p: *Dermatophagoides pteronyssinus* (HDM); Equ c: *Equus caballus* (horse); Gad c: *Gadus callarias* (codfish); Gly m: *Glycine max* (soy); Gal d: *Gallus domesticus* (egg); Fel d: *Felis domesticus* (cat); Hev b: *Hevea brasiliensis* (rubber tree or latex); Jug r: *Juglans regia* (walnut); Lep d: *Lepidoglyphus destructor* (storage mites); Mal d: *Malus domestica* (apple); Mer a: *Mercurialis annua* (plant pollen); Mus m: *Mus musculus* (mouse); Ole e: *Olea europaea* (olive tree); Par j: Parietaria Judaica (pellitory); Pen m: *Penaeus monodon* (shrimp); Phl p: *Phleum pratense* (timothy grass); Pla l: *Platanus acerifolia* (plane tree); Pru p: *Prunus persica* (peach); Sal k: *Salsola kali* (plant); Ses i: *Sesamum indicum* (sesame); Tri a: *Triticum aestivum* (wheat).

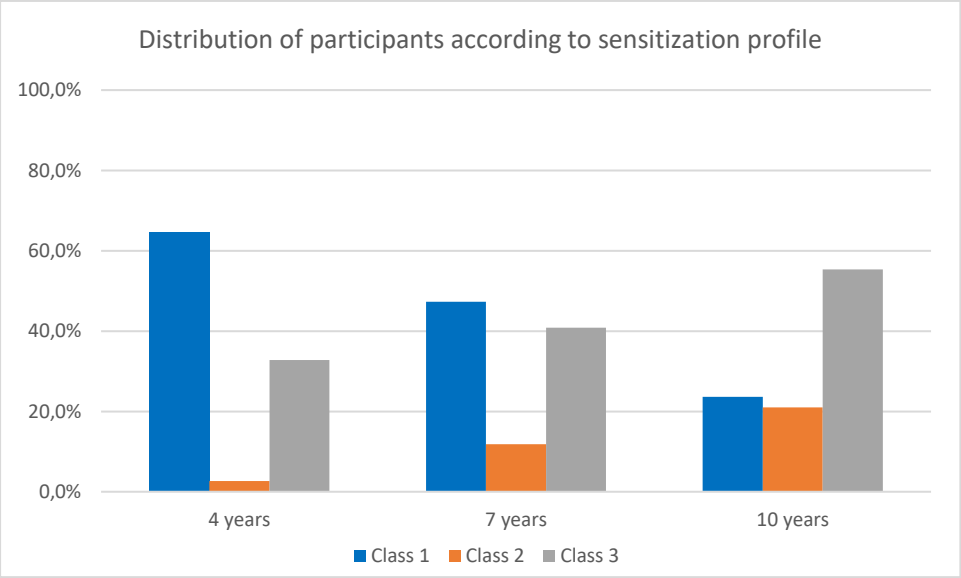

Figure S3 – Distribution of participants according to sensitization profile at 4, 7, and 10 years old. Class 1: "no or few sensitizations"; Class 2: "grass pollen"; Class 3: "HDM".

Table S3 – Characterization of sensitization trajectories defined by stability of clusters identified in the LCA and by moving between clusters (most prevalent transitions).

Trajectories	N	Latent class by age			N
		4 years	7 years	10 years	
No or few sensitizations [stable in class 1 over time or finishing at 10 years in class 1]	**44**	1	1	1	41
		1	3	1	1
		3	1	1	1
		3	2	1	1
Early persistent HDM [stable in class 3 over time or starting at 4 years and finishing at 10 years in class 3]	**52**	3	3	3	50
		3	2	3	1
		3	1	3	1
HDM and grass pollen [stable in class 2 over time or moving from class 3 to class 2 at any time and remain in class 2]	**15**	1	3	2	3
		3	3	2	3
		3	2	2	4
		2	2	2	5
Late grass pollen [moving from class 1 to class 2, regardless of time, and remain in class 2]	**24**	1	2	2	11
		1	1	2	13
Late HDM [moving from class 1 to class 3, regardless of time, and remain in class 3]	**51**	1	3	3	19
		1	1	3	32

*The classes identified by LCA are represent numerically as following: 1 represents “no/few sensitizations”, 2 represents “grass pollen”, 3 represents “HDM”. HDM: house dust mites, LCA: Latent class analysis.

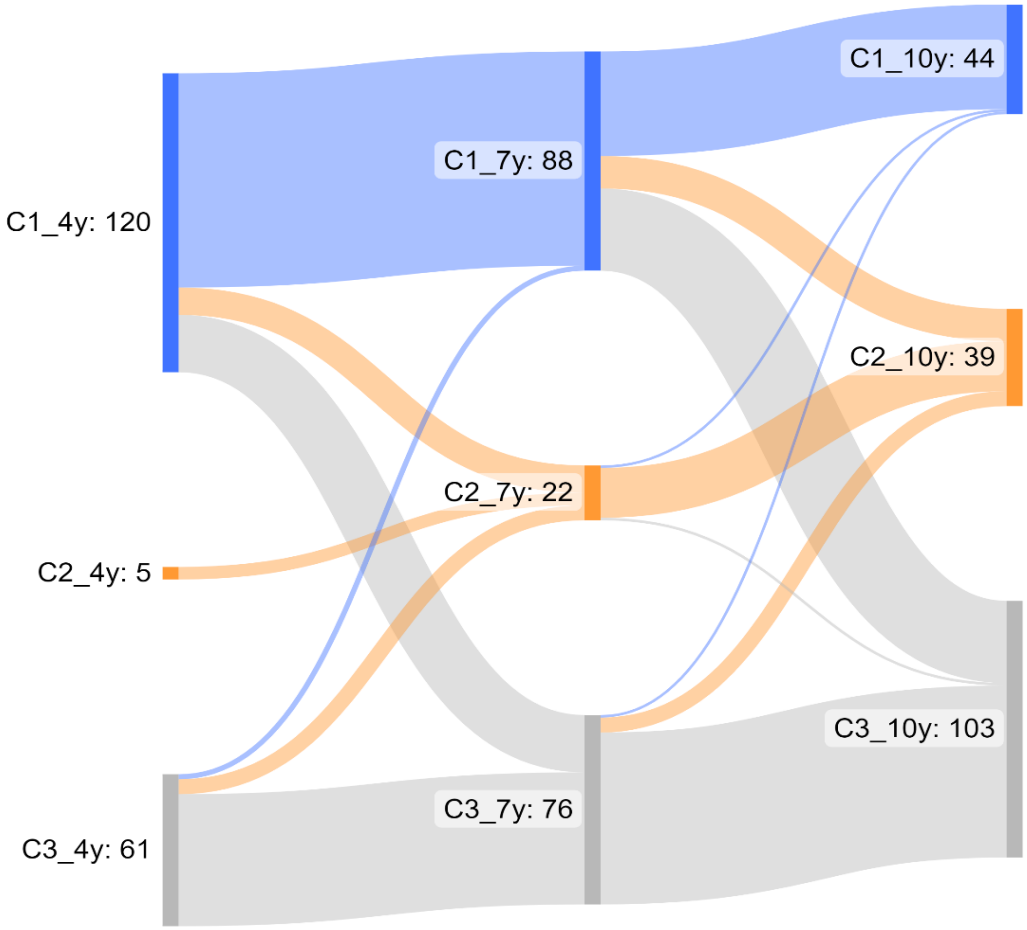

Figure S4 - Sankey plot presenting the transitions observed across the follow-ups (also represented in Table S3). C1: Class 1 (no/few sensitization); C2: Class 2 (grass pollen); C3: Class 3 (HDM); 4y: 4 years; 7y: 7 years; 10y: 10 years. The values after each class (defined as C1_4y, for example) represents the number of children within that class, at a specific follow-up. Blue colour represents transitions to class 1. Orange colour represents transitions to class 2. Grey colour represents transitions to class 3.

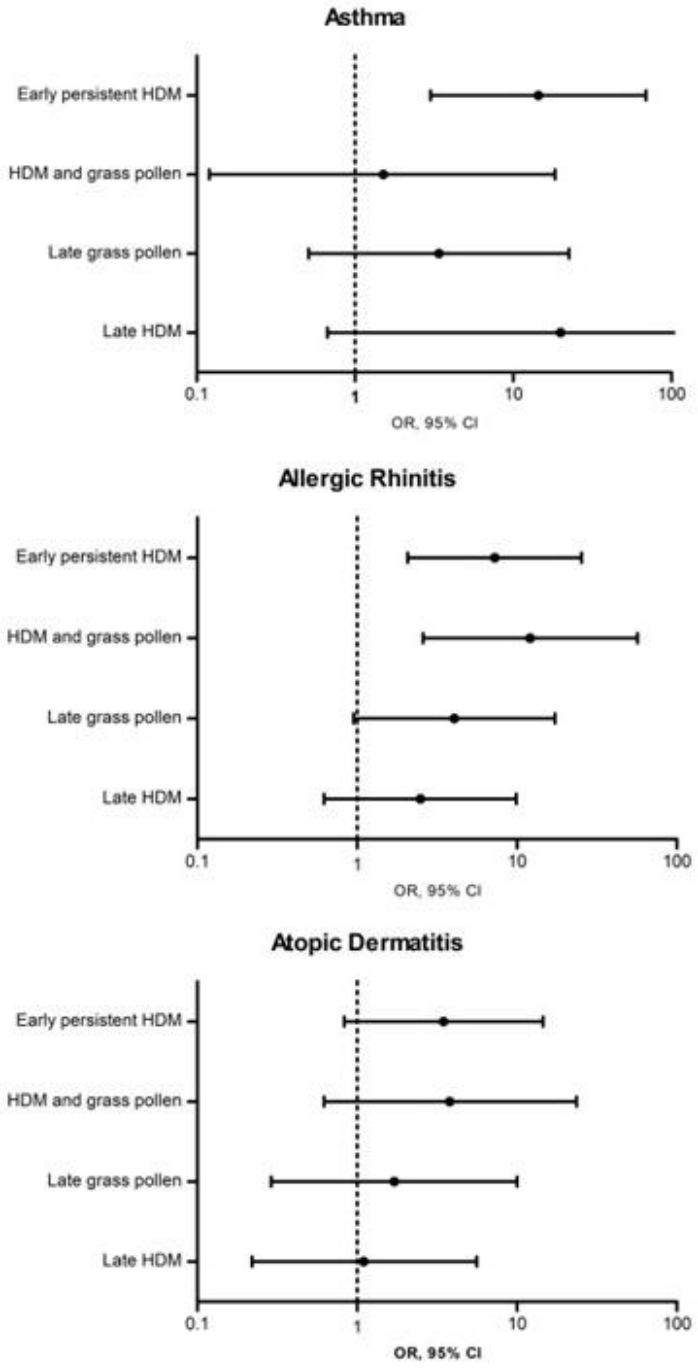

Figure S5 - Associations between the sensitization trajectories and allergic outcomes (asthma, allergic rhinitis and atopic dermatitis) assessed at 13 years old evaluation, adjusted for sex, parents' history of allergy, and maternal education. Results are expressed as odds ratios (OR) and 95% confidence intervals (CI). The reference category is the "no/few sensitizations" trajectory.

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REVIEW ARTICLE

Allergen immunotherapy for asthma prevention: A systematic review and meta-analysis of randomized and non-randomized controlled studies

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Abstract

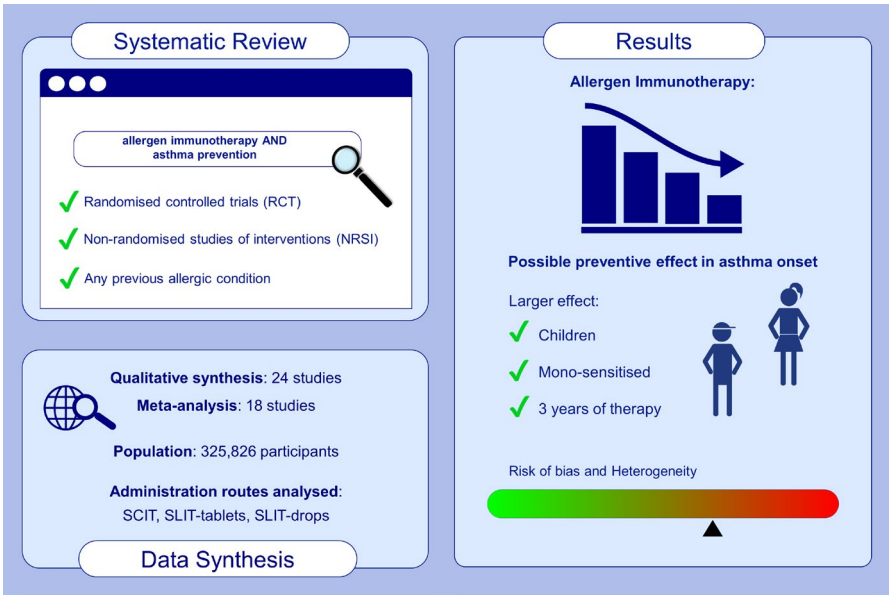
Background: Allergen immunotherapy (AIT) is a disease-modifying treatment for IgE-mediated diseases. Randomized controlled trials (RCTs) support AIT's potential role in asthma prevention but evidence from non-randomized studies of interventions (NRSI) and longitudinal observational studies has been poorly addressed. Therefore, we aimed to conduct a systematic review and meta-analysis to assess clinical data from all study types to evaluate quantitatively the preventive role of AIT in asthma onset.

Methods: We search three databases. Studies were screened, selected and evaluated for quality using risk-of-bias (ROB) tools. Data were descriptively summarized and meta-analysed using random effects. We performed a sensitivity, influence and subgroup analyses. Publication bias and heterogeneity were assessed.

Results: From the 4549 identified studies, 24 (12 RCTs and 12 NRSI) were included in the qualitative synthesis and 18 underwent meta-analysis. One study was at low ROB, seven had moderate ROB, and 15 were proven of high ROB. Random-effects analysis showed a significant decrease in the risk of developing asthma following AIT by 25% (RR, 95% CI: 0.75, 0.64–0.88). This effect was not significant in the sensitivity analysis. Publication bias raised concerns, together with the moderate heterogeneity between studies (I^2 = 58%). Subgroup analysis showed a remarkable preventive effect of AIT in children (RR, 95% CI: 0.71, 0.53–0.96), when completing 3 years of therapy (RR, 95% CI: 0.64, 0.47–0.88), and in mono-sensitized patients (RR, 95% CI: 0.49, 0.39–0.61).

Conclusions: Our findings support a possible preventive effect of AIT in asthma onset and suggest an enhanced effect when administered in children, mono-sensitized, and for at least 3 years, independently of allergen type.

KEYWORDS
allergen immunotherapy, allergy treament, asthma, prevention, rhinitis



GRAPHICAL ABSTRACT

This systematic review and meta-analysis included 18 studies (RCT and NRSI) in the quantitative synthesis. Our findings support a possible preventive effect of AIT in asthma onset. This effect might be enhanced when administered in children, mono-sensitised, and for at least three years, independently of allergen type. NRSI, non-randomised studies of intervention; RCT, randomised controlled trial; SCIT, subcutaneous immunotherapy, SLIT, sublingual immunotherapy.

1 | INTRODUCTION

Allergen immunotherapy (AIT), administered either by the sublingual (SLIT) or the subcutaneous (SCIT) route, has been used to treat some IgE-mediated allergic diseases for many decades.[1,2] Recent literature has highlighted the importance of studying allergic conditions using a multimorbidity framework instead of the classic transition from one disease to another, allowing for a mechanism-based treatment approach, such as AIT.[3–5] However, it is not fully understood how IgE profiles and other underlying allergic (and non-allergic) mechanisms in multimorbidities relate to better treatment response and prognosis, including the risk of developing other allergic conditions.[3,5,6] Atopic sensitization is a risk factor for the development of allergic multimorbidities,[4,6,7] and the presence of allergic diseases, such as food allergy, atopic dermatitis, rhinitis and rhinoconjunctivitis, is a significant risk factor for asthma development.[8] Children with allergic rhinitis have a higher risk of developing asthma later in life, and the co-existence of these diseases is very frequent since both share common pathophysiological features.[7,9]

Allergen immunotherapy induces allergen-specific immune tolerance and suppresses allergic inflammation by inducing changes in the profile of T- and B-cell responses to allergens, by decreasing activation and degranulation of effector cells, and by reducing effector cells pro-inflammatory mediators in mucosal tissues.[10,11] Studies evaluating the effectiveness of AIT in patients with allergic rhinitis suggest a reduction in the risk of developing asthma.[12,13]

Asthma affects more than 300 million people worldwide and it is expected to increase in the upcoming years, being one of the most common non-communicable diseases with a high impact on patient's lives and healthcare systems.[14] Thus, there is interest in gathering information from all studies investigating this potential preventive effect of AIT in asthma onset. In 2017, the European Academy of Allergy and Clinical Immunology (EAACI) conducted a systematic review (SR) to assess the effectiveness of AIT in the prevention of asthma and the development of new sensitizations using randomized controlled trials (RCTs).[12] This study showed a significant reduction in the short-term risk of patients with allergic rhinitis developing asthma (RR: 0.40; 95% CI: 0.29–0.54) when data from six RCTs were combined.

In this SR, we aimed to assess and update the current knowledge in this field by assembling and presenting new evidence, not limited to RCTs, but including non-randomized studies of interventions (NRSI) and longitudinal observational studies. The outcome of interest in this SR is the development of asthma. Different outcome definitions were considered to achieve a comprehensive analysis.

2 | METHODS

2.1 | Search strategy and study selection

A search was performed in Pubmed, Scopus and Cochrane databases for articles published until 6 April 2021 using the following search terms: (immunotherapy OR vaccine OR allergy shot) AND asthma* AND (prevent* OR risk). During the search, we selected studies conducted in humans and published in English, Spanish or Portuguese. No data restrictions were employed, and the search algorithm was repeated in the final stage of the review (date: 30.12.2021) to confirm whether new papers were

available. Duplicates were removed, and two independent reviewers screened the search results based on the title and abstract. Any original study that evaluated the effect of AIT on the development of asthma in healthy subjects or subjects with other allergic diseases was included. Two independent reviewers read the full text of the previous selected studies and those accomplishing the inclusion criteria were selected for data extraction. In case of disagreement, a third reviewer was consulted. References were managed with EndNote X9 Software (Clarivate). A manual search of the reference lists from all the relevant original studies and review articles to identify additional eligible studies was performed.

2.2 | Inclusion criteria

Inclusion criteria were defined following the PICOS framework. We were interested in studies conducted on subjects of any age, either healthy or with allergic conditions such as rhinitis, atopic dermatitis or rhinoconjunctivitis. AIT studies including subjects with comorbid asthma were included if data were available for the outcome of interest in those without asthma. The intervention was AIT with any allergen administered through any route, SLIT or SCIT. The comparator group included placebo or no intervention (control group). The outcome of interest was the development of asthma. Different asthma definitions were considered for the inclusion of studies to guarantee a comprehensive analysis: asthma symptoms and/or asthma medication, and GINA-based or medical diagnosis. RCTs and NRSI (cross-sectional, retrospective or prospective) were considered for selection and data extraction.

2.3 | Data extraction and synthesis

One reviewer extracted the main characteristics of eligible studies (study design, population characteristics, intervention, comparator, follow-up, baseline allergic conditions, asthma definition and outcome of interest) onto a customized data extraction sheet in Microsoft Excel. A descriptive summary and data tables were produced to summarize the literature. The outcome of interest was extracted, when possible, as the absolute number of subjects developing asthma (event rate data). When not reported, this number was estimated if the percentage of subjects developing the outcome was available or by contacting the authors of the study. If none of the previous data was reported, the study was only considered for the qualitative synthesis. The data available for the intention-to-treat population were preferable and, if not reported, the per-protocol population was extracted.

2.4 | Quality assessment

The revised Cochrane Risk of Bias tool (RoB2) for randomized trials[15] was used to assess the risk of bias (ROB) of the included RCTs. The ROB was judged as low, moderate, high or unclear for each domain: randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome, selection of the reported result and overall bias. This assessment was performed independently by the two reviewers and discussed if results differed across the evaluations. The Risk of Bias tool for non-randomized studies of interventions (ROBINS-I) was used to assess the ROB of the included observational studies.[16] The domains included in this tool are divided according to the stage of the intervention: pre-intervention (bias due to confounding, bias in selection of participants into the study), at intervention (bias in classification of interventions) and post-intervention (bias due to deviations from intended interventions, bias due to missing data, bias in measuring outcomes and bias in a selection of the reported result). The domains and the overall ROB were judged as low, moderate, high (included serious and critical judgements) or unclear.

2.5 | Data analysis

Meta-analysis using the random-effects model approach was performed in the available data provided by 18 studies and using RStudio software v3.4.2 (R Foundation). For dichotomous variables, the event rate data were pooled as risk ratio (RR) with a 95% confidence interval using the Der Simonian-Laird estimator.[17,18] The magnitude of heterogeneity between the included studies was estimated using the Higgins I^2 statistic, Cochran's Q, tau-squared and prediction interval. Outlier and influential analyses were performed to improve the robustness of the results. Publication bias was assessed through the creation of a funnel plot and using Egger's test.[19] In case of publication bias, Thuval & Tweedie's trim-and-fill procedure was applied to estimate the effect size if missing small studies have been published.[20]

Sensitivity analysis was performed according to the risk of bias/quality of evidence by comparing the summary estimates obtained by excluding studies to be at high risk of bias with those considered at low and moderate risk. Subgroup analyses were undertaken according to the type of study (RCT vs. NRSI), AIT administration route (SLIT tablets vs. SLIT drops; any SLIT vs. SCIT), population (children vs. adults), asthma definition, follow-up and therapy duration, type of outcome analysis (primary or secondary outcomes/endpoints), allergen (grass pollen vs. house-dust mites), history of sensitization (mono- vs. poly-sensitized) and funding (pharmaceutical industry vs. academic/other).

2.6 | Registration and reporting

This systematic review was reported per the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) checklist 2020 and the Cochrane Handbook for Systematic Reviews of Interventions Version 6.1.[17,21] This review is registered with PROSPERO registration number: CRD42021251166.

3 | RESULTS

3.1 | Study selection and characterization

In this systematic review, we identified 4549 potentially eligible studies after the removal of duplicates. During the screening of titles and abstracts using the prespecified inclusion criteria, 4482 studies were rejected, yielding 67 studies for a complete revision. Each of those studies was then reviewed. Forty-three studies were later excluded. Thus, 24 studies were included for the qualitative analysis and 18 for the quantitative analysis. Figure 1 shows the flow diagram of the search and selection process.

Regarding study design, 12 RCTs were included.[22–33] Of these, three publications are from the PAT study (the Preventive Allergy Treatment study) but with different updates according to the follow-up duration; after 3 years of therapy,[28] and after 2 and 7 years of therapy discontinuation.[26,27] Twelve studies were NRSI.[34–45] Of these, six studies used secondary data from healthcare databases; one analysed real-world data from a population-based administrative healthcare database,[36] while five used real-world data from a health insurance prescription database.[35,42–44,46] Three studies were not eligible for meta-analysis; one due to the single-arm design (no comparator group)[40] and two studies used a mixed population (with asthma and/or rhinitis) but did not present the data for those only with rhinitis at baseline.[25,32] Additionally, due to different follow-up times of the same population, three studies were also not included in the main meta-analysis.[26,27,41]

The population in each study varied significantly, ranging from 28 subjects (14 in the intervention group vs. 14 in the comparator group) up to 118,754 subjects (2431 in the intervention group vs. 116,323 in the comparator group). Eleven studies were performed only in children, one in adolescents, one in adults and eleven in a mixed population (children and adults). Most studies were performed in a population with allergic rhinitis (n = 12), followed by rhinoconjunctivitis (n = 9), atopic dermatitis (n = 2) and healthy individuals (n = 1). Eleven studies also included participants with allergic asthma; nine presented the data for the subpopulation without asthma at baseline. Eight only included participants mono-sensitized to the specific allergen under investigation. Asthma onset was the primary outcome/endpoint only in seven publications.

SLIT was evaluated in thirteen studies, SCIT in eight, and both in three studies of which two studies presented data separately according to the administration route. The allergens used for AIT were grass pollen (7 studies), house dust mites (5 studies), birch pollen (2 studies) and *Parietaria judaica* (1 study). Nine studies evaluated more than one allergen but results were not presented separately

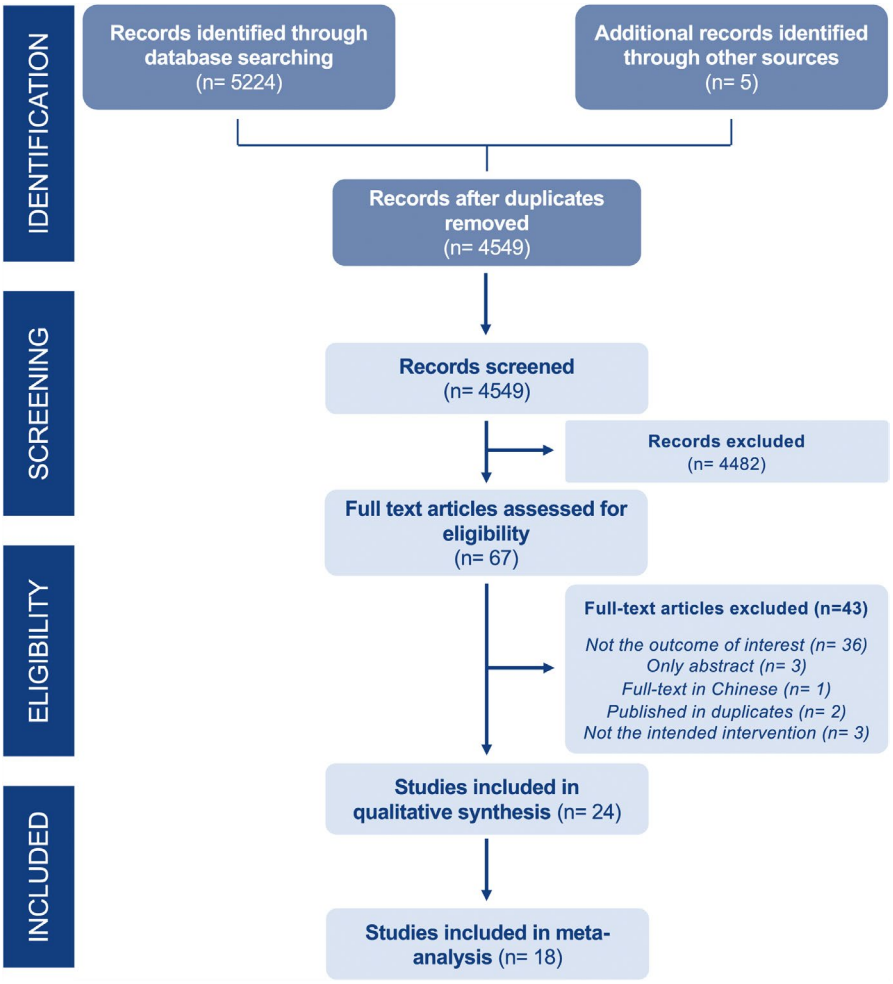


FIGURE 1 PRISMA flow diagram of the studies included

according to the type of allergen used for AIT. The follow-up after treatment discontinuation varied from 9 months to 12 years, while some studies did not conduct a follow-up of participants after discontinuation.

The definition of the outcome (asthma) varied between studies, and it was grouped as follows: a) asthma symptoms, presence of one or more symptoms of asthma (nine studies); b) asthma medication, prescription and use of asthma medication (5 studies); c) asthma symptoms and medication use; d) the combination of the previous definitions and the presence of both to define asthma (4 studies); e) and mixed criteria, according to symptoms, lung function and medication use or following the GINA guidelines (5 studies).

The key characteristics and main findings are presented in Table 1. Risk-of-bias results are detailed in the Appendix S1.

3.2 | Random-effects meta-analysis and sensitivity analysis

Random-effects meta-analysis of studies demonstrated a significant decrease in the risk of developing asthma following AIT by 25% (RR, 95% CI: 0.75, 0.64–0.88; $N = 17$) (Figure 2). The three indicators of between-study heterogeneity show moderate-to-high heterogeneity between the pooled studies (Figure 2). The broad prediction interval (0.53–1.08), which surpasses the value one, does not allow to overly confidence in the preventive effect of the intervention, meaning that AIT may not be protective in every context. In addition, after excluding studies with high risk of bias the result did not remain significant (RR, 95% CI: 0.76, 0.45–1.27; $N = 7$; $I^2 = 27\%$). When including the study considered an outlier (Friztching et al, 2021) and when excluding influential studies from the analysis (Friztching et al, 2021 and Wahn et al, 2018), the results remained significant and similar (Figures S3, S4, S5, S6) showing the robustness of these findings. The evidence of publication bias needs to be considered since the funnel plot (Figure 3) shows substantial asymmetry and the Egger's test is statistically significant ($p < .01$), denoting that small studies without a significant effect might be missing. When performing the trim-and-fill analysis to impute data from missing studies, the result remained marginally significant (RR, 95% CI: 0.82, 0.68–0.98) and the heterogeneity remained moderate-to-high ($I^2 = 59.7\%$). The analysis by study design evidenced the protective effect of AIT in preventing asthma onset in RCTs (RR, 95% CI: 0.59, 0.40–0.88; $I^2 = 17\%$), while in NRSI studies, the effect was not significant (RR, 95% CI: 0.82, 0.66–1.02; I^2: 86%). The between-study heterogeneity in RCTs was low ($I^2 = 17\%$), and the prediction interval remained wide (0.31–1.12); thus, these results need to be interpreted with caution.

3.3 | Subgroup analysis

Given the number of studies needed to perform the subgroup analysis to evaluate AIT in different contexts, we decided to perform the analysis combining all types of studies independently of the design. This analysis showed that AIT is beneficial in specific circumstances (Table 2): a) studies conducted only in children (RR 95% CI: 0.71, 0.53–0.96; N studies = 8); b) mono-sensitized patients (RR 95% CI: 0.49, 0.39–0.61; $N = 6$); c) AIT administration for at least 3 years (RR 95% CI: 0.64, 0.47–0.88; $N = 8$); d) follow-up after AIT discontinuation for up to 3 years (RR 95% CI: 0.74, 0.62–0.88; $N = 14$); and e) evidence from academic/or studies (RR 95% CI: 0.68, 0.53–0.88; $N = 10$). When asthma was defined by symptoms, medication and lung function (mixed criteria) or only by medication, the results did not remain significant. The AIT administration route did not influence the results since results were significant for both SCIT and SLIT. We were able to pool the results for grass pollen and HDM studies and results were significant for both. Concerning SLIT administration, both drops and tablets were statistically significant, with a larger effect observed when administering SLIT in drops. The subgroup analysis was further stratified according to SLIT and SCIT administration, but there were some limitations due to the low number of studies considered in each group, and results need to be interpreted cautiously (Table 2).

4 | DISCUSSION

This meta-analysis suggests a preventive effect of allergen immunotherapy in asthma development, confirming the association found in previous studies. The preventive effect of AIT was noteworthy in children, mono-sensitized patients, and when administered for at least 3 years, independently of the allergen (grass pollen or HDM) and administration route (SCIT, SLIT tablets or SLIT drops), indicating an overall effect of this therapy on the outcome of interest. The results were robust for the short-term (3 years of follow-up). This is the first meta-analysis combining all the evidence, independently of study design, since both RCTs and NRSI were reviewed, analysed and included in the analysis whenever possible. Thus, it was possible to pool the data from eighteen studies, achieving a relevant number of participants while having moderate heterogeneity.

There are several strengths of this study. First, the comprehensive search strategy and eligibility criteria with the inclusion of evidence from NRSI provide new insights on the preventive effect of AIT supported by real-world evidence (RWE) data from a larger number of studies and, consequently, participants. The inclusion of non-randomized studies, specifically retrospective cohorts of prescriptions and/or routine healthcare databases, was important to counterpart evidence from RCTs because these studies reflect the complexity of clinical settings.[47] AIT results in clinical practice depend on the population's characteristics such as patients' preferences, comorbidities, lifestyle, personal expectations and priorities, and the accessibility to correct diagnosis and treatment. Second, the overall heterogeneity between studies was moderate, supporting the meta-analysis using a random-effects model methodology. The heterogeneity may be explained by differences in study design, therapy duration, time of follow-up and population considered across studies. Third, the detailed subgroup analysis allowed the scrutiny of

TABLE 1 Characteristics and main results of the original studies including in the systematics review

Author, year, country	Design	Participants, allergic disease(s), sensitization	Intervention group	Comparator group
Zhou et al, 2021, China[45]	NRSI	Mixed (adults and children, 5–45 years old); Atopic dermatitis and Rhinitis; mono- or poly-sensitized	*N* = 91. SCIT (HDM, Allergovit, maintenance dose: 5000 TU/ml)	*N* = 116. control
Fritzsching et al (REACT study), 2021, Germany[44]	NRSI	Mixed (children and adults); Rhinitis; mono- or poly-sensitized	*N* = 27,039. SCIT, SLIT drops, or SLI tablets (against HDM, grass, tree, or other).	*N* = 27,039. Controls, selected using propensity matching scores
Jutel et al, 2020, Germany[43]	NRSI	Mixed (adults and children, 5–50 years old); Rhinitis; mono- or poly-sensitized	[a]*N* = 1492. SCIT (HDM extract, Acaroid®, 1000 or 10 000 TU/ml)	[a]*N* = 38,219. Control
Alviani et al, 2020, UK[22]	RCT	Children (aged 6–12 months); Healthy; No sensitization	*N* = 54. SLIT drops (HDM extract, ALK Abelló, 2000 standard treatment units per day)	*N* = 53. Placebo
Devillier et al, 2019, France[34]	NRSI	Mixed (adults and children over 5 years old); Rhinitis, asthma; mono- or poly-sensitized	[a]*N* = 686. SLIT tablets (grass pollen; oralair, Stallergenes Greer; or grazax, ALK Abelló)	[a]*N* = 16,699. Control
Wahn et al, 2018[42]	NRSI	Mixed (adults and children over 5 years old); Rhinitis, asthma; mono- or poly-sensitized	[a]*N* = 6349. SLIT drops (*N* = 634) or SCIT (*N* = 5715) (birch pollen; natural SLIT drops, natural SCIT or allergoid SCIT preparations)	[a]*N* = 31,683. Control
Zielen et al, 2018, Germany[35]	NRSI	Mixed (adults and children over 5 years old); Rhinitis, asthma; mono- or poly-sensitized	[a]*N* = 2191. SLIT tablets (grass pollen, grazax or oralair, ALK Abelló or Stallergenes Greer)	[a]*N* = 53,718. control

Therapy duration; follow-up	Asthma, *N* of events	Asthma definition	Risk of bias	Main findings
Therapy: 3 years; Follow-up: 2 years	Int: 19; Cp: 45	Allergic asthma (no more specifications)	High	New asthma onset was a secondary endpoint. Among patients with atopic dermatitis and rhinitis at baseline (but not asthma), the RR of asthma diagnosis for the control group was 1.86 (95% CI: 1.17–2.95), considering an interval of 5 years (3 of intervention +2 follow-up).
Therapy: at least 1 year; Follow-up: at least 1 year (and up to 9 years)	Int: 1148; Cp: 950	At least one asthma diagnosis (coded at database) and two prescriptions of SABA/ICS	High	New asthma onset was a secondary endpoint. Time-to-first onset of asthma showed an increased risk for the AIT group (HR: 1.22; 95% CI: 1.12–1.32). In the non-asthma cohort, the number of prescriptions for asthma medication over time was low and remained stable for the AIT group across the 9 follow-up years, but prescriptions tended to increase in the control group (indicating reporting bias). Data were not presented separately according to administration route, allergen or population (children vs. adults).
Therapy: at least 1 year; Follow-up: at least 2 years	Int: 208; Cp: 6829	Prescription data of asthma medication	High	New asthma onset was a secondary endpoint. After covariate adjustment in the regression analysis, the probability of asthma development was significantly lower in the patients receiving HDM allergoid (OR = 0.81; 95% CI: 0.70–0.95). When considering only children, the adjusted OR was 0.83 (95% CI: 0.69–1.00). Number of asthma events presented separately for children (used in meta-analysis, for the sensitivity analysis).
Therapy: 1 year; Follow-up: 5 years	Int: 1; Cp: 5	Based on symptoms, medication, and lung function	Mod.	Asthma development was the primary outcome. Early-life administration of HDM SLIT may reduce childhood asthma. Only RCT investigating primary prevention of asthma and atopy using AIT.
Therapy: at least 2 years; Follow-up: at least 1 year	Int: 94; Cp: 2836	Prescription data of asthma medication	High	New asthma onset was a secondary endpoint. The number of patients with a dispensed prescription for asthma medication (those who had not been treated for asthma at index date) was lower in the SLIT group (13.7%) than in the control group (17.0%). The data presented is for the primary analysis reported by the authors (No age-matching). For the secondary analysis, with age-matching of participants, adjusted OR was 0.549 (95% CI: 0.431–0.700). SLIT tablets on AR may have a potential effect in reducing asthma onset and asthma progression in routine clinical practice, using medication dispensing as a proxy.
Therapy: at least 2 years; Follow-up: at least 2 years	Int: 793 (SLIT = 61; SCIT: 732); Cp: 4159	Prescription data of asthma medication	High	New asthma onset was a secondary endpoint. During treatment, new-onset asthma risk was significantly reduced in the AIT vs. non-AIT group (OR: 0.83; $p = .001$). During the follow-up/post-treatment period, the OR for the AIT group was close to equality and therefore not significant (OR: 1.02, $p = .77$). Number of asthma events were presented separately for SCIT and SLIT drops (used in meta-analysis, for the sensitivity analysis). Birch pollen AIT demonstrated real-world benefits up to 6 years post-treatment cessation through significantly decreased risk of new-onset asthma medication use on-treatment.
Therapy: at least 2 Tx cycles; Follow-up: at least 2 years	Int: 208; Cp: 6222	Prescription of asthma medication	High	New asthma onset was a secondary endpoint. After adjustment for covariates, the odds ratio for new asthma onset evidenced a reduction in the risk of asthma onset in the SLIT tablet group in all analytical periods (OR: 0.696, 95% CI: 0.552–0.877). Real-world treatment of AR patients with grass pollen SLIT tablets was associated with less frequent asthma onset.

TABLE 1 (Continued)

Author, year, country	Design	Participants, allergic disease(s), sensitization	Intervention group	Comparator group
Valovirta et al (GAP study), 2018, Europe (11 countries)[23b]	RCT	Children (5–12 years old); RC; mono- or poly-sensitized	*N* = 398. SLIT tablets (grass pollen, grazax, ALK Abelló)	*N* = 414. Placebo
Schmitt et al, 2015, Germany [36]	NRSI	Mixed; Rhinitis; mono- or poly-sensitized	*N* = 2431. Any SLIT, SLIT drops (*N* = 248) or SCIT (*N* = 2030) (any allergen type was included in the analysis)	*N* = 11,6323. Control
Peng et al, 2013, China[37]	NRSI	Mixed; Rhinitis, asthma; only HDM sensitized	[a]*N* = 55. SCIT (standardized mite depot-allergen extract, Allergopharma)	[a]*N* = 23. Control
Holt et al, 2013, Australia and USA[24]	RCT	Children (12 months old); Atopic dermatitis; sensitization to ≥1 food allergen	*N* = 25. SLIT drops (mixture of soluble allergens of HDM, cat, and timothy grass)	*N* = 25. Placebo
Milani et al (EFESO trial), 2008, Italy[38]	NRSI	Adolescents (mean aged 22 years old); Rhinitis; mono- or poly-sensitized	*N* = 154. SLIT drops (SLITone, any allergen type, ALK Abelló)	*N* = 151. control
Marogna et al, 2008, Italy[25c]	RCT	Children (5–17 years old); Rhinitis, intermittent asthma; mono- or poly-sensitized	*N* = 144. SLIT (Any allergen type, Anallergo)	*N* = 72. Control
Jacobsen et al (PAT study, 10 years), 2007, Europe[26d]	RCT	Children (6–14 years old); RC, mild seasonal asthma; only sensitized to grass or birch	[a]*N* = 79. SCIT (grass and/or birch pollen, Alutard or Aquagen, ALK Abelló). Sample size after follow-up: 64	[a]*N* = 72. Control. Sample size after follow-up: 53
Niggermann et al (PAT study, 5 years), 2006, Europe[27d]	RCT	Children (6–14 years old); RC, mild seasonal asthma; only sensitized to grass or birch	[a]*N* = 79. SCIT (grass and/or birch pollen, Alutard or Aquagen, ALK Abelló). Sample size after follow-up: 95.	[a]*N* = 72. Control. Sample size after follow-up: 88.
Moller et al (PAT study, 3 years), 2002, Europe[28d]	RCT	Children (6–14 years old); RC, asthma; only sensitized to grass or birch	[a]*N* = 79. SCIT (grass and/or birch pollen, Alutard or Aquagen, ALK Abelló)	[a]*N* = 72. Control

Therapy duration; follow-up	Asthma, *N* of events	Asthma definition	Risk of bias	Main findings
Therapy: 3 years; Follow-up: 2 years	Int: 34; Cp: 39	Based on symptoms, medication, and lung function	Low	Asthma onset was the primary outcome. Asthma according to symptoms or medication intake was a secondary outcome. Treatment reduced the risk of experiencing asthma symptoms and using asthma medication. HR (95% CI) for asthma onset: 0.90 (0.57–1.43). OR (95% CI), and for asthma symptoms and medication used: 0.67 (0.46–0.97). Treatment with SLIT tablets reduced the risk of experiencing asthma symptoms and using asthma medication.
Therapy: at least 1 prescription; Follow-up: up to 6 years	Int: 33 (SLIT drops: 3; SCIT: 25); Cp: 1613	Diagnoses according to the ICD–10 code and at least 2 prescriptions of ICS	Mod.	Asthma onset was de primary outcome. AIT demonstrated an RR of 0.60 (95% CI: 0.42–0.84), indicating a 40% risk reduction for patients with AR for the development of asthma. Our data suggest that Tx duration of 3 years or more have slightly better preventive effects on the development of asthma. Number of asthma events presented separately for SCIT and SLIT drops (used in meta-analysis, for the sensitivity analysis).
Therapy: 3 years; Follow-up: 2 years.	Int: 6; Cp: 7	Based on GINA guidelines	High	The outcome of asthma development showed an OR of 3.57 (1.05–12.19; $p < .05$) in favour of the hypothesis that SCIT can prevent the development of asthma meaning that the risk of developing asthma was 3.57 times higher in the control group.
Therapy: 1 year; Follow-up: 3 years	Int: 4; Cp: 4	Asthma symptoms.	Mod.	Asthma onset was a secondary endpoint. It is not possible to draw firm conclusions concerning the potential efficacy of this overall approach for allergy prophylaxis. OR (95% CI): 1.00 (0.16–6.1).
Therapy: 2 years; Follow-up: 0	Int: 13; Cp: 30	Asthma symptoms (ISAAC questionnaire: 'Have you had wheezing or whistling in the chest in the last 12 months?')	High	Asthma onset was a secondary outcome. SLIT was associated with a lower incidence of asthma-related symptoms, such as wheezing, and new sensitizations at the end of the observational period in comparison with allergic patients not treated with SLIT. Age, sex distribution, duration of allergy symptoms and type of AR and poly-sensitized patients were equally distributed in the two groups.
Therapy: 3 years; Follow-up: 0	No data for subjects without asthma at baseline	Based on GINA guidelines (intermittent or mild asthma).	High	Asthma onset was the primary outcome. This study shows a preventive effect on the onset of mild persistent asthma and remained statistically significant even after the worst-case analysis.
Therapy: 3 years; Follow-up: 7 years	Int: 16; Cp: 24.	Asthma symptoms and response to B2-agonists.	High	Asthma development was the primary outcome. This 10-year follow-up study demonstrates that SCIT for 3 years shows the persistent long-term effect on clinical symptoms and a preventive effect on the later development of asthma in children with seasonal RC. Asthma development showed an OR of 2.5 (95% CI: 1.1–5.9) in favour of the hypothesis that SCIT could prevent the long-term development of asthma.
Therapy: 3 years; Follow-up: 2 years	Int: 15; Cp: 29.	Asthma symptoms and response to B2-agonists.	High	Asthma development was the primary outcome. This follow-up study indicates that SIT for 3 years had a long-term effect on symptoms and a reduction of the risk of developing asthma in children. OR (95% CI) of 2.68 (1.3–5.7, $p < .05$) in favour of the hypothesis that SCIT can prevent the long-term development of asthma.
Therapy: 3 years; Follow-up: 0	Int: 19; Cp: 32.	Asthma symptoms and use of B2-agonists.	High	Asthma development was the primary outcome. A 3-year course of SIT in children with allergic RC significantly reduces the risk of developing clinical asthma and improves BHR.

(Continues)

TABLE 1 (Continued)

Author, year, country	Design	Participants, allergic disease(s), sensitization	Intervention group	Comparator group
Eng et al, 2006, Switzerland[39e]	NRSI	Children (5–16 years old); RC; only sensitized to grass or tree	*N* = 14. SCIT (grass pollen, Allergovit, Allergopharma)	*N* = 14. Control
Eng et al, 2002, Switzerland[41e]	NRSI	Children (5–6 years old); RC; only sensitized to grass or tree	*N* = 14. SCIT (grass pollen, Allergovit, Allergopharma)	*N* = 14. Control
Crimi et al, 2004, Italy[29]	RCT	Adults (20–54 years old); Rhinitis; mono-sensitized only	*N* = 15. SCIT (Parietaria judaica, Alutard, ALK Abelló)	*N* = 15. Placebo
Novembre et al, 2004, Italy[30]	RCT	Children (5–14 years old); RC; only mono-sensitized	*N* = 54. SLIT drops (grass pollen, ALK Abelló). After follow-up: 47	*N* = 59. Control. After follow-up: 50
Madonini et al, 2003, Italy[40b]	NRSI	Mixed (2–68 years old); Rhinitis, asthma	*N* = 281. SLIT (Any allergen type, Allergopharma)	No comparator
Grembiale et al, 2000, UK[31]	RCT	Mixed (10–38 years old); Rhinitis; mono-sensitized only	*N* = 22. SCIT (HDM, Conjuvac, Bayer)	*N* = 22. Placebo
Pradalier et al, 1999, France[32b]	RCT	Mixed (>6 years old); Seasonal rhinitis, RC, mild asthma; sensitized to grass pollen only	*N* = 62. SLIT (5- grass pollen, Stallergenes Greer)	*N* = 61. Placebo
Moller et al, 1986, Sweden[33]	RCT	Children (8–16 years old); RC; mono or poly-sensitized	*N* = 16. SLIT tablets (birch pollen capsules)	*N* = 16. Placebo

Note: Studies presenting quantitative results regarding the effects of AIT on asthma were included in the meta-analysis (if data available on the column 'Asthma, *N* of events').

Abbreviations: AIT, Allergen immunotherapy; AR, allergic rhinitis; CI, confidence interval; Cp, Comparator group; HDM, house-dust mite; ICD-10, International Statistical Classification of Diseases; ICS, inhaled corticosteroids; Int, Intervention group; Mod., Moderate; *N*, number of cases; NA, not applicable; NRSI, non-randomized studies of interventions; OR, odds ratio; RC, rhinoconjunctivitis; RCT, randomized controlled trial; SABA, short-acting beta agonists; SCIT, subcutaneous immunotherapy; SLIT, sublingual Immunotherapy; Tx, treatment.

[a] Data were presented (sample size) for individuals without asthma at baseline.

[b] The primary outcome data were used in the meta-analysis.

[c] Not included in the meta-analysis.

[d] PAT study articles. For the meta-analysis, we used Moller et al, 2002. When considering the subgroup analysis by time of follow-up, we considered also Jacobsen 2007 due to the longer follow-up time.

[e] Studies with same population but different follow-up times (both, long-term follow-up, >6 years). For the meta-analysis, we used Eng et al, 2006.

Therapy duration; follow-up	Asthma, *N* of events	Asthma definition	Risk of bias	Main findings
Therapy: 3 years; Follow-up: 12 years	Int: 4; Cp: 7	Asthma symptoms.	High	Asthma onset was a secondary endpoint. This prospective controlled long-term follow-up study demonstrates that both the clinical efficacy and the preventive capacity of grass pollen SCIT are evident 12 years after the end of SIT when compared with seasonal pharmacotherapy alone. This study is the continuation *of Eng* et al, *2002*.
Therapy: 3 years; Follow-up: 6 years	Int: 3; Cp: 7	Asthma symptoms.	High	Asthma onset was a secondary endpoint. It demonstrates that SIT in children with monovalent pollen allergy prevents the development of new sensitization, reduces the prevalence of subsequent asthma and therefore has the potential to modify the natural course of the allergic disease.
Therapy: 3 years; Follow-up: 0.	Int: 2; Cp: 7	Asthma symptoms.	Mod.	Asthma onset was a secondary outcome. The preventive effect of SIT did not reach significance ($p = .056$) but a trend was apparent; 47% of subjects in the placebo group developed asthma symptoms by the end of the study, compared to 14% in the SIT group.
Therapy: 3 years; Follow-up: 0.	Int: 8; Cp: 18	Asthma symptoms and medication use.	High	Asthma development was not defined as the primary or secondary outcome. The results of this study show that it can prevent the onset of asthma in children with RC.
Therapy: 26 (mean) months; Follow-up: 9 months (mean)	Int: 2	Asthma symptoms.	NA	Not specified if asthma is a primary or secondary outcome. Despite the limitations of a retrospective, uncontrolled study, the results point to the long-term and preventive effects of SLIT benefits.
Therapy: 2 years; follow-up: 0.	Int: 0; Cp: 2	Asthma symptoms.	Mod.	Asthma was a secondary outcome. This pilot study, although not designed to detect differences in the incidence of asthma between the SCIT and placebo groups, suggests that individuals with allergic rhinitis should undergo testing of their bronchial reactivity to identify those with BHR and to provide a window of opportunity in which SCIT may be effective at preventing progression to asthma.
Therapy: 6 months. Follow-up: 0.	No data for subjects without asthma at baseline	Asthma symptoms.	Mod.	Asthma onset was a secondary outcome. The proportion of patients who experienced asthma symptoms at least once during the pollen season was significantly smaller ($p = .005$) in the active treatment group (15%) than in the placebo group (38%). Adjusting for a personal history of asthma did not abolish the significant between-group differences.
Therapy: 10 months; Follow-up: 0.	Int: 0; Cp: 5	Lung symptoms.	Mod.	Asthma onset was a secondary outcome. AIT would appear to have protected against the development of asthma during this period.

data, unravelling important clinical implications and supporting the consistency of the main results. For the first time, a meta-analysis pooled separate effect estimates for SLIT tablets and SLIT drops. Finally, the influence analysis was important to identify high impact studies driving the results and to increase the robustness of findings.

The main limitation is the low number of high-quality studies; specifically, only one was considered of low risk of bias. The sensitivity analysis supports the importance of this gap since it led to imprecise results. However, the biological plausibility of AIT effects in allergic diseases and the absence of studies pointing for a statistically significant increased risk of using AIT as a prevention strategy support the results presented. Most RCTs were conducted using small samples contributing to imprecision (wider confidence intervals) of individual studies. The effect estimate generated in NRSI was based on the raw results presented in each study. However, four studies presented the adjusted relative risk ratio which was systematically lower than the relative risk obtained in this meta-analysis; thus, the results provided by observational studies on the pooled meta-analysis might be underestimated. Nevertheless, NRSI results should be analysed cautiously having in mind the risk-of-bias and confounder factors. Another limitation is the fact that in very few studies asthma onset was a primary or a secondary endpoint, highlighting the need for studies specifically designed for the assessment of asthma onset. Additionally, asthma definition should follow GINA guidelines whenever possible to guarantee precise results and allow better comparisons across studies; specifically, when using a more complete definition of asthma (including symptoms, medication and lung function assessments), in a subgroup analysis, the results on asthma prevention were not significant, although the results suggest publication bias further evaluation using a trim-and-fill method to compute and estimate the impact of small studies missing in the analysis did not change the estimate, supporting their consistency. Finally, the subgroup analysis according to SLIT administration routes (drops vs. tablets) was hampered by the small number of studies.

The evidence provided by RCTs is more robust when compared to NRSI studies because of the randomization process which make results less prone to bias compared to NRSI. Furthermore, therapy compliance and treatment duration according to recommendations tended to be better accomplished and evaluated in RCTs, which could contribute to the observed positive effects of asthma prevention in these studies. However, some RCTs included in this review raise some concerns due to the unblinding of participants and outcome assessors, contributing to a lower quality of the evidence.[26–28,30,39,41] Authors justified the lack of use of controls due to ethical issues concerning the use of placebo in children for a long time and the difficulties in justifying its use to parents when asthma is a possible outcome.[30,41] However, more studies in this age group are needed despite the anticipated limitations and challenges regarding blinding. Still, the recent high-quality RCT conducted by *Valovirta* et al. in children used a large placebo group.[23] The study was primarily designed to detect differences in asthma onset according to different criteria. Despite not being significant for the time of asthma onset strictly defined by symptoms, medication and lung function, the adjusted odds ratio was statistically significant when considering asthma defined by symptoms and medication use, indicating a reduction of 33% in the risk of developing asthma, which corroborates with our findings.[23]

Non-randomized studies of interventions reflect better the real-world effectiveness of AIT but raises serious concerns regarding the selection of participants, reporting bias and confounders. Allergic patients receiving AIT are generally from higher socioeconomic status, with facilitated access to healthcare specialists, overestimating the effect. However, better access to the healthcare system reduces the likelihood of undiagnosed asthma in the intervention group, which may be the opposite in patients with less access or in the control group, underestimating the results. Another limitation is the incomplete or absence of data on relevant prognostic variables, especially in retrospective studies, such as multiple sensitization status, severity and duration of allergic disease for which AIT is being prescribed, and previous history of asthma. The previous variables may underestimate the results because patients requesting AIT usually have a longer and more severe disease, a previous or familiar history of asthma, and are multiple sensitized. The retrospective study conducted by *Schmitt* et al. was considered of moderate quality due to efforts in minimizing bias and confounding, such as the criteria to select controls and adjustment of the effect measure for *a priori*-defined confounder (age, sex, healthcare use, antihistamines use), as well as the outcome definition reliably.[36] Asthma was defined according to reporting of the ICD-10 code on the administrative database, at least two times, and prescription of asthma medication. At baseline, AIT patients were more likely to have higher prescriptions of antihistamines and healthcare use because of allergic rhinitis, being more likely to be diagnosed with asthma, which may underestimate the results.[36] Some studies might have reported overestimated results since control patients had more severe disease (higher number of allergic rhinitis prescriptions).[34,35] The study by *Schmitt* et al. reported a markedly decrease in the adjusted relative risk of 40% in developing asthma compared with the conservative result calculated by the random-effects meta-analysis that was based on raw event counts without any adjustments for covariates. Still, the adjusted association measure highlighted a higher preventive effect for SCIT, independently of allergen (seasonal or perennial) and duration of AIT.[36] Finally, we cannot rule out indication bias, which is inherent to these studies designs, as well as reporting bias since patients adhering to AIT are more carefully accompanied by physicians over time and asthma diagnosis can be reported more frequently. The study considered as an outlier showed different effects on asthma prevention.[44] The results were explained by the authors to be due to reporting bias since patients from the intervention group visit more frequently specialists and asthma definition required a medical diagnosis coded onto the database. This reasoning was supported by the major conclusions of the study that pointed a preventive effect of AIT in asthma progression from mild to severe.[44]

Treating allergic multimorbidities may prevent the onset of asthma because of induced allergen-specific immune tolerance, impacting underlying mechanisms of asthma development.[11] However,

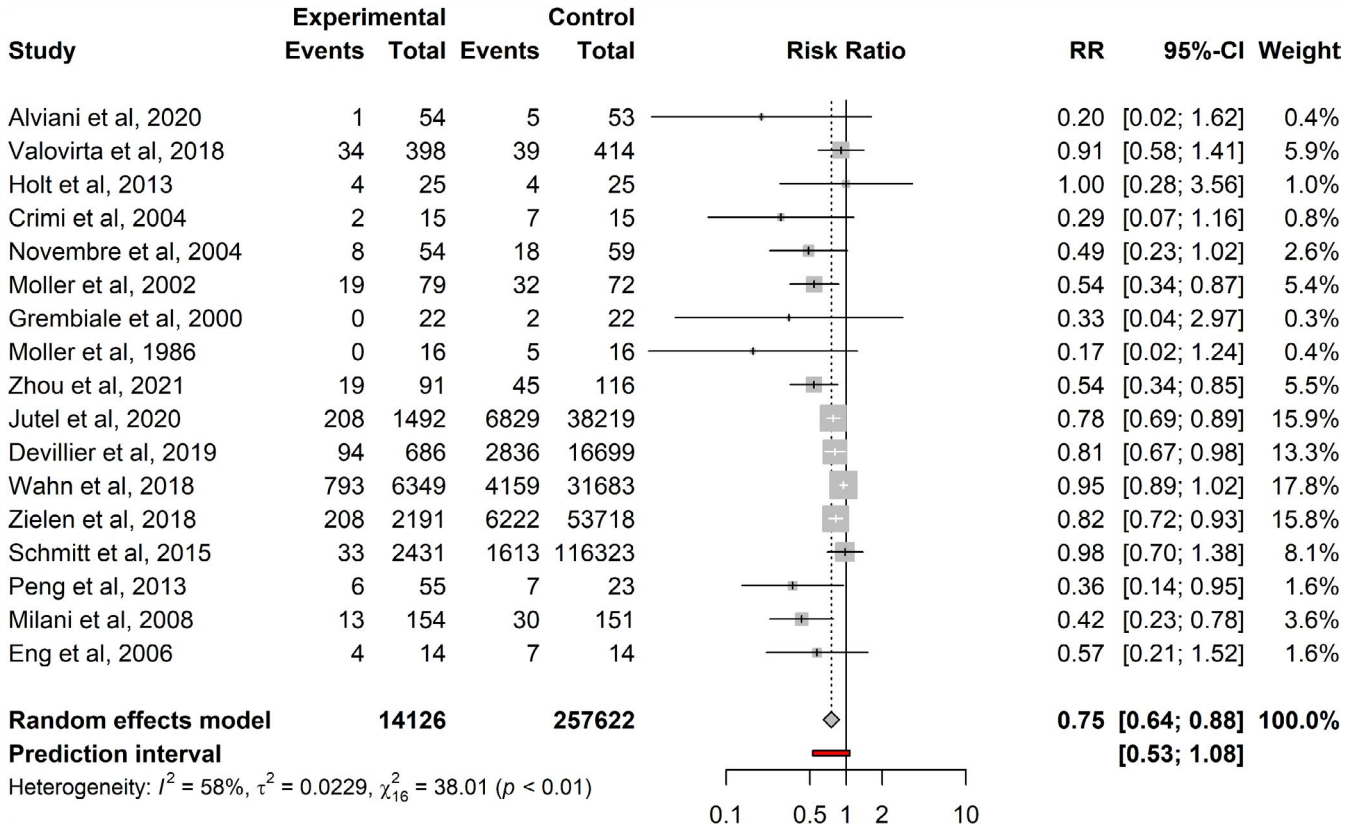
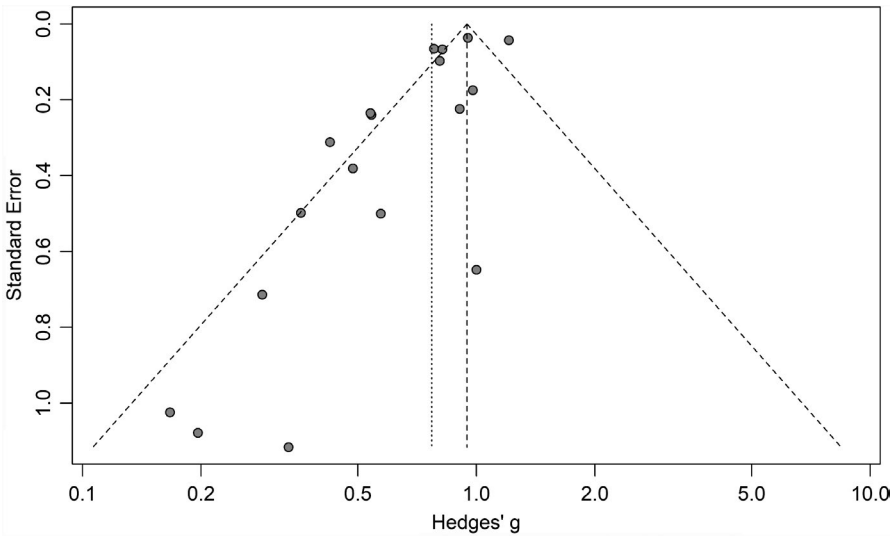


FIGURE 2 Random-effects meta-analysis of effectiveness of AIT in the prevention of asthma (*N* of studies =17). Diamond: pooled effect; grey square: represents study weight; black horizontal line: study confidence interval

FIGURE 3 Publication bias assessed by funnel plot



it is important to unravel the underlying mechanisms conferring higher risk of developing asthma and who will better respond to AIT, highlighting the importance of discovering treatable traits using the multimorbidity framework. Furthermore, it should be noted that AIT is not currently recommended in healthy individuals nor with atopic dermatitis because the link between atopic dermatitis and asthma development is not clear and studies are generally inconclusive.[48] Still, this work included evidence from studies conducted in the aforementioned population to ensure a comprehensive analysis of the literature.

Our data have important clinical and research implications. In terms of clinical practice, they may contribute to the further improvement of guidelines by extending the efficacy results of AIT in treating allergic diseases to its secondary preventive role in asthma.[49] Moreover, it might be an additional reason for patients to initiate AIT and to be more compliant, although future expectations

TABLE 2 Meta-analysis when combining all studies, all studies except the outlier (*Fritzsching* et al, *2021*), and by AIT administration route (SLIT or SCIT)

	All studies	All studies except the outlier	SLIT	SCIT
Meta-analysis, RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	**0.77 (0.64; 0.92)** **79.5%; 20.63** **18**	**0.75 (0.64; 0.88)** **58%; 38.01** **17**	**0.77 (0.66; 0.89)** **17%; 10.79** **10**[a]	**0.72 (0.56; 0.93)** **70%; 26.37** **9**[a]
Sensitivity, RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	0.76 (0.45; 1.27) 27%; 8.25 7	**0.76 (0.45; 1.27)** **27%; 8.25** **7**	0.77 (0.39; 1.54) 12%; 4.56 5	0.62 (0.13; 2.94) 33%; 2.97 3
Subgroup analysis				
Study design				
RCT, RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	**0.59 (0.40; 0.88)** **17%; 8.44;** **8**	**0.59 (0.40;0.88)** **17%; 8.44** **8**	0.63 (0.29; 1.35) 33%; 5.93 5	**0.50 (0.27; 0.93)** **0%; 0.87** **3**
NRSI, RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	0.82 (0.66–1.02) 86%; 65.41; 10	**0.79 (0.66; 0.94)** **67%; 24.08** **9**	**0.78 (0.66; 0.93)** **15%; 4.71** **5**	0.78 (0.59; 1.04) 73%; 18.41 6
Outcome				
Primary analysis, RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	0.84 (0.67; 1.06) 64%; 13.89 6	0.84 (0.67; 1.06) 64%; 13.89 6	0.76 (0.57; 1.03) 0%; 2.35 4	0.83 (0.59; 1.17) 78%; 13.75 4
Secondary analysis, RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	**0.67 (0.50; 0.89)** **83%; 63.74** **12**	**0.65 (0.52; 0.82)** **38%; 16.08** **11**	0.73 (0.54; 1.00) 40%; 8.38 6	**0.48 (0.36; 0.65)** **0%; 1.34** **5**
Population				
Children, RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	**0.71 (0.53; 0.96)** **30%; 10.04** **8**	**0.71 (0.53; 0.96)** **30%; 10.04** **8**	0.63 (0.29; 1.35) 33%; 5.93 5	0.71 (0.37; 1.38) 48%; 3.81 3
Adults, RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	0.75 (0.56; 1.01) 45%; 10.89 7	0.75 (0.56; 1.01) 45%; 10.89 7	0.78 (0.58; 1.05) 30%; 4.28 4	0.54 (0.21; 1.35) 43%; 5.24 4
Therapy duration				
<3 years, RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	0.85 (0.67; 1.07) 85%; 60.37 10	**0.81 (0.68; 0.96)** **62%; 20.82** **9**	**0.76 (0.61; 0.94)** **32%; 8.77** **7**	0.87 (0.59; 1.36) 79%; 9.69 3
≥3 years, OR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	**0.64 (0.47; 0.88)** **42%; 12.01** **8**	**0.64 (0.47; 0.88)** **42%; 12.01** **8**	0.78 (0.35; 1.74) 2%; 2.04 3	**0.60 (0.42; 0.85)** **22%; 6.43** **6**
Follow-up after treatment discontinuation				
Short-term (≤3 years), RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	**0.74 (0.62; 0.88)** **63%; 34.92** **14**	**0.74 (0.62; 0.88)** **63%; 34.92** **14**	**0.77 (0.65; 0.91)** **23%; 9.12** **8**	**0.70 (0.50; 0.97)** **77%; 25.55** **7**
Long-term (>3 years), RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	0.88 (0.51; 1.49) 67%; 11.93 5	0.73 (0.38; 1.40) 33%; 4.47 4	Not enough data	0.76 (0.42; 1.37) 0%; 1.62 3
Funding				
Pharma, RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	0.84 (0.64; 1.11) 87%; 51.91 8	0.79 (0.62; 1.02) 68%; 18.54 7	**0.77 (0.63; 0.95)** **32%; 7.37** **6**	Not enough data
Academic/others, RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	**0.68 (0.53; 0.88)** **29%; 12.58** **10**	**0.68 (0.53; 0.88)** **29%; 12.58** **10**	0.59 (0.27; 1.31) 0%; 2.43 4	**0.69 (0.53; 0.91)** **24%; 7.94** **6**
Asthma definition				
[b]Mixed criteria, RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	0.77 (0.34; 1.73) 46%; 5.57 4	0.77 (0.34; 1.73) 46%; 5.57 4	0.85 (0.36; 2.03) 0%; 1.95 3	Not enough data

TABLE 2 (Continued)

	All studies	All studies except the outlier	SLIT	SCIT
Symptoms + medication, RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	**0.52 (0.45; 0.60)** **0%; 0.07** **3**	**0.52 (0.45; 0.60)** **0%; 0.07** **3**	**0.50 (0.40;0.61)** **0%; 0** **2**	Not enough data
Medication, RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	0.91 (0.72; 1.14) 92%; 47.42 5	**0.85 (0.73; 0.99)** **70%; 9.96** **6**	**0.80 (0.71; 0.91)** **0%; 0.65** **3**	Not enough data
Symptoms, RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	**0.45 (0.29; 0.72)** **0%; 3.24** **6**	**0.45 (0.29; 0.72)** **0%; 3.24** **6**	0.47 (0.10; 2.24) 20%; 2.51 3	0.44 (0.16; 1.16) 0%; 0.73 3
SLIT administration route				
Drops, RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	-	-	**0.65 (0.48; 0.89)** **5%; 5.28** **6**	-
Tablets, RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	-	-	**0.82 (0.70; 0.96)** **0%; 2.65** **4**	-
Previous sensitization				
Only mono-sensitized, RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	**0.49 (0.39; 0.61)** **0%; 1.36** **6**	**0.49 (0.39; 0.61)** **0%; 1.36** **6**	Not enough data	**0.49 (0.36; 0.66)** **0%; 1.36** **5**
Mono- or poly-sensitized, RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	0.85 (0.70; 1.03) 84%; 63.43 11	**0.81 (0.70; 0.95)** **61%; 22.85** **10**	**0.79 (0.69; 0.90)** **15%;7.53** **8**	0.83 (0.58; 1.17) 79%; 14.04 4
Allergen				
Grass pollen, RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	**0.79 (0.68; 0.92)** **5%; 5.28** **6**	**0.79 (0.68; 0.92)** **5%; 5.28** **6**	**0.81 (0.71; 0.94)** **0%; 2.09** **4**	**0.55 (0.42; 0.72)** **0%; 0.01** **2**
House dust mite, RR (95% CI) Heterogeneity: I^2; Q *N* of studies analysed	**0.60 (0.38; 0.96)** **40%; 6.63** **5**	**0.60 (0.38; 0.96)** **40%; 6.63** **5**	Not enough data	0.63 (0.38; 1.05) 41%; 5.10 4

Note: For each output, we report the corresponding RR (95% CI), the heterogeneity parameters, and the number of studies included. Results for sensitivity analysis and subgroup analysis are also included.

Abbreviations: CI, confidence interval; I^2, Higgins & Thompson's I^2 Statistic; *N*, number of studies; NRSI, non-randomized studies of interventions; Q, Cochran's Q; RCT, randomized controlled trial; RR, risk ratio; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy.

[a]Two studies presented data for both SLIT and SCIT administration routes. The study by *Fritzching* et al, *2021*. presented mixed data for both SLIT and SCIT but not separated by each administration route and, thus, was not included in these analyses.

[b]Mixed criteria were defined as a combination of symptoms, medication and lung function assessments to diagnose asthma; thus, in these studies, asthma diagnosis was more accurate because it followed a more complete and strict criteria. One of these studies reported an asthma diagnosis based on the GINA guidelines.

Bold values denote statistical significance at the $p < 0.05$ level.

must be managed carefully as it is not fully understood how to select patients that will benefit the most from the preventive effect of AIT. Concerning research implications, our study reveals the lack of high-quality well-powered studies, independent of study design, especially for the long-term effects after AIT discontinuation. Evidence for implementation is also lacking. RWE provided by health-related nationwide and representative databases may overcome such limitations; however, researchers must ensure a suitable and comparable control group of patients and minimize, whenever possible and employ appropriate methodologies, confounding effects.[47] This study also provides data and highlights the importance of including asthma prevention outcomes in cost-effectiveness models.[50]

5 | CONCLUSIONS

This systematic review with meta-analysis suggests a possible preventive role of AIT in the short-term reinforcing the body of evidence and highlights a more pronounced effect in children, mono-sensitized, and for the patients completing 3 years of therapy, independently of allergen used for AIT. However, we should be aware that this effect was observed when considering the diagnosis of asthma based on symptoms or medication, which may indicate that AIT can prevent the onset of asthma-like symptoms and the use of asthma medication. It may be argued that secondary prevention of asthma using AIT has its best chance in stopping the progression

of allergic multimorbidities when administered early in life and at the beginning of the allergic disease and sensitization. Results were more noticeable for SCIT and SLIT drops, but SCIT tablets also demonstrated protective effects.

This work highlights the importance of gathering data from different sources, both RCTs and NRSI, allowing for a comprehensive analysis of different perspectives and reflecting the real-world complexity and preferences of populations. Future implications of using AIT as a secondary allergy prevention strategy while treating allergic rhinitis, atopic dermatitis, or rhinoconjunctivitis, depends on the availability of high-quality evidence, compliance and adherence of participants, and costs.

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CONFLICT OF INTEREST

None of the other authors report any conflict of interest in relation to this work.

AUTHOR CONTRIBUTIONS

AM, JCR and MF conceptualized the work and performed the literature search. AM, IA and MS discussed and approved the clinical aspects and conclusions as medical experts in this field. MF and JCR selected, extracted the data and evaluated the risk of bias of the studies. MF summarized data and conducted the meta-analysis. All authors discussed the results and were involved in the manuscript drafting and writing. All authors revised critically the manuscript.

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SUPPORTING INFORMATION

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Allergen immunotherapy for asthma prevention: A systematic review and meta-analysis of randomized and non-randomized controlled studies

Appendix S1

Results

1. Assessment of Risk of Bias

During the risk of bias evaluation, only one study was classified as having high quality (low risk of bias), followed by seven of moderate quality, and 15 of low quality (high risk of bias). Regarding RCTs, we considered five to be at high risk of bias mainly due to the use of a control group instead of a placebo group, which raises concerns due to unblinding of participants and assessors when measuring the outcome; and missing data of participants that were lost to the follow-up, usually without specifying the reasons and if the dropouts were balanced or unbalanced between the groups. As expected, the risk of bias was higher in non-randomised studies of intervetion (NRSI) mainly due to indication, selection, and detection bias reflecting the real-world nature of these studies, and consequently, confounding. We considered one non-randomized study of moderate quality due to the appropriate measures taken to minimize bias and confounding (matching, multivariate modelling) and the proper definition and assessment of the outcome reliably. A risk-of-bias diagram is presented in figure S1 and S2.

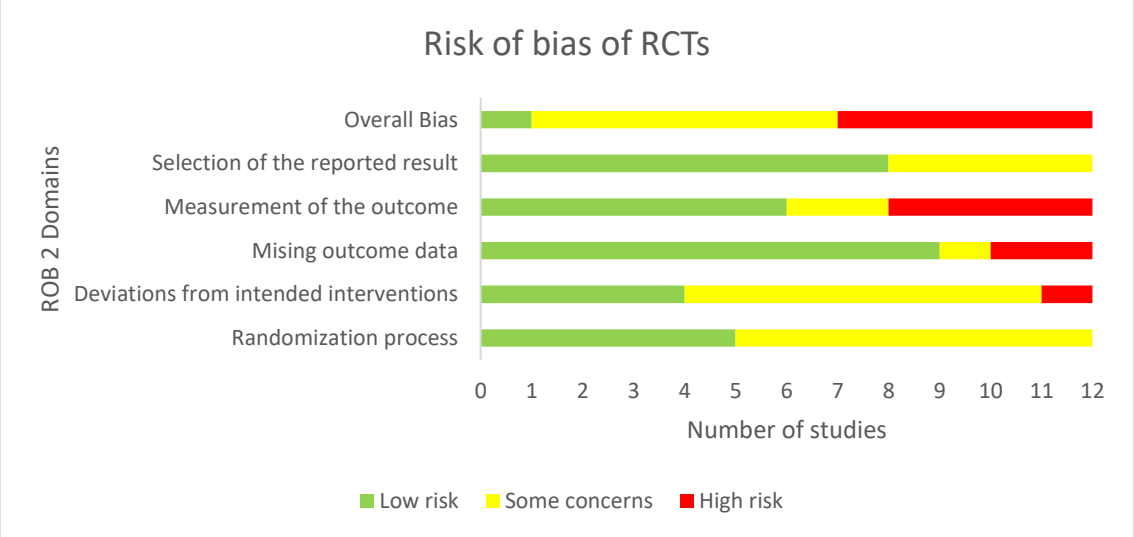

Figure S1 - Risk of Bias Assessment of randomised clinical trials (RCTs) using the ROB 2 Tool.

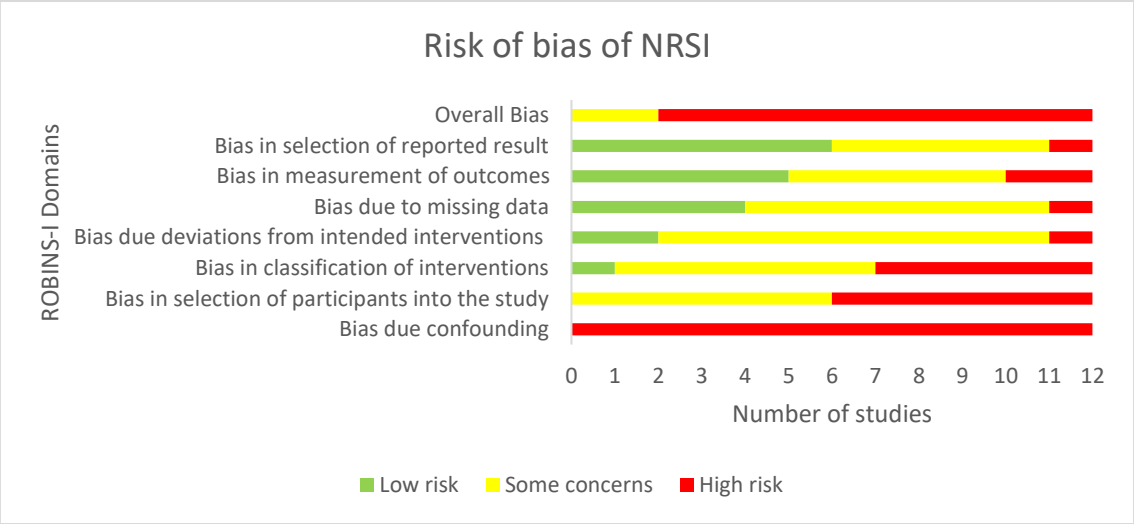

Figure S2 - Risk of Bias Assessment of non-randomised studies of intervention (NRSI) using ROBINS-I Tool.

2. Meta-analysis

2.1. Outlier analysis

By visual inspection, a study can be an outlier if its confidence interval does not overlap with the confidence interval of the pooled effect. In this meta-analysis, we can identify one outlier case: Fritzsching et al, 2021. The pool effect size with that study is presented in the figure below.

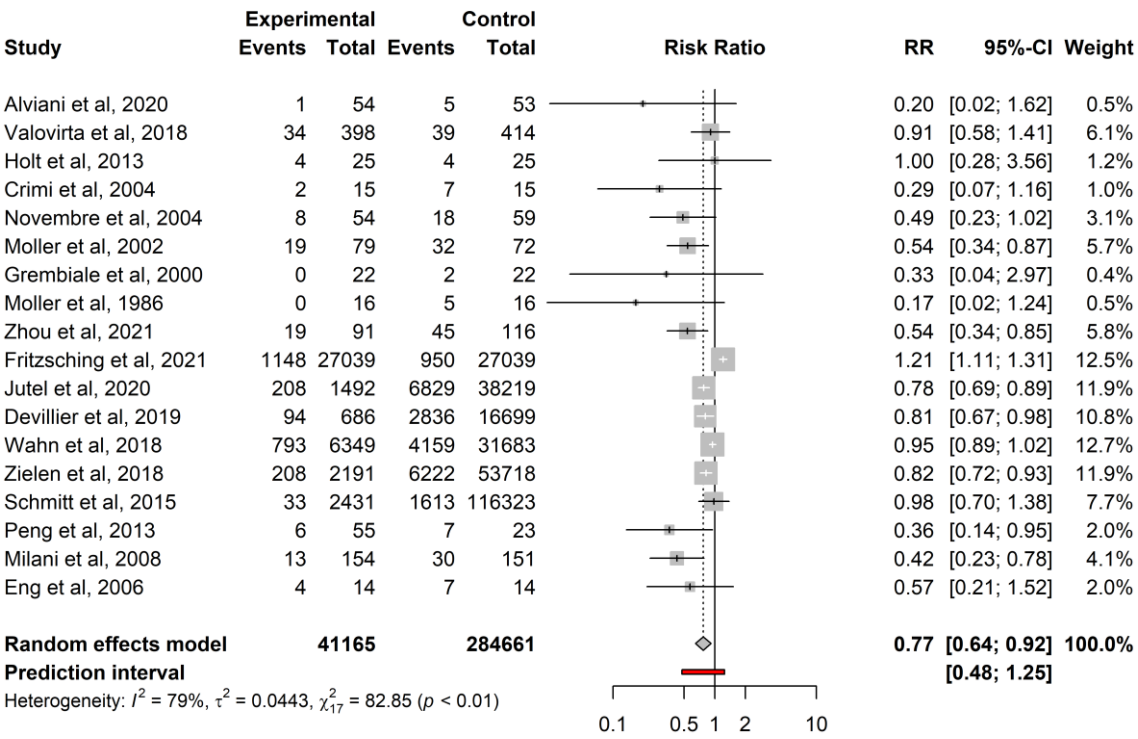

Figure S3 – Forest plot including all studies in the meta-analysis. Fritzsching et al, 2021. is an outlier case.

2.2. Influential analysis

Influential analysis is used to know if the pooled effect estimate we found is robust, meaning that it does not depend heavily on one single study. Therefore, we also want to know whether there are studies which heavily push the effect of our analysis into one direction. The following graphs highlight two influential studies: Fritzsching et al, 2021. and Wahn et al, 2018.

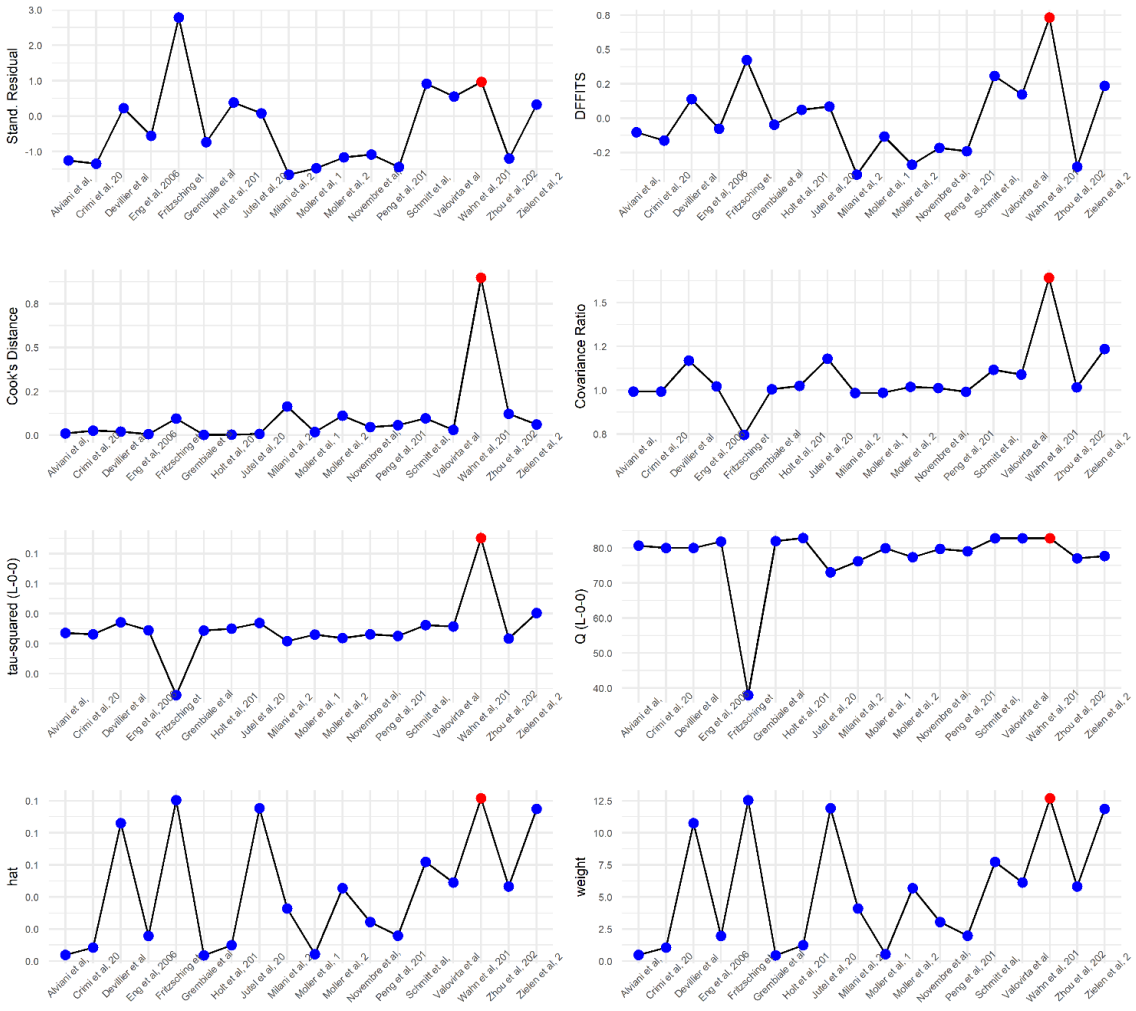

Figure S4 - Influence analysis according to different influence measures. Influential cases (in red) are those with very extreme values in graphs.

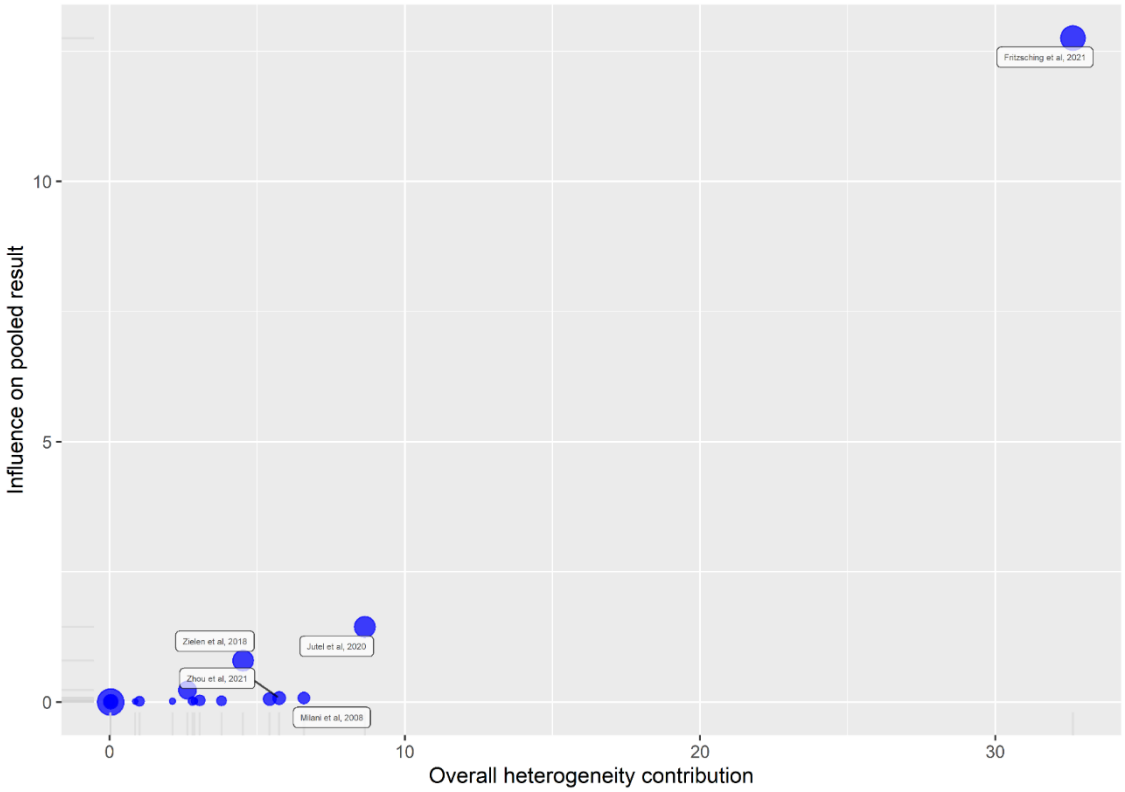

Figure S5 - Baujat Plot. Diagnostic plot to detect studies contributing to heterogeneity (those at the right side of the plot), Fritzsching et al, 2021.

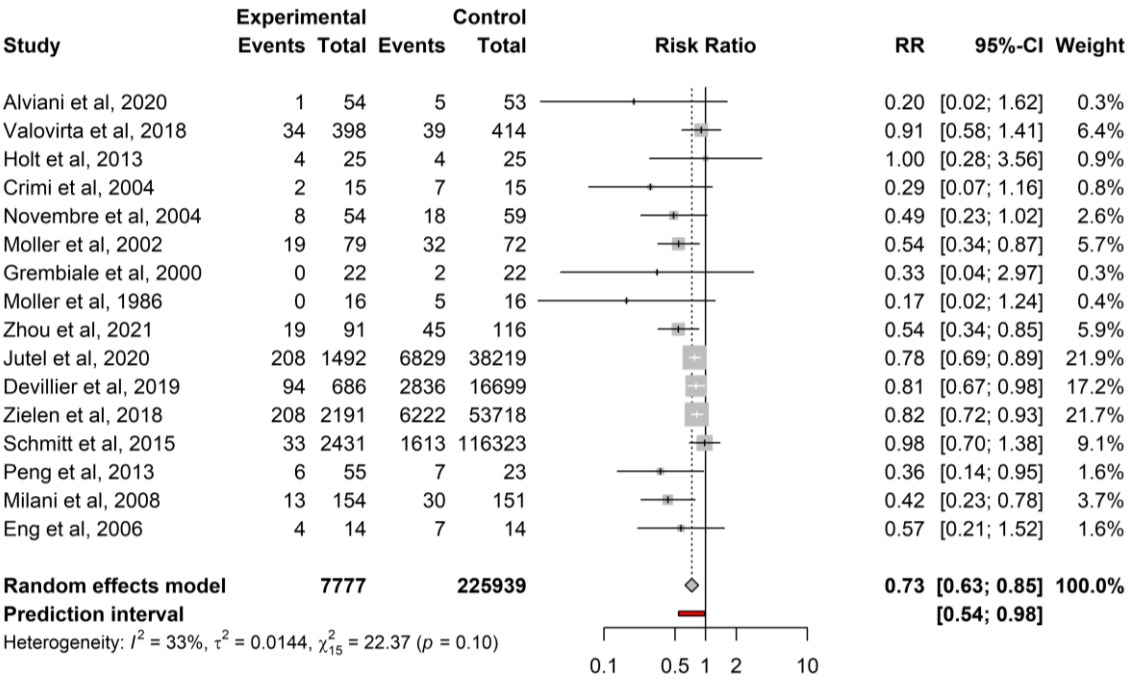

Figure S6 – Forest plot witout influential cases (Fritzsching et al, 2021. and Wahn et al, 2018.).

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Scientific Publication IV

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ORIGINAL ARTICLE

Asthma and Lower Airway Disease

Cost-effectiveness analysis of house dust mite allergen immunotherapy in children with allergic asthma

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Abstract

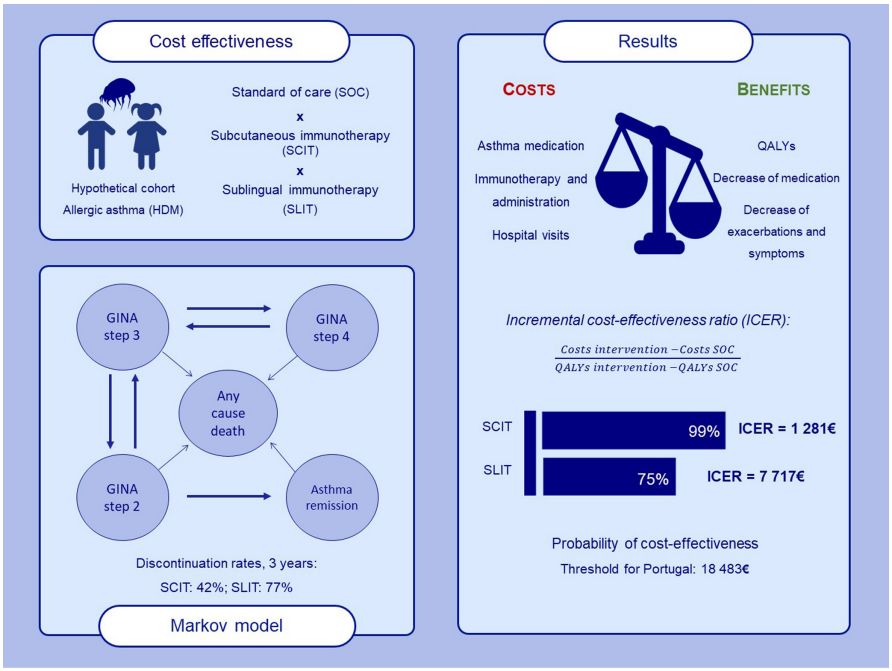
Background: Cost-effectiveness studies evaluating allergen immunotherapy (AIT) in children are limited but needed to drive clinical and policy-making decisions such as reimbursement of new interventions. In this study, we compared the cost effectiveness of subcutaneous (SCIT) and sublingual immunotherapy (SLIT) tablets to the standard of care (SOC) treatment in children with house dust mite-driven (HDM) allergic asthma.

Methods: We developed a hypothetical Markov model based on the Global Initiative for Asthma (GINA) severity steps to compare the three strategies over a 10-year horizon divided by cycles of 6 months. SOC was used as a reference to calculate the incremental cost-effectiveness ratio (ICER). Deterministic and probabilistic sensitivity analyses were used to assess models' uncertainty. Other scenarios were evaluated to strengthen the presentation of results.

Results: The ICER for SCIT and SLIT tablets was 1281€ and 7717€, respectively. The cost-effectiveness threshold for Portugal was 18,482.80€; both treatment approaches were below this limit. The major contributors to these results were the AIT effects on reducing moderate and severe exacerbations and asthma controller medication. In the sensitivity analysis, SCIT revealed a higher probability of cost-effectiveness than SLIT. When including allergic rhinitis as comorbidity, ICER values reduced markedly, especially for SCIT intervention.

Conclusions: AIT was cost effective in children with HDM-driven allergic asthma, especially when given by the subcutaneous route. The high probability of cost effectiveness, especially for SCIT, may drive future policy decisions and AIT-prescribing habits. AIT adherence greatly influenced the results highlighting the value of implementing strategies to promote adherence rates.

KEYWORDS
allergen immunotherapy, asthma, children, cost effectiveness, house dust mites



GRAPHICAL ABSTRACT

We used a Markov model, based on GINA steps, to compare the cost-effectiveness of subcutaneous and sublingual tablet immunotherapy against standard of care in children with house dust mite allergic asthma. Both therapies diminished asthma medication and exacerbations while quality of life increased. Both therapies were cost effective, but SCIT showed a higher probability of cost effectiveness.

Abbreviations: GINA, global initiative for asthma; HDM, house dust mite; ICER, incremental cost-effectiveness ratio; QALYs, quality-adjusted life-years; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; SOC, standard of care

1 | INTRODUCTION

Asthma is a chronic health problem that is increasing in prevalence and substantially impacting the quality of life and healthcare costs.[1–3] The worldwide trends in disease published by *The Lancet* in 2019 ranked asthma as 24th in the leading causes of disease burden measured as years of life lived with disability (YLD).[2] Both in children and adults, uncontrolled asthma has a significant effect on the quality of life, and daily activities are associated with loss of productivity at school and work.[4] Children with asthma miss 2.5 school days each year than children without asthma.[5] Asthma affects 224–309 million people worldwide, and its prevalence varies according to geographic region and age[2]; in Portugal, it is estimated to affect 5.3% of the population.[6] In children, asthma is the most common chronic disease.[2] Allergen sensitization is present in up to 50% of all cases of asthma in adults and up to 80% in the pediatric population.[7,8]

The standard of care (SOC) pharmacotherapy to achieve symptoms' control follows the Global Initiative for Asthma (GINA) guidelines.[9] It includes the prescription of inhaled corticosteroid (ICS) plus long-acting β-agonists (LABA). Sublingual tablets of house dust mite (HDM) allergen immunotherapy (AIT) are also recommended by GINA guidelines for mild to moderate cases of patients with asthma not obtaining adequate control of the disease. HDM is a common and relevant inhalant allergen worldwide; specifically, HDM such as *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* are a vital source of indoor allergens associated with rhinitis and asthma[10,11] and, usually, the development of sensitization to HDM occurs before polysensitization.[12] Around 26% of people in Portugal are sensitized to HDM.[13] AIT has a disease-modifying effect and induces allergen-specific immune tolerance. It confers prolonged benefits if administered for at least 3 years.[11,14] Thus, it becomes decisive to evaluate the economic benefits and health gains of HDM AIT compared with SOC in respiratory allergic diseases. There are few studies investigating the cost-effectiveness of AIT, both for subcutaneous (SCIT) and sublingual (SLIT, as tablets or drops) administration routes, in children with HDM allergic asthma. Children are an essential group to assess cost-effectiveness because AIT benefits may remain long-term. Moreover, there are no studies performed within the context of the Portuguese healthcare system.

Cost-effectiveness analysis (CEA) is a recognized approach to estimate short- and long-term consequences on costs and health gains of a specific treatment.[15] In Portugal, AIT is an expensive therapy that is not reimbursed, causing high nonadherence rates and health inequalities in terms of access to therapies.[16,17] The results provided by these hypothetical models are essential to guide policy decisions, such as the reimbursement of therapies and to improve quality of life while reducing health inequalities.

We compared SCIT and SLIT tablets in children with HDM-driven allergic asthma using a mathematical modeling approach and parameters from multiple sources, including randomized controlled trials and real-world data, according to the Portuguese healthcare system.

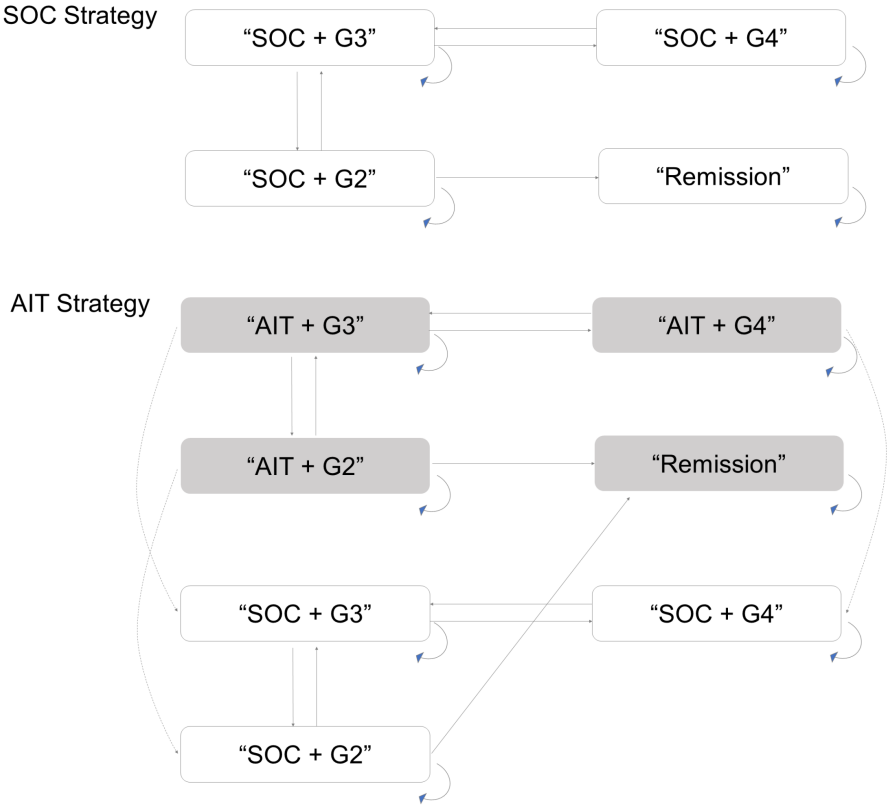


FIGURE 1 Basic structure of the Markov model. The risk of death is not shown (in order to simplify representation), but all patients, in any health state, are in risk of any-cause death. Patients under intervention (AIT administration—SCIT or SLIT) may discontinue treatment at any time (dashed arrows); at that time, patients follow the transitions and health states represented for the SOC arm. Patients achieving asthma remission do not return to any other health state (single arrow). AIT represents SCIT and SLIT strategies. All patients under the AIT strategy start, at baseline, in "AIT+G3" health state; all patients under the SOC strategy start, at baseline, in "SOC+G3" health state. AIT, allergen immunotherapy; Gx, GINA steps; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; SOC, standard of care treatment

2 | METHODS

We developed a hypothetical Markov model to evaluate the costs and health outcomes of HDM allergen immunotherapy, for both SLIT tablets and SCIT, in children with allergic asthma plus pharmacotherapy compared with pharmacotherapy alone (SOC). The adopted framework used the stepwise approach of GINA (Global Strategy for Asthma Management and Prevention of the Global Initiative of Asthma) to define health states and was based on a previous cost analysis study conducted by *Parra-Padilla* et al.[18] For the mathematical model; we simulated a hypothetical cohort of 12-year-old children, 1000 per strategy, over a 10-year horizon divided into 6-month cycles and following the Portuguese healthcare system perspective. The cycle length was defined to represent the average of medical visits scheduled in a year and allow adjustments in asthma therapy as in real-life. Each health state had an associated cost and utility value. Moderate and severe exacerbations were incorporated into the model as a relevant health outcome. Thus, the effectiveness of AIT was captured through changes in (a) health-related quality of life, quantitively measured as utilities gained (positive scale, from zero to one); (b) asthma exacerbations, included as disutilities (negative scale, opposite of utilities); and (c) medication requirements, as patients moved along health states according to treatment-specific probabilities of step-up or step-down controller medication. The inclusion criteria of patients were a diagnosis of moderate-persistent asthma according to GINA guidelines (corresponding to Step 3) and sensitization to HDM with clinically relevant symptoms.

Patients were allowed to move across five health states according to the intervention prescribed (SOC or AIT—SCIT or SLIT tablets): GINA Step 3 ("AIT+G3" or "SOC+G3"), all patients started at this health state in the first Markov cycle defined as a low dose of inhaled corticosteroid plus long-acting beta-agonists (ICS/LABA); GINA Step 4 ("AIT+G4" or "SOC+G4"), characterized as an increase in asthma severity requiring a medium dose of ICS/LABA; GINA Step 2 ("AIT+G2" or "SOC+G2"), described an improvement of asthma by ICS step-down protocol (low dose of ICS); remission of asthma ("AIT+R" or "SOC+R"), defined as the withdrawal of asthma therapy for the remaining time horizon; and any-cause death ("death"), every participant was at risk for death during all the time horizon independently of the health state taken in the model (Figure 1). In both SLIT and SCIT strategies, patients were allowed to discontinue AIT as in real-life,[17] maintaining the standard of care treatment for the remaining time horizon; after treatment discontinuation, patients were not allowed to return to any AIT health state. For those patients, AIT effects on medication, exacerbations, and utilities were not considered when treatment was discontinued, and SOC values were considered instead. AIT was ideally administered for 3 years, as recommended,[11] and patients completing AIT intervention then continued pharmacotherapy alone for the remaining cycles; the effectiveness of AIT on medication, exacerbations, and utilities remained the same for the remaining years. When completing 3 years of AIT, the probability of asthma remission for these patients increased.[19,20] For all strategies, patients achieving asthma remission continued in that state until the end of the 10-year time horizon.[21]

Each health state reflected a specific asthma severity level or disease remission. In each Markov cycle, costs and utilities were accumulated according to the re-distribution of participants until the end

TABLE 1 Parameters included in the Markov model (base case analysis) for the three strategies (SOC, SCIT, and SLIT)

Parameter	SOC	SCIT and SLIT	DSA parameters range		Distribution	Ref.
			Lower	Higher		
Time horizon	10 years					
Age at baseline	12 years					
Initial Health State (t0)	"SOC+G3" ($N = 1000$)	"SCIT+G3" ($N = 1000$); "SLIT+G3" ($N = 1000$)	-	-	-	-
Annual discount rate (costs/QALYs)	3% (range 0%–6%)					
Duration of a Markov cycle	6 months					
Transition probabilities (6 months)						
Disease progression (SOC+G3 -> SOC+G4)[a]	0.048160		0	0.159762	Binomial	29
Disease progression (AIT+G3 -> AIT+G4)	-	SCIT: 0.030979 SLIT: 0.040833	SCIT: 0 SLIT: 0	SCIT: 0.140348 SLIT: 0.151472	Binomial	29,30
Disease progression (SOC+G2 -> SOC+G3)[a]	0.133975		0.025321	0.258380	Binomial	29
Disease progression (AIT+G2 -> AIT+G3)	-	SCIT/SLIT: 0.105572	SCIT/SLIT: 0	SCIT/SLIT: 0.175985	Binomial	29
Disease Improvement (SOC+G3 -> SOC+G2)[a]	0.133975		0.025321	0.258380	Binomial	29
Disease Improvement (SOC+G4 -> SOC+G3)[a]	0.056602		0	0.169338	Binomial	31
Disease Improvement (AIT+G3 -> AIT+G2)	-	SCIT: 0.183299 SLIT: 0.169337	SCIT: 0.068872 SLIT: 0.056602	SCIT: 0.316626 SLIT: 0.300000	Binomial	29,32
Disease Improvement (AIT+G4 -> AIT+G3)	-	SCIT/SLIT: 0.056602	SCIT/SLIT: 0	SCIT/SLIT: 0.169338	Binomial	31
AIT discontinuation (for any AIT health state during the first 3 years of SCIT or SLIT)	-	SCIT: 0.086788 SLIT: 0.217253	SCIT: 0.040564 SLIT: 0.131216	SCIT: 0.148932 SLIT: 0.400000	Binomial	17
Remission (SOC+G2 ->R)[b,c]	0.012468		0.002575	0.025348	Binomial	33
Remission (point estimate for AIT completion—3 years) (AIT+G2 ->R)[c]	-	SCIT: 0.500000 SLIT: 0.340000	SCIT: 0.300000 SLIT: 0.140000	SCIT: 0.700000 SLIT: 0.540000	Binomial	19,30
Annual mortality						
All-cause mortality	0.000061				-	34

(Continues)

TABLE 1 (Continued)

Parameter	SOC	SCIT and SLIT	DSA parameters range		Distribution	Ref.
			Lower	Higher		
Costs (6 months)						
AIT acquisition price	-	SCIT: 127.28€ SLIT: 450.00€	SCIT: 101.82€ SLIT: 360.00€	SCIT: 152.74€ SLIT: 540.00€	-	Market price
Administration of SCIT	-	126€	100.8€	151.2€	-	35
Scheduled visits+ additional tests	75.00€		60.00€	90.00€	-	35
Medication Gina 4	108.66€		74.10€	128.37€	-	36
Medication Gina 3	102.57€		89.43€	118.11€	-	36
Medication Gina 2	23.88€		14.70€	29.76€	-	36
Moderate exacerbation (ED visit)	127.67€		102.14€	153.20€	-	35
Severe exacerbation (hospitalization)	2759.00€		2207.20€	3310.80€	-	35
Utilities						
Step Gina 3	0.789		0.631	0.947	Beta	37,38
Step Gina 2	0.781		0.625	0.937	Beta	37,38
Step Gina 4	0.725		0.580	0.870	Beta	37,38
Remission	0.812		0.650	0.974	Beta	37,38
Disutilities						
Moderate exacerbations	−0.117		−0.114°	−0.121°	LogNormal	39
Severe exacerbations	−0.179		−0.153°	−0.217°	LogNormal	39
Exacerbations (6 months probabilities)						
Moderate exacerbations (ED visits)	0.206275	SCIT: 0.0536315 SLIT: 0.072196	SOC: 0.145510° SCIT: 0.037832° SLIT: 0.050928°	SOC: 0.278890° SCIT: 0.072511° SLIT: 0.097611°	Binomial	40–42
Severe exacerbations (Hospitalization)	0.007025	SCIT: 0.001826 SLIT: 0.002458	0	SOC: 0.035635° SCIT: 0.009265° SLIT: 0.012472°	Binomial	40–42

Note: DSA range defined according to a margin error of ±20%, unless otherwise stated. $^{\circ}$95% confidence interval.

Abbreviations: AIT, allergen immunotherapy; DSA, deterministic sensitivity analysis; ED, emergency department; SCIT, subcutaneous immunotherapy; SLIT, sublingual (tablets) immunotherapy; SOC, standard of care.

[a]Probabilities from the SOC arm were also used for patients in AIT strategies that discontinued SLIT tablets or SCIT earlier.

[b]Remission in AIT strategies assumed the value as in SOC arm for all the time horizon; however, those patients completing 3 years of AIT, at that point, had a higher probability of asthma remission corresponding to the values presented in this table.

[c]Patients achieving remission remained in this state until the end of the analysis.

of the analysis. The distribution of participants according to specific probabilities was different for each strategy, representing the real-life flow of patients taking AIT or SOC alone. Transition probabilities followed some assumptions: patients started in the "SOC+G3" or "AIT+G3" health state; step-down or step-up of treatment was gradual; for example, patients in GINA step 4 were not allowed to transit directly to GINA step 2 or remission but were allowed to decrease to GINA step 3, while in step 3 they could move again to step 4 or improve to step 2; when a patient achieved remission was not allowed to move into other health states; the probability of moderate and severe exacerbations was determined according to the probability of emergency department (ED) visits and hospitalizations due to asthma, respectively, and were possible to occur in all health states except in Gina Step 2 in which was allowed only ED visits (moderate exacerbations); AIT discontinuation was considered in SLIT tablets and SCIT strategies, the parameters of therapy effectiveness for these patients was equal to those in the SOC arm from the time of discontinuation; death was an adsorbing state.

TABLE 2 Base case results of costs and health gains for SOC, SLIT, and SCIT strategies at the end of the model calculation (10-year horizon)

Variables base case scenario	SOC	SCIT	SLIT
Asthma medication+ scheduled visits	2,915,378€	2,476,179€	2,760,857€
Intervention costs (SLIT or SCIT+ administration)	0€	1,176,098€	1,327,165€
Moderate exacerbations costs	506,673€	264,369€	409,105€
Number of moderate exacerbations	3969	2071	3205
Severe exacerbations costs	243,756€	118,214€	189,383€
Number of severe exacerbations	88	43	69
Total cost	3,665,807€	4 034 859€	4,686,510€
Total cost (discount rate)	3,209,552€	3 647 624€	4,231,070€
Utilities	17,785	17,949	17,842
Disutilities	480	250	387
QALYs	17,305	17,699	17,455
QALYs (discount rate)	15,096	15,438	15,228
Cost difference	Reference	438€	1021€
Effect difference	Reference	0.342	0.132
ICER	Reference	1281€	7717€

Abbreviations: ICER, incremental cost-effectiveness ratio; QALYs, quality-adjusted life-years; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; SOC, standard of care.

The input data used in our model are presented in Table 1 and are explained in detail in the Supporting Information of this article. All data were retrieved from literature and discussed with medical specialists. We extensively described the sensitivity analysis and other scenarios taken into account to strengthen the base case results presented in this work (see the Supporting Information for these details).

2.1 | Model calculation

Cost-effectiveness was established by calculating the incremental cost-effectiveness ratio (ICER) as incremental costs divided by incremental quality-adjusted life-years (QALYs), assuming SOC strategy as the reference. Costs and QALYs were discounted at 3% per year as performed in previous studies.[18,22] The cost-effectiveness threshold (CE threshold) was based on WHO recommendations as one to three times the gross domestic product (GDP) per capita of the country.[23] We considered the value corresponding to the lowest category suggested by WHO—thus, up to one time the GDP per capita.[23,24] Therefore, in Portugal, the GDP per capita in 2020 was set at 22,488,62USD (18,482,80€; conversion on June 7th, 2021). All the analyses were performed in RStudio Software version 3.4.2 (R Foundation for Statistical Computing, Vienna, Austria) using the *Heemod* package.[25]

3 | RESULTS

3.1 | Base case results

The base case results of this economic analysis indicate an ICER of 1281€ and 7717€, per QALY, gained, for SCIT and SLIT tablets compared with the SOC strategy, respectively. The base case results are presented in Table 2. For SCIT and SLIT tablets, per patient, we observed an incremental cost of 438€ and 1021€ and an incremental QALY of 0.342 and 0.132. As we expect a CE threshold for Portugal of 18,482.80€, both treatment strategies are below this limit. The major contributor to these results was AIT effects on health outcomes by reducing moderate and severe exacerbations and asthma controller medication. For the SCIT strategy, we expected a reduction of 1898 moderate exacerbations and 45 severe exacerbations, corresponding to cost differences of 242,304€ and 125,542€, respectively. We anticipated a less optimistic scenario for the SLIT tablet strategy. A reduction of 764 moderate exacerbations and 19 severe exacerbations; still, the impact on asthma-related costs was relevant. The reduction in asthma medication and scheduled medical visits (for patients in remission) led to savings of 439,199€ and 154,521€ in SCIT and SLIT tablets strategies, respectively.

We have included AIT discontinuation rates to simulate what happens in the real world; 570 children completed 3 years of subcutaneous immunotherapy compared with 227 children in the sublingual strategy. Over the 10-year time horizon, the numbers of patients experiencing remission of asthma medication were estimated to be 79, 233, and 128, corresponding to SOC, SCIT, and SLIT strategies. At the end of the time horizon, SOC, SCIT, and SLIT led to

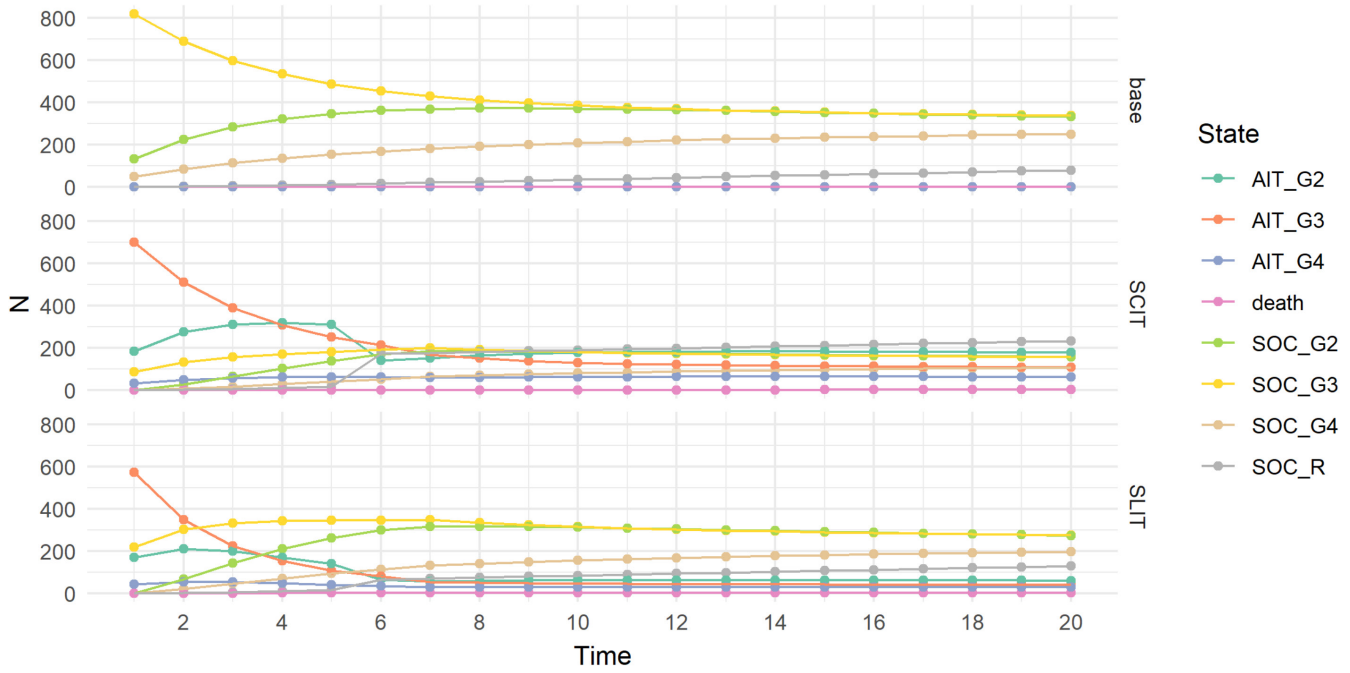


FIGURE 2 Number of participants in each health-state over a 10-year time horizon, corresponding to 20 Markov cycles, per strategy; namely, base (standard of care treatment), SCIT (subcutaneous immunotherapy), and SLIT (sublingual immunotherapy)

an increase in asthma medication (Gina Step 4) in 250, 170, and 225 children, respectively (Figure 2 and Table S1).

3.2 | Sensitivity analysis

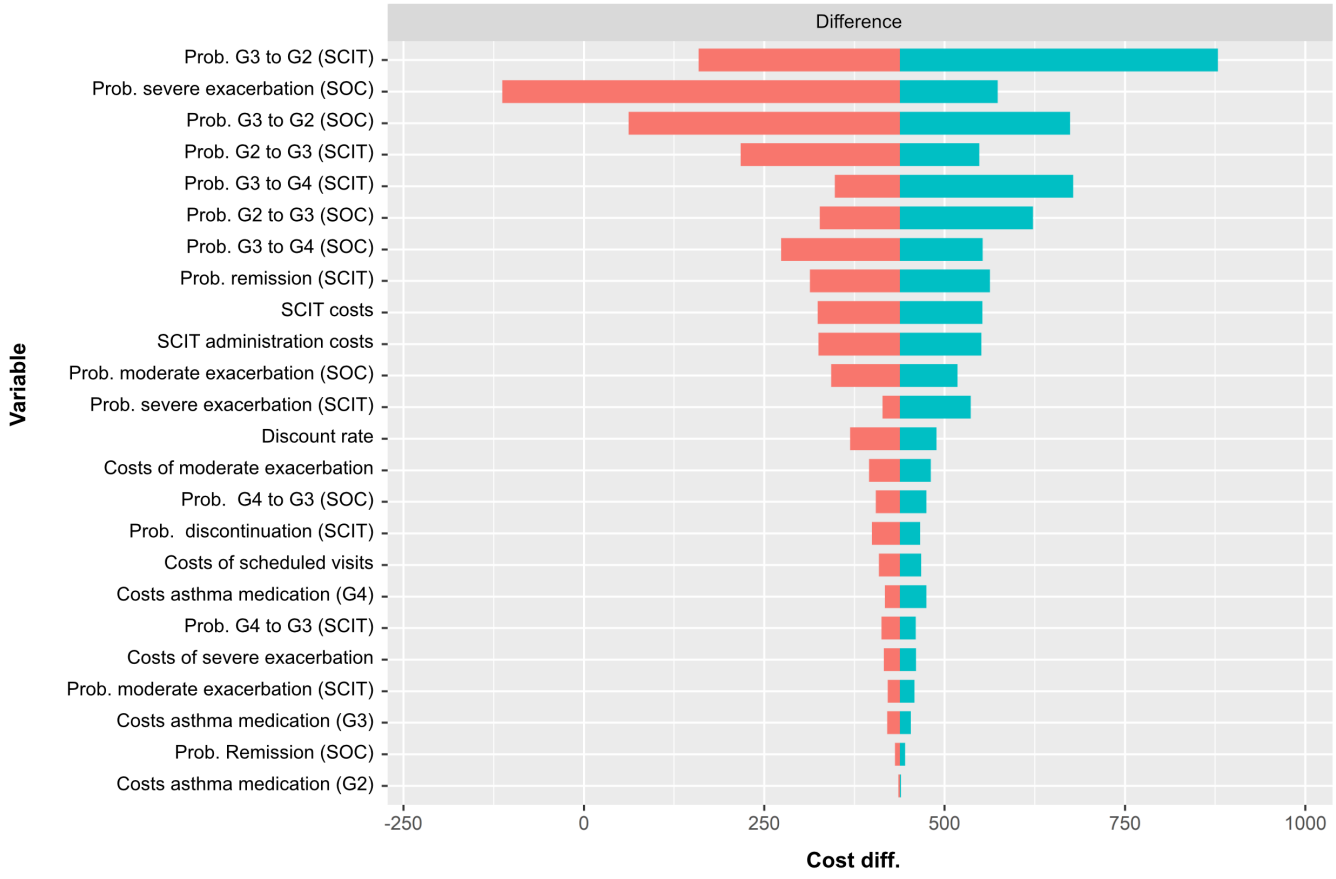
The robustness of the model was assessed through deterministic sensitivity analysis (DSA) and probabilistic sensitivity analysis (PSA). The DSA (Figure 3) showed which parameters contributed to cost differences between the AIT and SOC strategies. Differences in utilities due to parameters uncertainty are shown in the (Figure S1). These variations caused differences in ICER results (Figure S2 and Table S2). Considering the lowest and highest boundaries for each parameter uncertainty, ICER values ranged between 138€ and 6628€ for SCIT, and 3273€ and 46,710€ for SLIT tablets (Table S2). Additionally, one of the analyses of the SCIT strategy led to a scenario of cost savings meaning that the new treatment dominated the old one resulting in a negative ICER (Table S2). On the other hand, one of the analyses of the SLIT strategy led to a scenario of no health gains (negative effect difference), and consequently, a negative ICER; meaning that the intervention was not cost-effective when considering an increase in the probability of moving from "SLIT GINA step 3" into "SLIT GINA step 4".

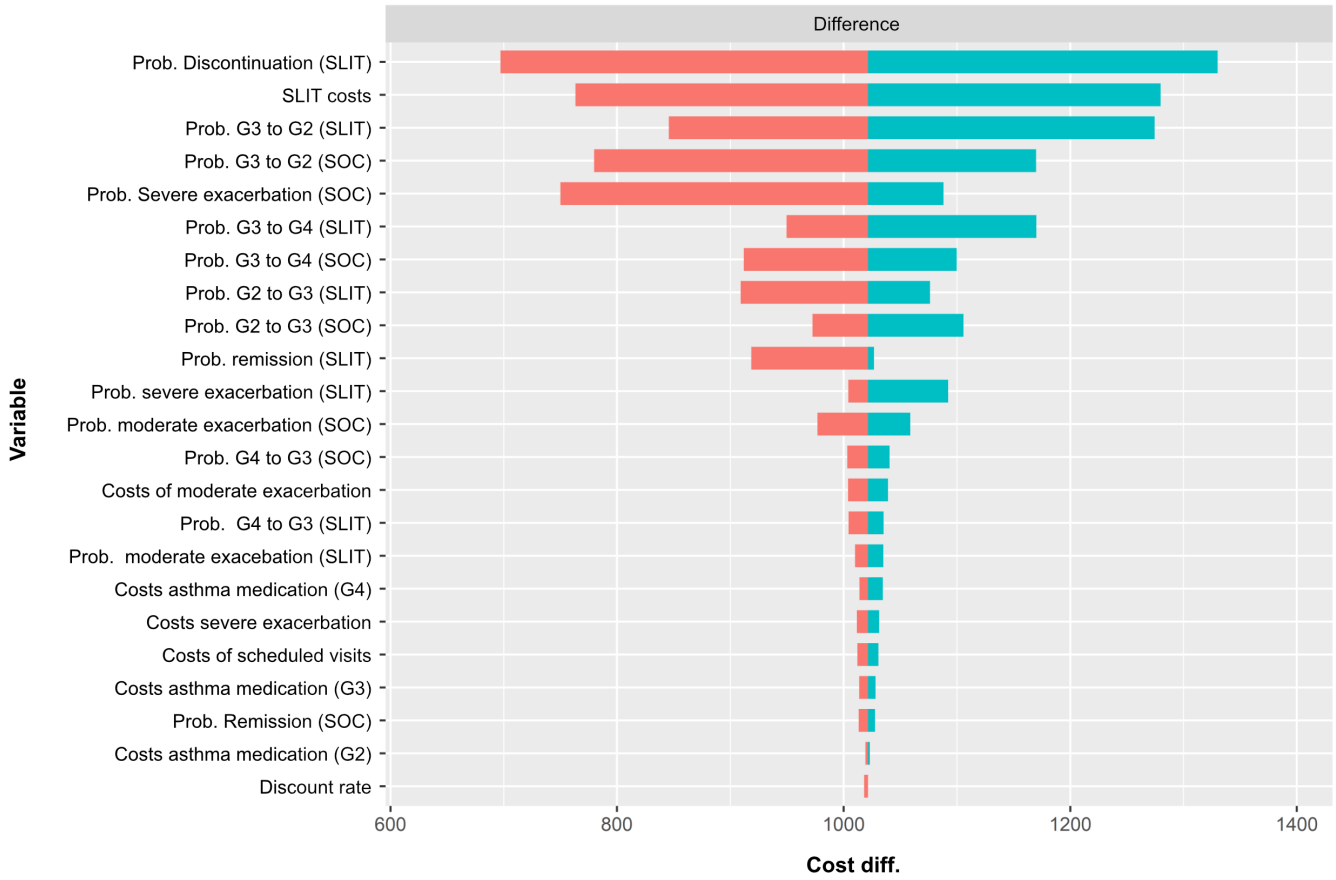
The PSA analysis (Figure 4) showed a linear correlation trend evidencing the cost-effectiveness of both interventions because as QALYs increased, the costs decreased. However, for the SLIT tablets strategy, some simulations were plotted in a non-cost-effectiveness region (upper left quadrant of the graph), and costs were markedly higher for most simulations when compared to SCIT. The average ICER after 1000 simulations was 1387€ for SCIT and 8498€ for SLIT; the SLIT strategy presented a higher uncertainty compared with base case results. The Monte Carlo simulation results were used to compute cost-effectiveness acceptability curves (CEAC) according to a range of WTP thresholds. The graphs show the probability of cost-effectiveness considering different threshold values; specifically, considering a limit of 10,000€ the probability of being cost-effective was around 58% (SLIT tablets) and 98% (SCIT), but increasing this limit to 20,000€, which is a similar value to the CE threshold that we assumed for Portugal, the values increased to 75% and 99%, respectively (Figure S3).

3.3 | Other scenarios

We also considered other possible scenarios, according to circumstances that might happen in the real world, to evaluate the robustness of the model while strengthening the recommendations provided by the base case results. The results are presented in Table S3. None of the ICER values hit the CE threshold considered in our analysis in all scenarios. However, we verified which variables

FIGURE 3 Tornado plot resulting from the deterministic sensitivity analysis. Blue and red bars represent an increase or decrease in costs, respectively, according to model's parameters uncertainty (the range of values is represented in each side of the bars and also in Table 1). Subcutaneous strategy (top plot) and sublingual strategy (bottom plot). To simplify y-axis, the reference to health states according to GINA steps is G4, G3, and G2 corresponding to GINA step 4, GINA step 3, and GINA step 2. Cost diff, cost difference; Prob., probability; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; SOC, standard of care


Difference
Prob. G3 to G2 (SCIT)
Prob. severe exacerbation (SOC)
Prob. G3 to G2 (SOC)
Prob. G2 to G3 (SCIT)
Prob. G3 to G4 (SCIT)
Prob. G2 to G3 (SOC)
Prob. G3 to G4 (SOC)
Prob. remission (SCIT)
SCIT costs
SCIT administration costs
Prob. moderate exacerbation (SOC)
Prob. severe exacerbation (SCIT)
Discount rate
Costs of moderate exacerbation
Prob. G4 to G3 (SOC)
Prob. discontinuation (SCIT)
Costs of scheduled visits
Costs asthma medication (G4)
Prob. G4 to G3 (SCIT)
Costs of severe exacerbation
Prob. moderate exacerbation (SCIT)
Costs asthma medication (G3)
Prob. Remission (SOC)
Costs asthma medication (G2)
Variable
-250
0
250
500
750
1000
Cost diff.


Difference
Prob. Discontinuation (SLIT)
SLIT costs
Prob. G3 to G2 (SLIT)
Prob. G3 to G2 (SOC)
Prob. Severe exacerbation (SOC)
Prob. G3 to G4 (SLIT)
Prob. G3 to G4 (SOC)
Prob. G2 to G3 (SLIT)
Prob. G2 to G3 (SOC)
Prob. remission (SLIT)
Prob. severe exacerbation (SLIT)
Prob. moderate exacerbation (SOC)
Prob. G4 to G3 (SOC)
Costs of moderate exacerbation
Prob. G4 to G3 (SLIT)
Prob. moderate exacebation (SLIT)
Costs asthma medication (G4)
Costs severe exacerbation
Costs of scheduled visits
Costs asthma medication (G3)
Prob. Remission (SOC)
Costs asthma medication (G2)
Discount rate
Variable
600
800
1000
1200
1400
Cost diff.

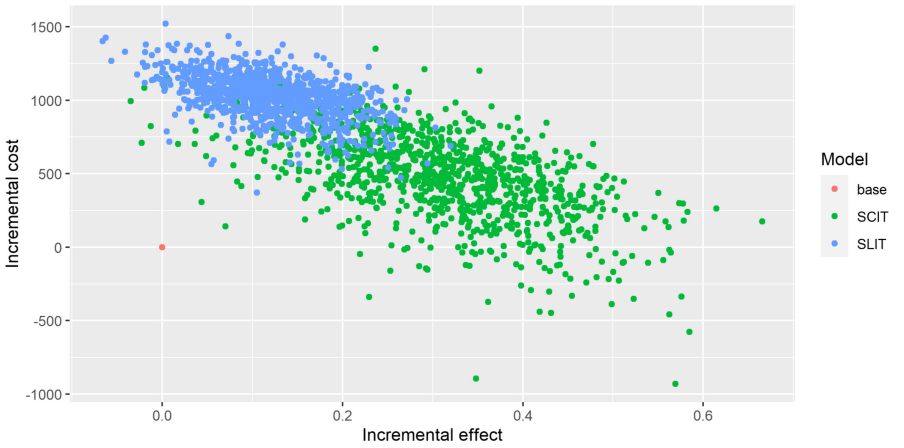


FIGURE 4 Results of the probabilistic sensitivity analysis graphically represented on a cost-effectiveness plan. Green: subcutaneous immunotherapy, Blue: sublingual immunotherapy, Orange: standard of care (reference)

had a higher impact on ICER. On the one hand, in a perfect scenario of full adherence to AIT or considering a 50% discount in AIT acquisition prices, we observed a marked reduction in the ICER.

On the other hand, if the effects of AIT in asthma remission were the same as in the SOC arm, the ICER increased. As expected, ICER values were higher when considering a short-time horizon and lower for a long-time horizon. When AR medication costs were included, the ICER decreased, particularly in the SCIT strategy because adherence rates to SCIT are higher and more children benefit from a reduction in AR drug use. If we do not take into account the effects of AIT on moderate and severe exacerbations for the entire time horizon, as expected, the ICER values increased. Still, SCIT results remained optimistic (3539€ and 17,437€ for SCIT and SLIT, respectively). For the remaining scenarios, ICER values were similar or slightly higher than the base case, evidencing the robustness of the results since these scenarios were less optimistic about the effects of AIT on asthma.

4 | DISCUSSION

Allergen immunotherapy, for both subcutaneous and sublingual administration routes, seems cost-effective in children with moderate HDM-driven allergic asthma, considering a CE threshold in Portugal of 18,482.80€. The core parameters contributing to these ICER values were the AIT effects on reducing exacerbations and asthma medication. Comparing both administration routes, SCIT was more cost effective and the sensitivity analysis demonstrated the robustness of the model. The cost-effectiveness of both strategies can be improved if AIT acquisition costs decrease and adherence rates increase.

To our knowledge, this is the first study estimating both SCIT and SLIT tablets' effects on health and costs in children with allergic asthma while addressing the limitations of previous economic analyses. Our model was adapted from a recent study conducted by Parra-Padilla and colleagues in children with asthma, but some new considerations were included to better reflect the real world.[18] First, the model has been restructured to allow the flow of patients across health states representing different medication requirements as defined in GINA guidelines; thus, medication step-up was allowed instead of medication step-down only as in the previous study. Second, we included AIT adherence rates since this is a major concern for clinicians, patients, and policymakers. Third, we conducted an extensive analysis considering another scenario that we believed to be relevant for interpreting the results among different contexts. Our results are in line with those obtained for SCIT in the aforementioned study[18]; the small differences may be due to the inclusion of compliance data and medication step-up in the model and the differences in the input data according to each country specifications. Another study evaluated the cost-effectiveness of SLIT in adults with asthma sensitized to HDM in three European countries.[22] Our ICER estimate was slightly lower when compared to Czech Republic, Poland, and Slovakia (7455€, 7492€, and 8814€, respectively). However, differences in the model assumptions, WTP thresholds, lack of compliance data and population's age might have led to these differences. Our results agreed with the previous ones and highlighted the higher likelihood of cost-effectiveness for the subcutaneous route due to lower acquisition prices and greater health and quality of life improvements that were enlarged by better adherence rates than SLIT.

This study has several strengths. We have included discontinuation rates based on real-world studies to surpass previous gaps from cost-effectiveness studies. As emphasized by *Parra-Padilla* et al.,[18] the definition of health states according to GINA steps allows its use in different contexts and captures both the complexity of the disease and the effects of interventions. Moreover, the length of each Markov cycle was chosen to represent the clinical response of patients and changes in medication requirements according to the average number of scheduled medical visits in Portugal. The base case model followed important conservative assumptions to avoid over-or underestimation of the results. First, moving across health states was gradual. Second, the probability of asthma remission was the same for all strategies and higher only for patients who completed 3 years of immunotherapy. Third, after AIT discontinuation, patients followed the odds and health states represented in the SOC strategy; thus, AIT had no effects in the subsequent years. Finally, whenever possible, input data were retrieved from real-world studies conducted in children with HDM-driven asthma. The sensitivity analyses were extensive to test the robustness of the base case model, while the alternative scenarios analyses were relevant to identify

core parameters that may contribute to improving cost-effectiveness in the real world and to drive political decisions. We considered an alternative scenario including the costs due to allergic rhinitis since this is a common comorbidity in children with asthma and an indication for AIT.[9] The results reinforced the cost-effectiveness of AIT, especially for SCIT, and were in line with literature findings.[18]

We should also address limitations. This model was applied to the Portuguese context, but it can be easily applied to other settings (hospital, region, or country, for example) that need to estimate the ICER of immunotherapy for patients with asthma. In practice, this can be achieved by changing the parameter values of the model according to the available specific data for that setting, such as adherence rate and drug costs, for example. Furthermore, these results provide relevant insights for clinicians and policymakers independently of the country. The transition probabilities were calculated based on studies performed in children under HDM SLIT tablets or SCIT except for two probabilities due to lack of published data; first, the transition probability from GINA step 4 into GINA step 3 was equal in all strategies because we did not find specific data for AIT, second, transition probability from GINA step 2 into GINA step 3 in the SLIT strategy was assumed to be the same as in SCIT strategy. In the DSA analysis, the variation of those specific parameters did not affect the ICER estimates significantly. Markov models are highly dependent on the underlying assumptions and data retrieved from the literature; this was shown by the DSA analysis since some parameters, especially transition probabilities, led to considerable variations in cost and effect differences and ICER. We also lack high-quality evidence of AIT effects on moderate and severe exacerbations in the short- and long-term since these are major drivers of costs; consequently, results should be interpreted cautiously, but SCIT remained cost-effective in an alternative scenario in which AIT effects on exacerbations were not considered. This study highlights the need for high-quality studies evaluating the long-term effects of AIT in children with asthma, particularly the steroid-sparing effects and asthma progression for those whose AIT is not effective. Additionally, we were unable to estimate the cost-effectiveness of SLIT-drops due to the lack of both steroid-sparing effect data and discontinuation rates. We did not incorporate nonmedical costs, such as transportation to the hospital for SCIT administration and the management of adverse events due to AIT, which may underestimate the ICER to some extent.[26] We did not consider the risk of death from SCIT, but the risk exists despite being very low.[27] We proposed a CE threshold based on the lower limit suggested by the WHO. However, this limit should be interpreted cautiously since the Portuguese authorities may consider another willingness to pay value for the health system.[23] Another possible threshold relies on the estimates of the individuals' willingness to pay (WTP) to improve their health; this approach defines the value of health consumption from a social perspective rather than that defined by the health system.[23] Since we did not find this estimate for Portugal, due to the proximity and similarities with Spain, we decided to compare the ICER with the individuals' WTP threshold defined for that country (which ranged from 10,119€ up to 28,187€) and we verified that both SCIT and SLIT tablets were cost-effective.[28] Nevertheless, the extensive presentation of the results allows the interpretation using different thresholds.

This study fills a gap in the literature in different ways. Firstly, in our model, we included adherence rates to represent what happens in the real world. Secondly, it was simulated in a pediatric population for different administrations routes of AIT. Thirdly, we applied the model considering a Portuguese context. Both strategies were cost-effective. However, SCIT showed more consistent results and seemed to have a greater impact on clinical and quality of life outcomes by reducing exacerbations leading to emergency department visits and medication requirements. The high probability of cost-effectiveness for both strategies may drive future policy decisions and AIT-prescribing habits. To perform reliable and accurate cost-effectiveness studies, AIT's long-term effects should be addressed in high-quality studies. We also conclude that AIT adherence rates significantly impact results, highlighting the value of implementing strategies to promote adherence rates.

AUTHOR CONTRIBUTIONS

AM, JCR, and MF conceptualized the study. AM, IA, and MS reviewed, discussed, and approved the clinical data included in the model as medical and scientific experts. MF conducted the literature review. MF and JCR implemented the model (data analysis). All authors interpreted, discussed the results, and finalized the manuscript.

CONFLICT OF INTEREST

None of the other authors report any conflict of interest in relation to this work.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Supporting Information: Cost-effectiveness analysis of house dust mite allergen immunotherapy in children with allergic asthma

Methods

In this section we describe in full detail the developed model and all the data retrieved from literature to support the model input that is presented the table 1 (main manuscript). We also describe extensively the sensitivity analysis and the other scenarios taken into account to strength the base case results presented in this work.

Model Parameters

Effectiveness parameters were retrieved from the literature and discussed with clinical experts. Cumulative probabilities were converted as rates using their periodicity and re-expressed as probabilities within six months (cycle length).[1, 2] The probabilities of increase or decrease asthma pharmacotherapy doses were retrieved from the control arm of a study performed in children with mild and moderate allergic asthma.[3] The probability of moving from "SOC+G4" into "SOC+G3" was determined using the data from a recent study performed in children with severe asthma.[4] The probability of asthma remission was defined as the proportion of patients that discontinue asthma medication in a study that followed up children for 12 years; 26% of children, initially diagnosed with mild to moderate asthma (N=879), reverted the use of medication and achieved asthma remission during the follow-up.[5] For the SCIT strategy, transition probabilities of increase or decrease medication requirements were determined from the SCIT arm of a study performed in children with HDM allergic asthma.[3] During all the analysis, the probability of asthma remission was assumed to be equal to the control arm. However, after completing three years of SCIT, this probability increased to 50% [6]; this point estimate was used in the sixth Markov cycle (t=6), when patients completed AIT, and those achieving remission remained in that state until the end of the analysis.[7] The cumulative probability of SCIT and SLIT tablets discontinuation for the recommended duration of three years was 42% and 77%, respectively.[8] Since we found no data of AIT effects in decreasing medication from Gina step 4 to Gina step 3, the probability calculated for the SOC arm was considered in both AIT strategies.[4] The transition probabilities for the steroid-sparing effects of SLIT tablets were calculated from a trial performed in children with HDM allergic asthma.[9, 10] The probability of transition from "SLIT+G2" to "SLIT+G3" was assumed to be qual to SCIT since we did not find specific data for SLIT tablets. The probability of disease remission after three years of HDM SLIT tablets was 32% and we assumed the previous assumptions as in SCIT strategy.[11] The data used for the input of model parameters are presented in Table 1.

Costs were defined for each health-state, *per* six months, to express differences in medication and healthcare resources used between strategies (Table 1). Asthma controller drug costs were calculated for each GINA step and according to the mean price of those drugs in the country; namely, for step 2 we considered a low dose of ICS while for steps 3 and 4 a combination of low or medium ICS-LABA, respectively.[12, 13] We assumed full-adherence to the symptomatic treatment in all strategies. We also considered costs related to moderate and severe asthma exacerbations according to the probability of ED visits and hospitalizations based on contracted values for Portuguese public hospitals.[14] The probability of ED visits and hospitalizations decreased by 74% and 65% for patients under SCIT and SLIT tablets strategies in comparison to SOC [15-17]; these probabilities remained for all the time horizon for patients completing three years of AIT. One scheduled visit every six months and medical tests were considered for all strategies.[14] Acquisition prices of SCIT and SLIT tablets were retrieved from a pharmaceutical company; namely, 255€ and 900€ for one year of intervention, respectively. SCIT strategy also included hospital administration costs for monthly injections.[14] The utilities were calculated from a study using the 5-dimension Asthma Quality of Life Questionnaire (AQL-5D) in adults.[18] However, utilities in children are generally lower.[19] We adjust the previous utilities according to the methodology described by *Di Bona et al*.[20]; thus, a multiplicative function using the "perfect health" utility value for children, according to the AQL-5D questionnaire, was applied to the previous values (0.870±0.005).[21] Disutilities values for moderate and severe exacerbations were used based on the AQL-5D questionnaire.[22] Both utilities and disutilities are presented in Table 1. Quality-adjusted life years (QALYs) was the outcome used to translate efficacy in health gains. QALYs for each GINA step were determined based on the corresponding utilities values and the subtraction of disutilities due to exacerbations[23]; disutilities due to moderate and severe exacerbations were considered in GINA steps 3 and 4 health-states while moderate exacerbations only were accounted for GINA step 2 health states.

Sensitivity analysis

To investigate uncertainty, we performed one-way deterministic sensitivity analysis (DSA) by varying the values of individual parameters while evaluating its impact on ICER, in comparison to the base case scenario.[24] Variations in disutilities and probabilities of exacerbations were assumed to take the range of the corresponding confidence intervals presented in literature, while the remaining variables varied according to a margin error of ±20% (Table 1). A Tornado diagram was developed to summarize the relative contribution of each parameter to costs and effects variation.[25]

A probabilistic sensitivity analysis (PSA) was performed by running 1 000 Monte Carlo simulations.[24, 25] These multiple repetitions of ICER calculations were drawn randomly according to the defined distribution for utilities and transition probabilities parameters.[25, 26] Utilities followed a beta distribution while transition probabilities followed a binomial distribution. Costs were point estimates since its calculation was based on market prices and did not follow any specific distribution. PSA results were represented graphically, in a cost-effectiveness plane, to evaluate the extent of uncertainty.[25] Based on those results, a cost-effectiveness acceptability curve (CEAC) was created showing the probability of the intervention being cost-effective, compared to the symptomatic arm, for different values of willingness-to-pay (WTP).[25]

Other scenarios

Finally, we varied specific parameters to account for possible scenarios that might happen in real-life or to understand the impact of some measures on the ICER: (1) different time horizon (5 years vs 15 years); (2) AIT effects on exacerbations only during treatment administration and not prolonged after AIT completion; (3) AIT effects on transition probabilities only considered for up to two years of follow-up after AIT completion instead of all time horizon; (4) no change in remission probability after AIT completion; (5) higher probability of asthma remission during the years of AIT administration instead of considering this probability only upon AIT completion as in the base case analysis; (6) full adherence to AIT; (7) 50% discount of acquisition AIT prices; (8) No AIT effects on exacerbations for all the time period (no short or long term effects); (9) all patients with allergic rhinitis as comorbidity. For the last scenario (#9), all patients were considered to have comorbid allergic rhinitis (AR) and rhinitis-related medication costs were included in all strategies.[13, 23] Symptomatic treatment of persistent AR followed the Allergic Rhinitis and its Impact on Asthma (ARIA) recommendations[27] and patients were under nasal corticosteroids and oral anti-histamines; thus, considering an annual adherence of 80%, we estimate a mean drug cost of 46€ (per six months, per person) for AR. Children allocated to SLIT and SCIT health-states and completing AIT were assumed to have a mean reduction of AR medication by 56% and 60%, respectively.[28, 29] Children achieving AA remission were assumed to maintain AR medication.

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Results (Supplementary tables and Figures)

Table S1 - Distribution of participants according to specific Markov cycles (cycles 0, 1, 6, and 20) by health state.

Cycles	AIT+G3	AIT+G2	AIT+G4	SOC+G3	SOC+G2	SOC+G4	Remission	Death
T=0								
SOC	-	-	-	1000	-	-	-	-
SCIT	1000	-	-	-	-	-	-	-
SLIT	1000	-	-	-	-	-	-	-
T=1								
SOC	-	-	-	818	134	48	0	0
SCIT	699	183	31	87	0	0	0	0
SLIT	573	169	41	217	0	0	0	0
T=6 (completion of AIT)								
SOC	-	-	-	455	361	168	16	0
SCIT	212	141	61	192	170	52	172	0
SLIT	79	65	34	346	299	112	65	0
T=20								
SOC	**-**	**-**	**-**	**337**	**333**	**250**	**79**	**1**
SCIT	**108**	**177**	**63**	**155**	**156**	**107**	**233**	**1**
SLIT	**39**	**60**	**30**	**274**	**273**	**195**	**128**	**1**

AIT: allergen immunotherapy, G2: GINA step 2, G3: GINA step 3, G4: GINA step 4, GINA: global initiative for asthma, SCIT: subcutaneous immunotherapy, SLIT: sublingual immunotherapy, SOC: standard-of-care.

Table S2 - Costs and QALYs differences (SOC as reference), *per* patient, and the respective minimum and maximum positive ICER results according to the deterministic sensitivity analysis, for SCIT and SLIT.

	Cost Difference	Effect Difference	ICER
SLIT (vs SOC)			
Minimum*	911€	0.278	3 273€
Maximum	1 099€	0.023	46 710€
SCIT (vs SOC)			
Minimum**	62€	0.449	138€
Maximum	678€	0.102	6 628€

ICER: incremental cost-effectiveness ratio, SCIT: subcutaneous immunotherapy, SLIT: sublingual immunotherapy, SOC: standard-of-care.

*In SLIT strategy, one of the analyses led to a result of no cost-effectiveness with a negative ICER, which reflects that SLIT was less effective with an increase cost (cost diff.: 1 170€; effect diff.: -0.027; ICER: -42 086€).

**In SCIT strategy, one of the analyses led to a cost saving scenario, meaning that SCIT was more effective and saved money compared with the SOC since the cost difference was negative (cost diff.: -113€; effect diff.: 0.378; ICER: -300€).

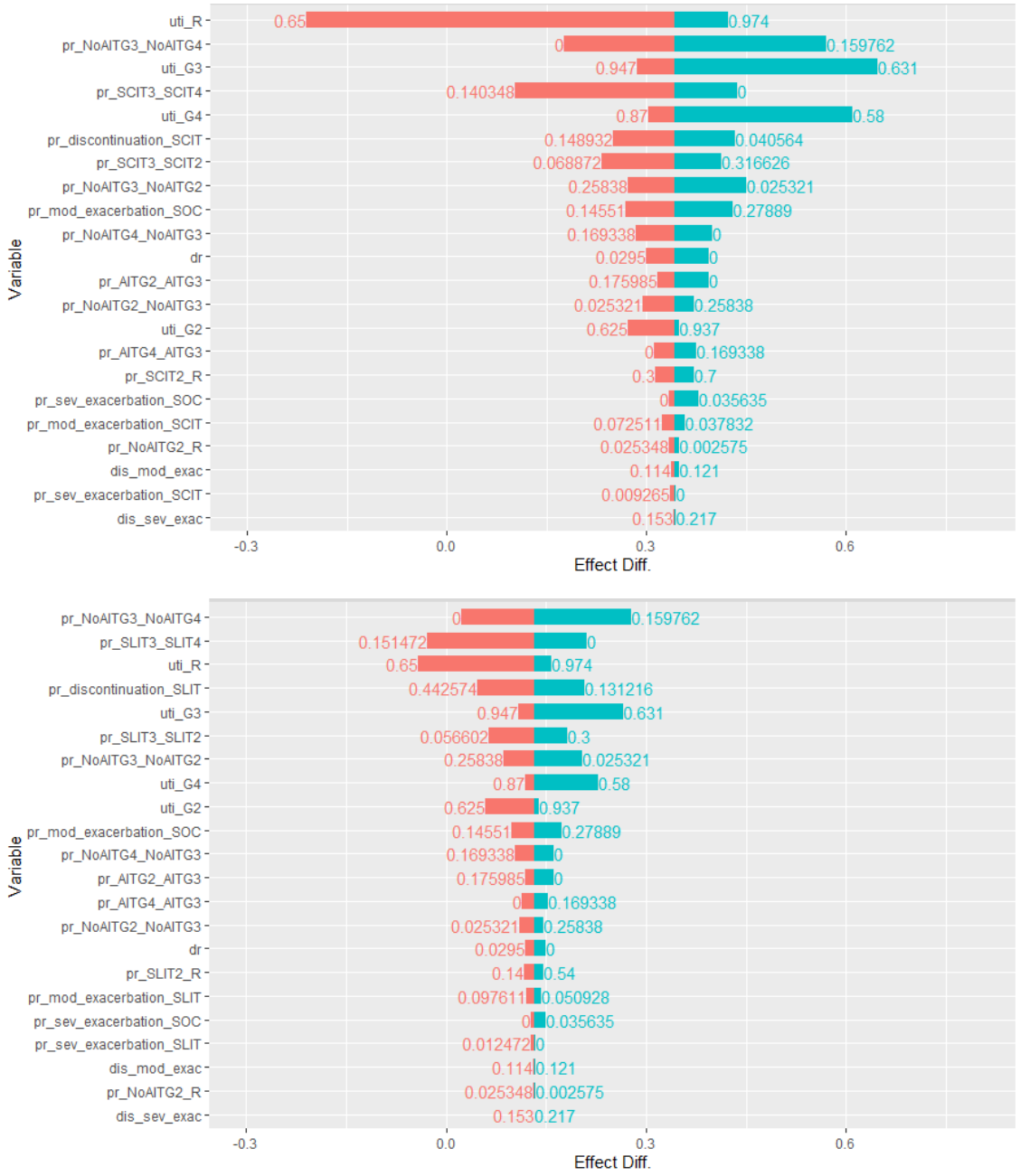

Figure S1 - Tornado plot resulting from the deterministic sensitivity analysis. Blue and red bars represent an increase or decrease in the health effects (represented as utilities), respectively, according to model's parameters uncertainty (the range of values is represented in each side of the bars and also in Table 1). Subcutaneous strategy (top plot) and sublingual strategy (bottom plot). Effect diff.: effect difference.

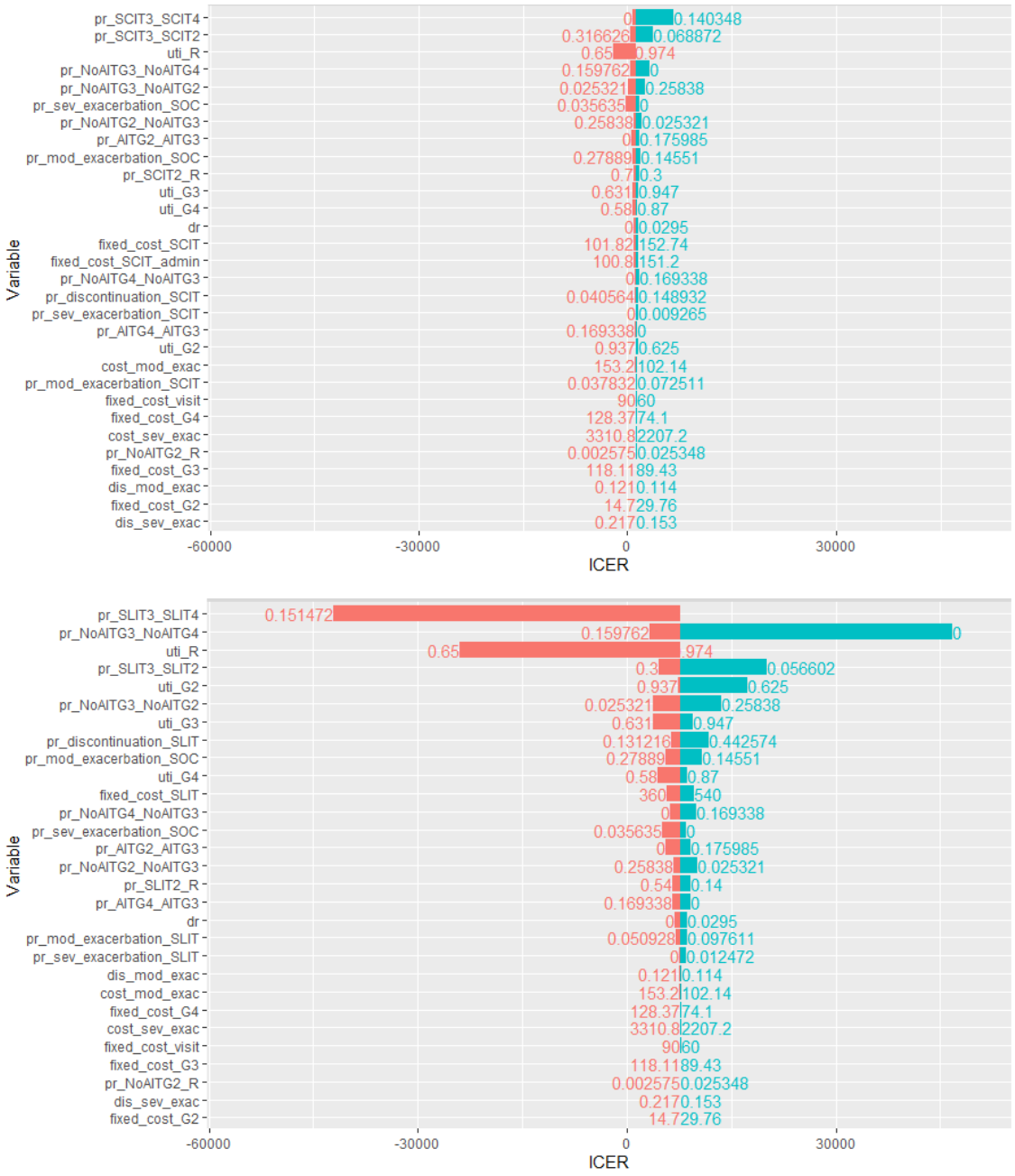

Figure S2 - Tornado plot resulting from the deterministic sensitivity analysis. Blue and red bars represent an increase or decrease in the ICER, respectively, according to model's parameters uncertainty (the range of values is represented in each side of the bars and also in Table 1). Subcutaneous strategy (top plot) and sublingual strategy (bottom plot). ICER: incremental cost-effectiveness ratio.

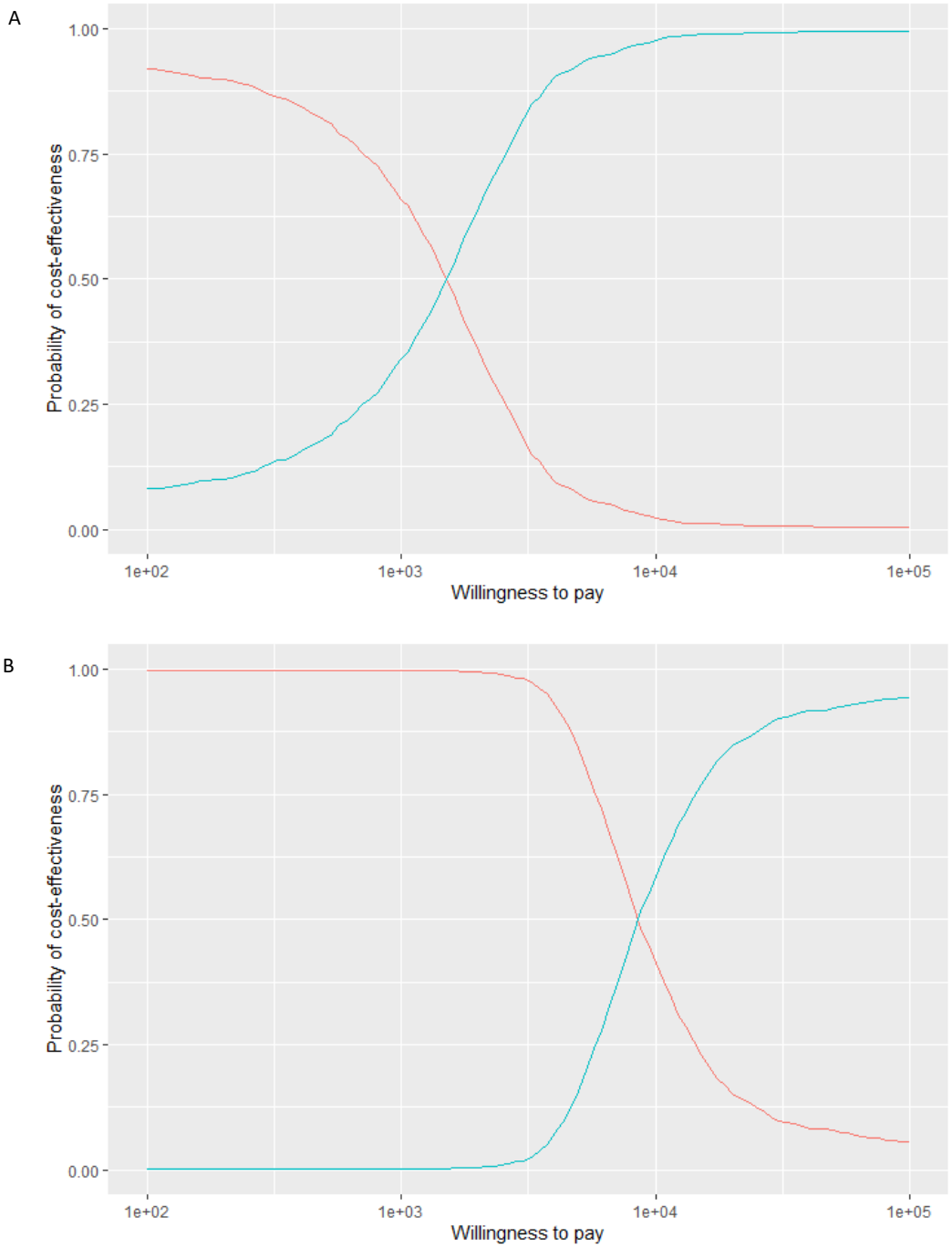

Figure S3 - Cost-effectiveness acceptability curve for different willingness-to-pay (WTP) threshold values. **A)** Subcutaneous strategy; **B)** Sublingual strategy. Red: standard-of-care (reference); Blue: SCIT or SLIT.

Table S3 - ICER results for SLIT and SCIT according to different scenarios (SOC as reference).

	Cost Difference	Effect Difference	ICER
Base Case			
SCIT	438€	0.342	1 281€
SLIT	1 021€	0.142	7 717€
#1 – Different time horizon			
5 years			
SCIT	793€	0.175	4 532€
SLIT	1 103€	0.083	13 319€
15 years			
SCIT	132€	0.488	271€
SLIT	839€	0.200	4206€
#2 - No effects of AIT on decreasing exacerbations after 3 years of therapy (only short-term effects)			
SCIT	542€	0.271	2 001€
SLIT	990€	0.126	7 871€
#3 - AIT effects on transition probabilities only for up to 2 years after completion AIT			
SCIT	530€	0.287	1 849€
SLIT	986€	0.131	7 529€
#4 - No AIT effect on asthma remission			
SCIT	742€	0.272	2 725€
SLIT	1 130€	0.109	10 403€
#5 - AIT effects on asthma remission spread over the three years of therapy instead of after three years of AIT			
SCIT	399€	0.347	1 149€
SLIT	858€	0.166	5 167€
#6 - Full adherence to AIT			
SCIT	489€	0.533	917€
SLIT	1 831€	0.454	4 032€
#7 - 50% discount on AIT acquisition prices			
SCIT	153€	0.342	447€
SLIT	324€	0.146	2 225€
#8 – No AIT effects on reducing moderate and severe exacerbations for all the time horizon			
SCIT	669€	0.189	3 539€
SLIT	1126€	0.065	17 437€
#9 - Allergic rhinitis			
SCIT	138€	0.342	402€
SLIT	882€	0.132	6 662€

AIT: allergen immunotherapy, SCIT: subcutaneous immunotherapy; SLIT: sublingual immunotherapy, ICER: incremental cost-effectiveness ratio, SOC: standard-of-care treatment.

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Farraia M, Paciência I, Castro Mendes F, Cavaleiro Rufo J, Delgado L, Moreira A. Cost-effectiveness analysis of grass pollen specific immunotherapy in children with allergic rhinitis compared to the standard of care symptomatic treatment in Portugal. Eur Ann Allergy Clin Immunol. 2023;55(5):212-228. doi: 10.23822/EurAnnACI.1764-1489.240.

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Cost-effectiveness analysis of grass pollen specific immunotherapy in children with allergic rhinitis compared to the standard of care symptomatic treatment in Portugal

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Allergen immunotherapy; allergic rhinitis; children; cost-effectiveness; grass pollen.

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Impact statement

Allergen immunotherapy is cost-effective in children with grass pollen allergic rhinitis due to an increase in quality-of-life and asthma prevention, especially for the subcutaneous route.

Summary

Background. *Cost-effectiveness studies evaluating allergen immunotherapy (AIT) in children are scarce. We aim to compare the cost-effectiveness of subcutaneous (SCIT) and sublingual immunotherapy (SLIT) against standard-of-care (SOC) treatment in children with grass pollen allergic rhinitis.* ***Methods.*** *We created a Markov model to compare the three strategies over a 10-year horizon. SOC was the reference to calculate the incremental cost-effectiveness ratio (ICER). Deterministic and probabilistic sensitivity analysis were used to assess models' uncertainty.* ***Results.*** *We obtained an ICER of 12,605€ and 6,318€ for SLIT and SCIT, respectively. In sensitivity analysis, SCIT was more cost-effective than SLIT.* ***Conclusions.*** *AIT is cost-effective in children with grass pollen allergic rhinitis, especially for the subcutaneous route.*

Introduction

Allergen specific immunotherapy (AIT) is a treatment aiming to improve the health and quality of life of patients suffering from allergic conditions such as rhinitis, rhinoconjunctivitis, food allergy, and asthma (1-4). Beyond the symptomatic treatment, AIT is an effective option in the long-term because it may alter the natural course of the disease by inducing allergen-specific immune tolerance and suppressing allergic inflammation (5, 6). The main routes of administration are subcutaneous injections (SCIT) and sublingual preparations (SLIT) as tablets or drops, hereafter referred together as AIT, which are ideally administered for at least three years to maximize efficacy (1, 4). But other delivery routes are emerging, such as oral mucosal, epicutaneous, and intralymphatic, that may also target other IgE mediated hypersensitivities besides aeroallergies while meeting patients' expectations of tolerability, effectiveness, and adherence (7, 8). However, the acquisition costs of AIT are not currently reimbursed by the Portuguese National Health System, except for persons under other subsystems or health insurances, compromising its use due to high costs and contributing to health inequalities (9).

Cost-effectiveness analysis (CEA) is a recognized approach to estimate short- and long-term consequences on costs and the health of a specific treatment (10). Pharmacoeconomics, a branch of health economics, is highly important in health policy making because it allows the comparison and analysis of the value of a certain policy or treatment with another. Quality-adjusted life years (QALYs) are used to quantify, on a scale from zero to one, the outcome of health in economic evaluations allowing comparisons across interventions since it can be applied in all models independently of the kind of drugs, interventions, or diseases (10). CEA studies are important to inform costs and health gains of an intervention, over a time-specific horizon, translating the knowledge of clinical efficacy trials and real-world studies (11). The results provided by these studies allow to drive political decisions, as reimbursement of therapies, and to implement policies contributing to the improvement of patients' lives while reducing health inequalities regarding drugs' assessment.

A review on CEA studies published in the literature of AIT effects on allergic rhinitis and asthma has been published recently (12). Briefly, although AIT shows to be cost-effective in most scenarios, the studies performed in children and incorporating real-life compliance information comparing SCIT and SLIT therapies are scarce (12). Moreover, there are no studies performed within the context of the Portuguese healthcare system. Children are an important population to assess because AIT can modify the natural course of the disease, especially at an early age, and prevent the development of asthma (6, 13, 14). The prevalence of allergic rhinitis is around 25% in Portuguese children and adolescents, with grass pollen being a relevant allergen in the country and 70% of cases also presenting conjunctivitis symptoms (15, 16). Having in mind the perspective of the Portuguese healthcare system, we aim to compare SCIT and SLIT therapies for children with grass pollen allergic rhinitis using a mathematical modelling approach and parameters from multiple sources, including randomized controlled trials and real-world data, such as compliance.

Materials and methods

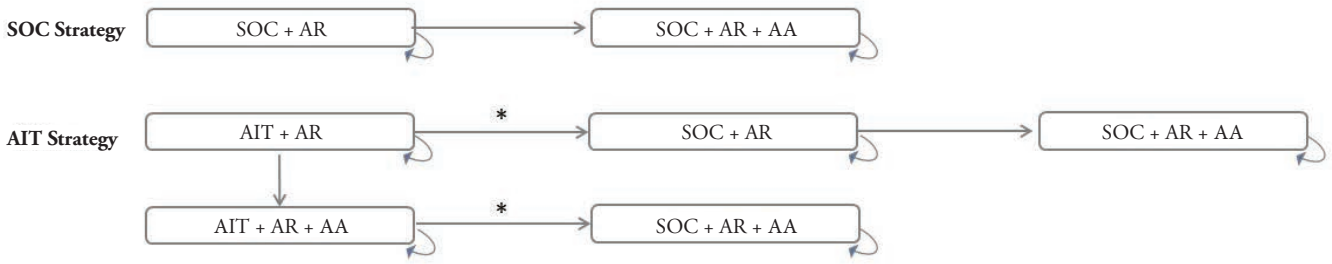
This cost-effectiveness analysis was based on previous modelling cost-analysis studies conducted in adults with allergic rhinitis/rhinoconjunctivitis (17). We developed a Markov model framework with three strategies to compare costs and health-related quality-of-life outcomes in children with grass allergic rhinitis treated with SLIT or SCIT plus symptomatic treatment *versus* children treated with pharmacotherapy alone, over a 10-year horizon, according to the Portuguese healthcare system perspective.

Model assumptions

For the Markov state-transition model, we simulated a hypothetical cohort of 8-years old patients, one thousand per strategy, over a 10-year time horizon divided into cycles of one year. The inclusion criteria of patients were a diagnosis of moderate persistent allergic rhinitis (AR) eligible for AIT (18), and a positive skin-prick test to grass pollen. Patients were modelled across different mutually exclusive health states. At baseline, none of the children had a diagnosis of asthma, however, throughout the model the probability of developing allergic asthma (AA) was considered and accounted as an effectiveness parameter. We assumed that AIT plus pharmacotherapy would generate a decrease in AR medication and symptoms, and a reduction of AA cases when compared to the standard-of-care (SOC) pharmacotherapy strategy. The SOC strategy had three possible health states: allergic rhinitis ("SOC + AR"), allergic rhinitis plus asthma ("SOC + AR + AA"), and any-cause death (**figure 1**). Allergen immunotherapy strategies, sublingual (SLIT) and subcutaneous (SCIT), were defined by five health states each: two states according to AIT administration, namely, AIT and rhinitis ("SLIT/SCIT + AR"), and AIT with rhinitis and asthma ("SLIT/SCIT + AR + AA"), plus the three health states mentioned for the SOC arm (**figure 1**).

At the beginning of the intervention, all patients were in the health state of "SOC + AR" or "SLIT/SCIT + AR", if assigned to any of the AIT strategies. In the two intervention strategies, the health states representing AIT administration were also characterized by the concomitant use of symptomatic SOC therapy for AR. We accounted for the possibility of therapy discontinuation as it happens in the real world (19). Patients who discontinued AIT were allocated to the corresponding health state as in the SOC arm, taking only symptomatic therapy ("SOC + AR" or "SOC + AR + AA"), and this therapy regimen was allowed for

Figure 1 - *Basic structure of the Markov model. The risk of death is not shown (in order to simplify representation) but all patients, in any health state, are in risk of any-cause death. Patients under intervention (AIT administration) may discontinue treatment at any time; at that time patients follow the transitions and health states represented for the SOC arm. The same happens when treatment ends (after 3 years). The previous situations are explained in the scheme with an asterisk (*). AIT represents SCIT and SLIT strategies. All patients under the AIT strategy start, at baseline, in "AIT + AR" health state; all patients under the SOC strategy start, at baseline, in "SOC + AR" health state.*



AA: allergic asthma; AIT: allergen immunotherapy; AR: allergic rhinitis; SOC: standard-of-care treatment; SCIT: subcutaneous immunotherapy; SLIT: sublingual immunotherapy.

the remaining time horizon. After treatment discontinuation, patients were not allowed to return to any AIT health-state. Patients were allowed to discontinue at any time of the year; thus, to account for this assumption, AIT costs corresponding to six months of administration were considered during the year before discontinuation. For these patients, AIT effects on medication and utilities were not considered once treatment was discontinued, and SOC values were considered instead. AIT was ideally administered for three years, as recommended, and patients who completed AIT continued pharmacotherapy alone for the remaining cycles; the AIT effects on costs and utilities remained the same after the end of therapy as described in literature (14). The effect of AIT on AA prevention was only considered for the years of AIT administration assuming, then, the value for the SOC strategy (6, 13, 20). When a patient developed asthma, it was not possible to return to a health state with AR only.

Model inputs

Each health state had an associated cost and utility value. After each Markov cycle, the cohort was re-distributed across the possible health-states based on transition probabilities derived from the literature. In the end, health-state costs and utilities were accumulated according to the number of patients, in each cycle, and over the time horizon. The effectiveness of strategies was measured by the reduction of symptomatic treatment and the development of asthma. The transition probabilities, disease-related costs, and utilities reflect the effect of strategies in the sample according to different sources for clinical data input. We conducted a literature review and meta-analysis to obtain the effectiveness parameters of both SLIT and SCIT on asthma prevention for grass pollen allergic patients to avoid the overestimation of effects if data were retrieved from a single study (**online supplements table IS**). The protective effect was higher for SCIT than for SLIT (OR, 95%CI, n studies: 0.50, 0.28-0.88, 3 *vs* 0.81, 0.67-0.97, 5) (**online supplements table IS**). For the SOC strategy, the probability of developing asthma was extracted from the control arm of the grass sublingual immunotherapy tablet asthma prevention (GAP) trial that was conducted in children with grass pollen allergy over 5 years (20). Asthma was reported if a patient had experienced asthma symptoms and medication use during the year leading up to the visit. The probability of discontinuation was retrieved from a recent real-world study conducted in German children with pollen allergic rhinitis taking SLIT or SCIT (19). Cumulative non-adherence for 3 years of therapy was 66% and 53% for SLIT and SCIT, respectively. Any-cause death probability was estimated based on country-specific data from 2019, for 8-years old children, using data from the Portuguese national institute of statistics (21). Cumulative probabilities were converted as rates using their periodicity and re-expressed as probabilities within 1 year (cycle length) (22, 23).

Costs were defined for each health state, per year, to express differences in medication and healthcare resources use between strategies (**table I**). AR treatment followed ARIA recommendations (18) and the duration of the pollen season was estimated at 4 months (120 days) (24). We assumed full adherence to the AR symptomatic treatment in all strategies. The cost of symptomatic treatment was calculated based on drug total costs for 4 months; patients were under nasal corticosteroids and oral antihistamines (18, 25). For both AIT strategies, the costs of AR symptomatic drugs were reduced by 27% according to the mean reduction effect found in the GAP trial and this effect remained after AIT completion (14, 20, 26). AIT costs for one year of SLIT (Sulgen) and SCIT (Allergovac Poliplus) were based on the price list of the pharmaceutical company Roxall, for Portugal (27). In the case of SCIT, we considered administration costs of the therapy at the hospital assuming the con-

tracted costs for Portuguese public hospitals in 2020 (28). In the case of AIT discontinuation, we considered a reduction of 50% in the AIT cost in the year in which treatment was discontinued (representing a mean of six months of immunotherapy the year before discontinuation). Asthma symptomatic drug costs were calculated according to the price of drugs in the country; patients were stratified by GINA guidelines in steps 2, 3, and 4 in equal proportions to calculate a mean value for drug's costs (25, 29). We also considered costs related to asthma moderate and severe exacerbations according to the probability of emergency department (ED) visits and hospitalizations, respectively, based on contracted values for Portuguese public hospitals (28). For both AIT strategies, asthma medication costs and exacerbations were reduced based on literature findings; the use of drugs was reduced by 34% and exacerbations leading to ED visits or hospitalizations due to asthma were reduced by 74% (20, 30). The previous effects on asthma were included in the model for patients taking and completing three years of immunotherapy. In all strategies, we considered two scheduled medical visits (including medical tests), per year, and SPT testing was considered only in SLIT and SCIT strategies, at the first visit, to confirm the diagnosis of grass allergy (31).

Quality-adjusted life years (QALYs) was the outcome used to translate efficacy in health gains. QALYs were extrapolated from a study conducted in children with grass pollen rhinoconjunctivitis with or without well to partially controlled allergic asthma (32). The effect was assumed to be the same for SLIT and SCIT since we did not find specific data for children by the AIT administration route. The QALYs are presented in **table I**. Alternative QALYs values were retrieved from another study conducted in adults with a grass pollen allergy that stratified SLIT and SCIT effects on symptoms (17). The authors applied the multi-attribute Rhinitis Symptom Utility Index (RSUI) to convert symptoms severity in utilities (33). Symptoms severity was evaluated through the rhinoconjunctivitis total symptom scores (RTSS) reported in a meta-analysis (34, 35). To adjust these data from a single condition, we further considered the patient's age and co-existing asthma utilities values to better describe our pediatric population using a multiplicative function (17, 36). Thus, we used as reference a value for "perfect health" valid for children (0.960) and incorporated asthma comorbidity into utilities by attributing a utility of 0.737, as described (17, 37). These adjusted QALYs were applied in an alternative scenario analysis.

Model calculation

Cost-effectiveness was established by the calculation of the incremental cost-effectiveness ratio (ICER) as incremental costs divided by incremental QALYs assuming the SOC strategy as reference. Costs and QALYs were discounted at 3% per year as performed in previous studies (10, 17, 38). The cost-effectiveness threshold was based on literature; the WHO recommends a threshold of up to three times the gross domestic product (GDP) per capita of the country (39). However, this threshold has been widely discussed because it is very high and a lower value, corresponding to the lower category suggested by WHO, specifically, up to one time the GDP *per capita*, was adopted in this analysis (39, 40). Therefore, in Portugal, the GDP *per capita* in 2020 was set at 22,488.62 USD (corresponding to 18,482.80€; data converted on June 7th, 2021). All the analyses were performed in RStudio Software version 3.4.2 (R Foundation for Statistical Computing, Vienna, Austria) using the heemod package (22).

Sensitivity analysis

A deterministic sensitivity analysis (DSA) was performed to check the uncertainty of each parameter, allowing to determine an ICER range due to lower and higher changes in the input parameters in comparison to the base case scenario (10). For the efficacy parameter of asthma development in both AIT strategies, the range of values for DSA was defined based on the 95% confidence interval. For QALYs and remaining parameters we considered a margin of error of ± 10% and ± 20%, respectively (**table I**). A Tornado diagram was developed to summarize the relative contribution of each parameter to ICER variation (11).

A probabilistic sensitivity analysis (PSA) was performed by running 1,000 Monte Carlo simulations (10, 11). These multiple repetitions of ICER calculations were drawn randomly according to the defined distribution for utilities and transition probabilities parameters (11, 41). Utilities followed a beta distribution while transition probabilities followed a binomial distribution. Costs were point estimates since their calculation was based on market prices and did not follow any specific distribution. PSA results were represented graphically, in a cost-effectiveness plane, to evaluate the extent of uncertainty (11). Based on those results, a cost-effectiveness acceptability curve (CEAC) was created showing the probability of the intervention being cost-effective compared to the symptomatic arm for different threshold values of willingness-to-pay (WTP) (11).

In the end, other plausible scenarios were considered to analyze alternative assumptions that might happen or be improved in clinical practice (23). Thus, we considered the following alternative scenarios (the values changed in the model for each scenario are shown in parentheses) to the base case model:

1. Asthma medication costs calculated based only on step 2 GINA guidelines, assuming that all patients who developed asthma were considered to be mild cases (SOC: 153€; AIT: 59€).
2. Equal asthma costs across all strategies (do not consider AIT effects on asthma) (SOC: 451€; AIT: 451€).
3. Adherence of 50% to AR symptomatic treatment across all strategies (SOC: 14€; AIT: 10€).
4. Different time horizons, 5 years to ensure the short-term effect found in the literature review, and 15 years assuming that effects are longer.

Table I *- Parameters of the Markov model for the three strategies (SOC, SLIT and SCIT).*

Parameter	Base case value (Symptomatic arm)	Base case value (AIT arm, SLIT or SCIT)	Range for DSA		Distribution	Ref.
			Lower	Upper		
Time horizon	10 years					
Age at baseline	8 years					
Number of health states	3	5	-	-	-	-
Initial Health State (t0)	SOC + AR (n = 1,000)	SLIT/SCIT + AR (n = 1,000)	-	-	-	-
Annual discount rate (costs/QALYs)	3% (range 0%-6%)					
Time of a Markov cycle	1 year					
Transition probabilities						
Disease progression (SOC + AR --> SOC + AR + AA)	0.021287		0	0.068850	Binomial	20
Disease progression (AIT + AR --> AIT + AR + AA)	-	SCIT: 0.010643 SLIT: 0.014262	SCIT: 0.005960* SLIT: 0.012985*	SCIT: 0.018732* SLIT: 0.020648*	Binomial	Meta-analysis
AIT discontinuation	-	SCIT: 0.22250 SLIT: 0.302047	SCIT: 0.124966 SLIT: 0.185674	SCIT: 0.353670 SLIT: 0.480750	Binomial	19
Annual mortality						
All-cause mortality (any state to death)	0.000066				-	21
Costs (1 year)						
SPT testing (t = 0)	-	31€	-	-	-	28
Scheduled visits + additional tests	150€ (2 visits/year)		-	-	-	28
Medication AR	28.86€	21.00€	SOC: 23.86€ AIT: 16.80€	SOC: 34.63€ AIT: 25.2€	-	18, 20, 25
Asthma costs	451€	180€	SOC: 361€ AIT: 144€	SOC: 541€ AIT: 216€	-	20, 25, 29
AIT	-	SCIT: 300€ SLIT: 860€	SCIT: 240€ SLIT: 688€	SCIT: 360€ SLIT: 1,032€	-	27
AIT administration	-	SCIT: 252€	-	-	-	28
QALYs						
SOC + AR	0.671		0.604**	0.738**	Beta	31
SOC + AR + AA	0.666		0.599**	0.733**	Beta	
SLIT/SCIT + AR	-	0.705	0.635**	0.776**	Beta	
SLIT/SCIT + AR + AA	-	0.677	0.609**	0.745**	Beta	
Death	0		-	-	-	-

*Value obtained based on the 95% confidence interval; **value obtained based on a margin error of 10%; the range for DSA analysis was calculated based on a margin error of 20% unless otherwise stated.

Table II *- Base case results of costs and health gains for SOC, SLIT, and SCIT strategies at the end of the model calculation (10-year horizon).*

Base case scenario	SOC	SLIT	SCIT
AR costs	288,495€	257,814€	248,095€
AIT costs	0€	1,571,120€	1,144,970€
AA costs	495,501€	334,882€	282,076€
Healthcare resource costs	1,500,002€	1,531,000€	1,531,000€
Total cost	2,283,999€	3,694,815€	3,206,141€
Total cost (discount rate)	1,988,279€	3,396,562€	2,921,615€
QALYs	6,702	6,827	6,868
QALYs (discount rate)	5,889	6,001	6,037
Cost difference	Ref	1,408€	933€
Effect difference	Ref	0.112	0.148
ICER	**Ref**	**12,605€**	**6,318€**

AA: allergic asthma; AIT: allergen immunotherapy; ICER: incremental cost-effectiveness ratio; QALY: quality-adjusted life-years; Ref: reference; SCIT: subcutaneous immunotherapy; SLIT: sublingual immunotherapy; SOC: standard-of-care.

Table III *- Distribution of participants according to specific Markov cycles (cycles 0, 3, and 10) by health state.*

Cycle	SOC + AR	SOC + AR+AA	AIT + AR	AIT + AR + AA	Death
t = 0					
SOC	1,000	-	-	-	0
SLIT	-	-	1,000	-	0
SCIT	-	-	1,000	-	0
t = 3					
SOC	937	63	-	-	0
SLIT	634	27	319	20	0
SCIT	511	19	451	19	0
t = 10					
SOC	806	193	-	-	1
SLIT	543	116	275*	65*	1
SCIT	439	90	388*	82*	1

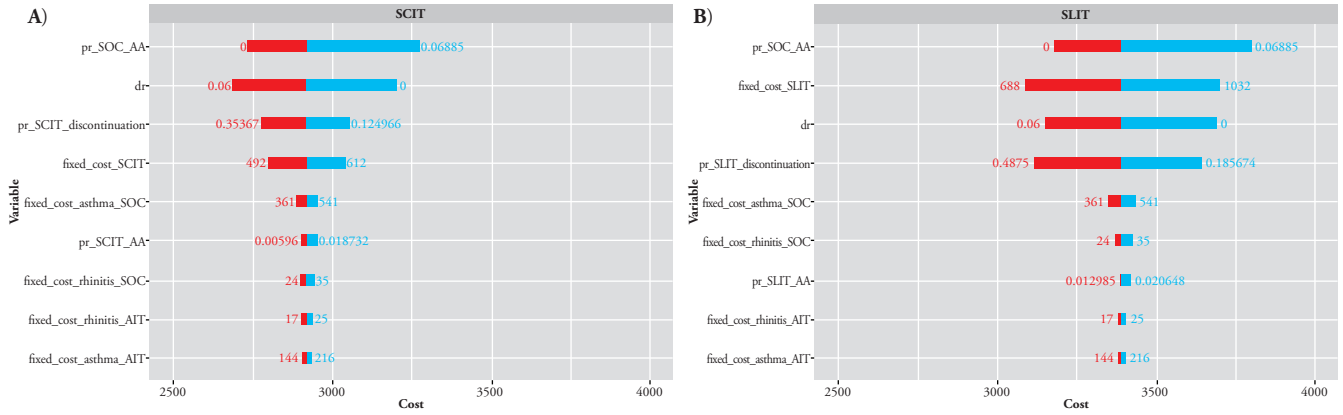
AA: allergic asthma; AIT: allergen immunotherapy; SCIT: subcutaneous immunotherapy; SLIT: sublingual immunotherapy; SOC: standard-of-care; *patients who completed AIT (t = 3) were assumed to remain in the same state to consider long-term effects of AIT in medication and symptoms, however, the probability of developing asthma was assumed to be equal to the SOC arm since long-term effects are still under evaluation.

5. Long-term effect of AIT on asthma prevention.
6. Full adherence to AIT (no possibility of AIT discontinuation).
7. Discount of 50% in AIT acquisition costs (SLIT: 430€; SCIT: 402€).
8. Different utilities values, adjusted for SLIT and SCIT ("SOC + AR": 0.748; "SOC + AR + AA": 0.551; "SLIT + AR": 0.797; "SLIT + AR + AA": 0.587; "SCIT + AR": 0.817; "SCIT + AR + AA": 0.602).

Results

The base case analysis shows an incremental cost of 1,408€ and 933€ per patient for SLIT and SCIT, respectively, and a correspondent incremental QALYs of 0.112 and 0.148 per patient, causing an ICER of 12,605€ and 6,318€ per QALY gained (**table II**). SCIT strategy was more cost-effective than SLIT, but both strategies are lower than the willingness-to-pay threshold assumed for Portugal. SCIT demonstrated to be less costly than SLIT mainly due to savings in asthma costs and AIT price. Over

Figure 2 *- Tornado plot resulting from the deterministic sensitivity analysis.*



Blue and red bars represent an increase or decrease in costs, respectively, according to changes in variables (the range of values is represented in each side of the bars). **(A)** Subcutaneous strategy; **(B)** Sublingual strategy. AIT: allergen immunotherapy; dr: discount rate; fixed_cost_asthma_AIT: asthma-related costs in AIT arm; fixed_cost_asthma_SOC: asthma-related costs in SOC arm; fixed _cost_rhinitis_AIT: rhinitis-related costs in AIT arm; fixed_cost_rhinitis_SOC: rhinitis-related costs in SOC arm; fixed_cost_SCIT/SLIT: cost of AIT according to the administration route, subcutaneous or sublingual; pr_SCIT/SLIT_AA: probability of developing asthma in AIT arm; pr_SCIT/SLIT_discontinuation: probability of AIT discontinuation; pr_SOC_AA: probability of developing asthma in the SOC strategy.

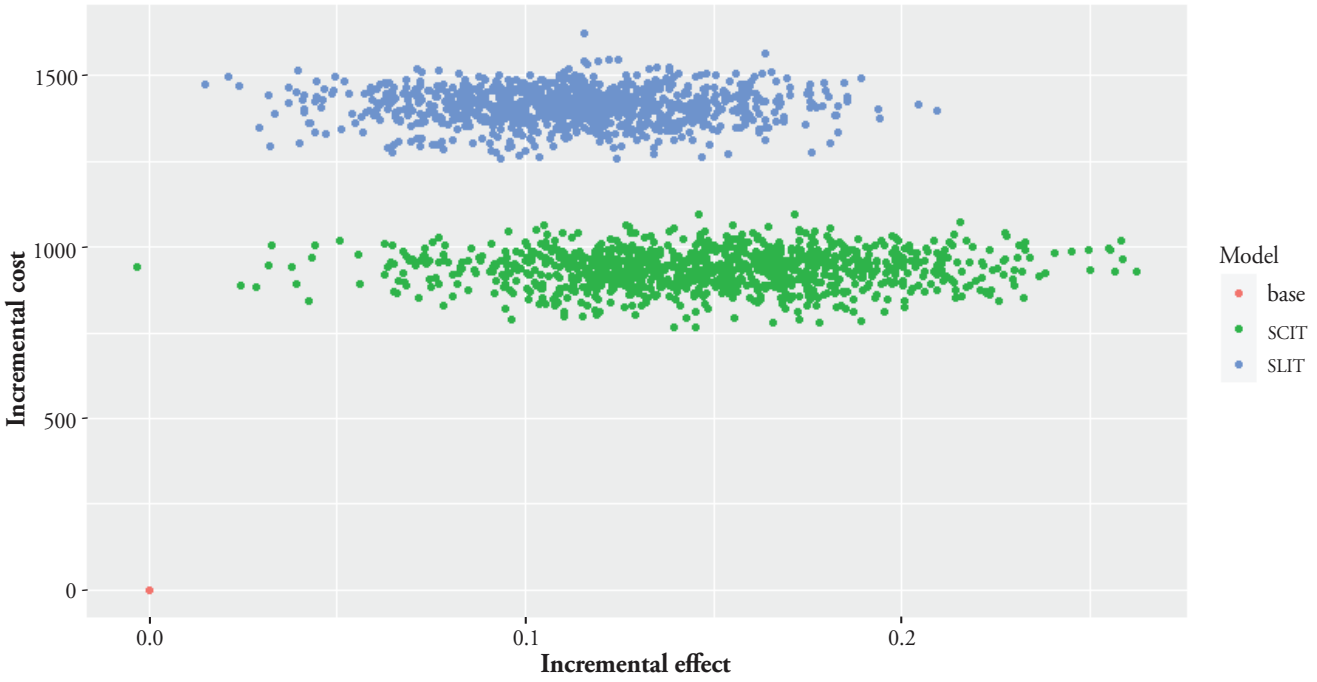
the 10-year horizon, the number of patients experiencing allergic asthma were 193, 181, and 172 in SOC, SLIT, and SCIT strategies, respectively (**table III** and **online supplements figure 1S**). According to the model, 339 and 470 patients completed three years of immunotherapy (SLIT and SCIT, respectively). For these patients, the reduction of medication and allergic symptoms remained the same until the end of the analysis.

The robustness of the results was assessed in a sensitivity analysis. The DSA showed the parameters with the greatest contribution for the estimation of costs; specifically, for both AIT strategies, the natural probability of asthma development (assumed in the SOC arm) over the years was the main driver for change in costs, followed by annual discount rate, treatment discontinuation, and AIT costs (particularly, in SLIT arm) (**figure 2**). The variation of model parameters resulted in a range of ICER values varying between 4,185€ and 20,290€ for SLIT, and 2,093€ and 8,417€ for SCIT. The full list of ICER values according to individual variation of parameters is presented in the supplemental material (**online supplements tables IIS, IIIS**).

The PSA showed the uncertainty surrounding the point estimates of the base case analysis and is graphically represented in **figure 3** (each dot represents a Monte Carlo iteration of PSA). Both strategies showed to be cost-effective as QALYs increase, the costs remain similar, decreasing ICER; still, SCIT presented higher values for QALYs and lower costs which translates to a lower ICER value. The mean ICER after Monte Carlo simulation was 12,599€ and 6,249€ for SLIT and SCIT interventions, respectively (**online supplements table IVS**). These values are very similar to those from the base case scenario. These data were used to compute the cost-effectiveness acceptability curves (CEAC) according to a range of WTP thresholds. The graphs are presented in the supplemental material (**online supplements figure 2S**) and show the probability of AIT to be cost-effective considering different WTP threshold values; for example, considering a WTP limit of 10,000€ the probability of being cost-effective is 20% (SLIT) and 90% (SCIT), but increasing this limit to 20,000€, which is a similar value to the WTP that we assumed for Portugal, the values increase to 60% and 98%, respectively.

Uncertainty of the model was also assessed by varying model parameters according to possible circumstances that might happen in the real-world (**online supplements table VS**). The ICER remained similar to the base case when AA and AR costs varied. As expected, ICER values were higher when considering a short-time horizon and lower for a long-time horizon; both strategies were cost-effective for a higher follow-up of patients mainly due to the accumulated QALYs over years since costs were marginally reduced compared to the base case. If the probability of AA prevention remains after AIT completion, ICER results did not vary significantly. The possible scenarios related to full-adherence to AIT and a 50% discount on AIT acquisition costs had a significant effect on ICER as the probability of SLIT and SCIT being cost-effective increase to 77% and 97% (full-adherence), and 92% and 98% (50% discount), respectively. The previous probabilities are for a WTP of 10,000€, which is almost half of the WTP threshold value considered for Portugal. The last scenario analysis considered different utility values as the values found in the literature vary (the values assumed are described in

Figure 3 *- Results of the probabilistic sensitivity analysis graphically represented on a cost-effectiveness plan.*



Green: subcutaneous immunotherapy; blue: sublingual immunotherapy; orange: standard-of-care (reference).

Methods section). These values were adjusted for the patient's age and administration route of AIT according to the method described previously. The results were significantly lower being both strategies cost-effective at a WTP of 10,000€.

Discussion

Over a 10-year time horizon, grass sublingual and subcutaneous immunotherapy seems to be cost-effective in children with grass pollen-induced allergic rhinitis considering a WTP threshold of 18,482.80€. Specifically, SCIT showed robust results for all sensitivity analyses and different scenarios. The key drivers were the reduced asthma-related costs due to the prevention of more asthma cases and the lower acquisition price of SCIT. Sensitivity analysis evidenced the core parameters that might improve the cost-effectiveness of both strategies; namely, a reduction in AIT acquisition prices and an increase of AIT adherence. The results were sensitive to changes in utilities showing the importance to improve evidence of AIT effects on QALYs in younger populations. Still, the conclusions remained the same for this alternative scenario.

To our knowledge, this is the first cost-effectiveness study conducted in children with grass pollen allergic rhinitis that evaluated two different administration routes of allergen immunotherapy relative to the standard symptomatic treatment. Vogelberg and colleagues conducted a similar analysis in children for sublingual immunotherapy (38). Our results were similar in terms of QALYs gained per patient and higher regarding costs resulting in a relatively higher ICER value. Differences in costs can be due to some assumptions that differed between studies; Vogelberg *et al.* (38) considered only mild cases of asthma and an additional health-state to account for improvement of rhinitis severity (mild rhinitis) which may result in a lower cost per patient treated with SLIT. Still, our study reinforces the result previously obtained for SLIT in children (38). Additionally, our study evaluated for the first-time grass pollen SCIT in a pediatric cohort and demonstrated to be more cost-effective than SLIT, especially when assessing sensitivity analysis and alternative scenarios. The main reasons for this effect are the lower costs of AIT and the larger effect on asthma prevention and, consequently, in asthma-related costs. This study assumes a higher relevance because it simulates a pediatric cohort of patients in which preventive effects might be more prominent; when evaluating studies conducted in adult patients, the ICER results usually are higher for both strategies evidencing the greater long-term effects if administered early in life (17, 42). QALYs estimation for children may also impact this hypothesis since values differed greatly from adults highlighting the scarcity of studies conducted in children (32).

This study has several strengths. The base case model outcomes can be considered conservative due to different assumptions

considered for model input. First, despite the productivity losses of children in school and absenteeism not being accounted, because we considered in the analysis direct costs, the inclusion of those parameters would reduce the ICER estimates which strengths the conclusions of this simulation (17). Second, the effect of AIT on asthma prevention was assumed only for the years of treatment, but if we assume this effect in an alternative scenario for the remain time-horizon, in patients completing three years of treatment, the results do not differ significantly. Third, asthma prevention effects of AIT were retrieved from a meta-analysis conducted by the team synthesizing multiple data sources to improve the precision of pooled estimates resulting in a less optimistic estimate when compared to previous studies avoiding the overestimation of the results (31, 38). Fourth, whenever possible, we included data from real-world studies, such as the discontinuation rates. Fifth, patients who discontinue AIT earlier than the recommended duration were assumed to not receive any benefit from AIT (medication, QALYs, asthma prevention). Lastly, we conducted an extensive sensitivity analysis (deterministic and probabilistic) to account for the uncertainty of parameters and considering different scenarios in alternative analysis to the base case model, strengthening the results and evidencing key parameters to improve the cost-effectiveness of strategies in practice and drive policy decisions.

There are limitations that we should address. As a direct limitation of Markov models, we should be aware that we calculated expected costs and health benefits by simulating disease progression based on literature findings and we are not following each patient since the model is memoryless (10). Although we proposed a WTP threshold based on the lower limit suggested by the WHO, this limit should be interpreted cautiously since the Portuguese authorities may consider another value (39). Nonetheless, the extensive presentation of the results allows the interpretation using different WTP thresholds. The efficacy of AIT on allergic rhinitis symptoms and medication use was assumed to be the same for both strategies based on a study conducted only for SLIT (20). However, a meta-analysis revealed no differences between SCIT and SLIT on standardized mean differences for rhinitis medication scores in children (26) and there are no head-to-head comparisons of SLIT and SCIT efficacy. We also assumed that AIT had effects on reducing moderate and severe asthma exacerbations in children completing three years of immunotherapy but still developed asthma (65 and 82 for SCIT and SLIT arms, respectively). The evidence to support this assumption is very limited and we should be aware that this effect may be not significant as we expected. The key assumption underpinning our choice are the known effects of AIT in patients with asthma while the extrapolation for the remaining time-horizon was the sustained efficacy of AIT on symptoms and medication demonstrated previously in GAP trial (20). In a complementary analysis, assuming no effects of AIT on reducing asthma medication and exacerbations in children who developed asthma, asthma-related costs would be 495,501€, 400,458€, and 437,790€ for SOC, SCIT, and SLIT, respectively, leading to an ICER value of 6,989€ (SCIT) and 13,384€ (SLIT). As discussed, the results are highly dependent on the underlying assumptions retrieved from the literature. Thus, the long-term effects of AIT on allergic rhinitis and asthma, in children, should be demonstrated in higher-quality studies since published studies are inconsistent and limited, especially for AR patients that develop asthma under AIT completion. Therefore, we varied model parameters to allow interpretation of the results under different assumptions. We did not incorporate nonmedical costs such as transportation to the hospital for SCIT administration which may underestimate the ICER for this strategy at some extent (17). The management of adverse events due AIT was not considered. We believe that the impact of this parameter would be low because the differences found in randomized controlled trials between AIT and placebo are not statistically significant (6, 20). The analysis is also limited to a 10-year time horizon, and we cannot predict the long-term effect of asthma development in adulthood as well as asthma severity of those who developed asthma. We assumed SPT to be enough to identify grass pollen patients eligible for AIT, but the pattern of the pollen season can be very heterogeneous as well as the sensitization profiles of patients (43, 44). These situations usually require the use of molecular diagnostic tests which may increase the costs associated to the AIT arms and the reported ICER. We assumed that SPT would be performed only in children of AIT strategies to confirm the diagnosis of grass allergy prior to AIT initiation and to allow the comparison with other studies (31). A complementary analysis assuming skin-prick testing in children of SOC group showed a decrease in ICER for both SLIT and SCIT (12,328€ and 6,108€, respectively). Finally, the analysis is limited to the Portuguese context but the model can be applicable to other countries and realities according to the available data to fulfil the model parameters.

Despite variations underlying model assumptions, we sought to assess which strategy is more cost-effective in a pediatric population. Different sensitivity and scenario analysis demonstrated a favorable result to SCIT mainly due to the lower acquisition costs, higher effect on asthma prevention and related costs, and lower discontinuation rates. However, SCIT is not always the preferred route of administration in children due to frequent hospital visits and discomfort and constraints intrinsically related to the administration of injections. New routes of AIT administration are being developed and evaluated, such as epicutaneous, intradermal, and intralymphatic (8), but there is limited evidence of effectiveness, especially in young children in which AIT is more likely to prevent new sensitizations and asthma.

The present study highlights the scarcity of cost-effectiveness studies conducted in pediatric populations and, considering the

Portuguese context for both children and adults. Despite the conservative framework adopted in this study, we cannot strongly conclude that both forms of AIT for grass pollen allergic rhinitis are cost-effective. However, SCIT showed consistent results across different scenarios and a high probability of being cost-effective which may drive future policy decisions and AIT prescribing habits. To perform reliable and accurate cost-effectiveness studies, AIT long-term effects should be addressed in high-quality studies as well as in head-to-head comparative studies. We also conclude that AIT adherence has a great impact on results highlighting the value of implementing strategies to promote adherence rates.

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Contributions

AM, JCR, MF: conceptualization. AM, LD: clinical data discussion and approval - as medical experts in the field. MF, IP, FCM: literature review. MF, JCR: implemented the model (data analysis). All authors: results interpretation and discussion, writing - original draft, writing - review & editing.

Conflict of interests

The authors declare that they have no conflict of interests.

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Figure 1S *- Distribution of participants in each health state per strategy.*

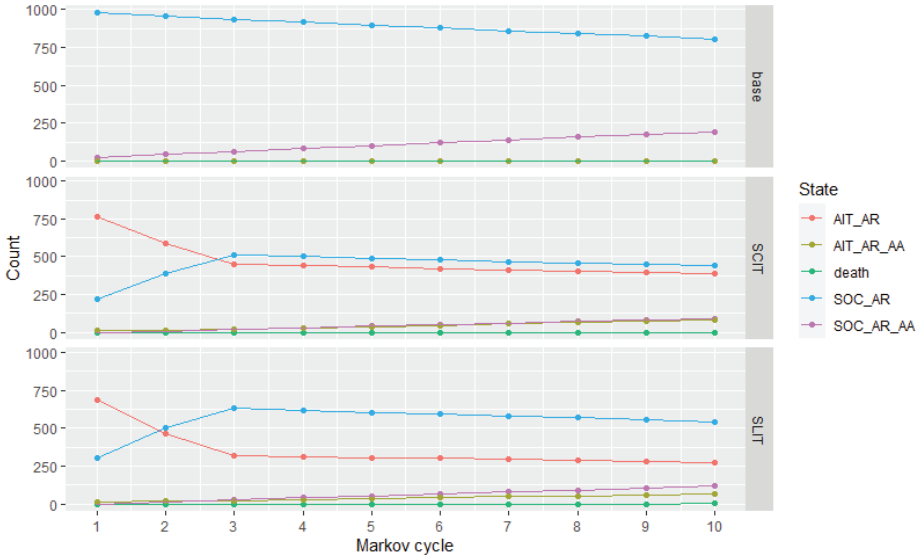
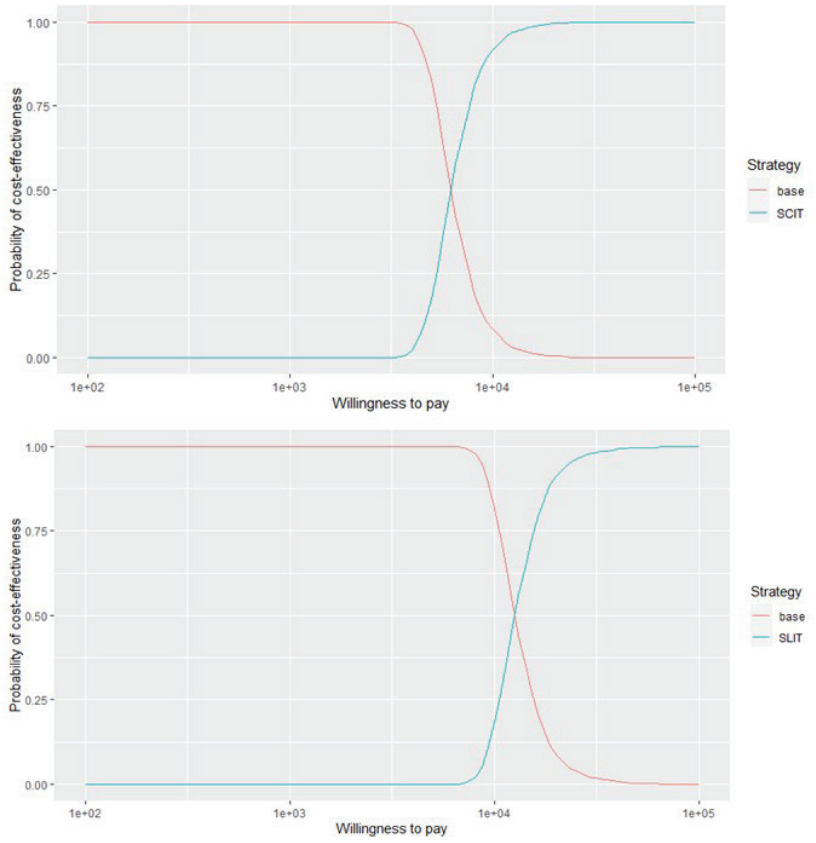


Figure 2S *- Cost-effectiveness acceptability curve for different willingness-to-pay (WTP) threshold values.*



(A) Subcutaneous strategy; **(B)** Sublingual strategy.

Table IS *- Results from the meta-analysis performed by our team to estimate the effect of AIT on asthma prevention. For each output is presented the corresponding OR (95%CI), heterogeneity parameters, and the number of studies included.*

Outcome	Meta-analysis, OR (95%CI)	Heterogeneity I2; Q (P-value)	n of studies analyzed
All studies (independently of allergen and population)			
SCIT	0.58 (0.37; 0.90)	26%; 6.72 (0.24)	6
SLIT	0.76 (0.62; 0.94)	23%; 10.45 (0.23)	9
Studies performed only in children			
SCIT	0.52 (0.18; 1.49)	0%; 0.14 (0.71)	2
SLIT	0.63 (0.29; 1.35)	33%; 5.93 (0.20)	5
Studies performed only in grass/ birch pollen allergic patients			
SCIT	0.50 (0.28; 0.88)	0%; 0.8 (0.67)	3
SLIT	0.81 (0.67; 0.97)	11%; 4.47 (0.35)	5

CI: confidence interval; I^2: Higgins I^2 statist; OR: Odds ratio; Q: Cochran's Q; SCIT: subcutaneous immunotherapy; SLIT: sublingual immunotherapy.

Table IIS *- Costs and QALYs per patient and the respective ICER results according to changes in key parameters (deterministic sensitivity analysis) for the sublingual immunotherapy strategy.*

SLIT strategy	Cost difference	Effect difference	ICER
Base case scenario	**1,408€**	**0.112**	**12,605€**
dr – no annual discount	1,411€	0.125	11,320€
dr – annual discount 6%	1,403€	0.101	13,858€
Cost asthma AIT	1,397€	0.112	12,502€
	1,420€	0.112	12,709€
Cost asthma SOC	1,447€	0.112	12,954€
	1,369€	0.112	12,257€
Cost rhinitis AIT	1,394€	0.112	12,481€
	1,422€	0.112	12,730€
Cost rhinitis SOC	1,425€	0.112	12,757€
	1,387€	0.112	12,414€
AIT discontinuation	1,659€	0.165	10,082€
	1,129€	0.056	20,290€
Pr AA (AIT arm)	1,402€	0.112	12,509€
	1,435€	0.110	13,095€
Pr AA (SOC arm)	1,610€	0.113	14,206€
	1,053€	0.108	9,739€
AIT cost	1,100€	0.112	9,845€
	1,717€	0.112	15,366€
QALY SOC + AR	1,408€	0.316	4,452€
	1,408€	0.092	15,162€
QALY SOC + AR + AA	1,408€	0.141	10,007€
	1,408€	0.093	17,026€
QALY SLIT + AR	1,408€	0.110	12,818€
	1,408€	0.336	4,185€
QALY SLIT + AR + AA	1,408€	0.090	15,669€
	1,408€	0.134	10,544€

AA: allergic asthma; AIT: allergen immunotherapy; dr: discount rate; ICER: incremental cost-effectiveness ratio; pr: probability; QALY: quality-adjusted life-years; Ref: reference; SCIT: subcutaneous immunotherapy; SLIT: sublingual immunotherapy; SOC: standard-of-care.

Table IIIS *- Costs and QALYs per patient and the respective ICER results according to changes in key parameters (deterministic sensitivity analysis) for the subcutaneous immunotherapy strategy.*

SCIT Strategy	Cost difference	Effect difference	ICER
Base case scenario	**933€**	**0.148**	**6,318€**
dr – no annual discount	922€	0.166	5,563€
dr – annual discount 6%	939€	0.133	7,058€
Cost asthma AIT	920€	0.148	6,228€
	947€	0.148	6,407€
Cost asthma SOC	983€	0.148	6,653€
	884€	0.148	5,982€
Cost rhinitis AIT	915€	0.148	6,194€
	952€	0.148	6,441€
Cost rhinitis SOC	956€	0.148	6,468€
	905€	0.148	6,128€
AIT discontinuation	1,068€	0.200	5,345€
	789€	0.094	8,413€
Pr AA (AIT arm)	914€	0.150	6,107€
	967€	0.145	6,690€
Pr AA (SOC arm)	1,163€	0.151	7,721€
	526€	0.142	3,715€
AIT cost	812€	0.148	5,493€
	1,055€	0.148	7,142€
QALY SOC + AR	933€	0.417	2,239€
	933€	0.121	7,693€
QALY SOC + AR + AA	933€	0.185	5,056€
	933€	0.111	8,417€
QALY SCIT + AR	933€	0.146	6,382€
	933€	0.446	2,093€
QALY SCIT + AR + AA	933€	0.123	7,598€
	933€	0.173	5,406€

AA: allergic asthma; AIT: allergen immunotherapy; dr: discount rate; ICER: incremental cost-effectiveness ratio; pr: probability; QALY: quality-adjusted life-years; Ref: reference; SCIT: subcutaneous immunotherapy; SLIT: sublingual immunotherapy; SOC: standard-of-care.

Table IVS *- Results from the probabilistic sensitivity analysis (mean values).*

Strategy	Cost difference	QALY difference	ICER
SLIT	1,409€	0.112	12,618€
SCIT	934€	0.149	6,249€

ICER: incremental cost-effectiveness ratio; QALY: quality adjusted life years; SCIT: subcutaneous immunotherapy; SLIT: sublingual immunotherapy.

Table VS *- ICER results for SLIT and SCIT according to different scenarios.*

Scenarios	Cost difference	Effect difference	ICER
#1 – Lower asthma costs			
SLIT			
Base case	1,498€	0.112	13,412€
PSA (average)	1,500€	0.112	13,338€
PSA CEAC probability (10,000 WTP)	-	-	12.5%
SCIT			
Base case	1,053€	0.148	7,127€
PSA (average)	1,054€	0.148	7,127€
PSA CEAC probability (10,000 WTP)	--	-	85%
#2 – Equal asthma costs for all strategies			
SLIT			
Base case	1,495€	0.112	13,385€
PSA (average)	1,492€	0.111	13,407€
PSA CEAC probability (10,000 WTP)	-	-	12.5%
SCIT			
Base case	1,033€	0.148	6,989€
PSA (average)	1,036€	0.150	6,882€
PSA CEAC probability (10,000 WTP)	--	-	87%
#3 – 50% of adherence to AR symptomatic treatment for all strategies			
SLIT			
Base case	1,422€	0.112	12,726€
PSA (average)	1,422€	0.111	12,765€
PSA CEAC probability (10,000 WTP)	-	-	17.5%
SCIT			
Base case	951€	0.148	6,437€
PSA (average)	951€	0.147	6,446€
PSA CEAC probability (10,000 WTP)	--	-	90%

Scenarios	Cost difference	Effect difference	ICER
#4.1 – Shorter time-horizon (5 years)			
SLIT			
Base case	1,501€	0.069	21,647€
PSA (average)	1,503€	0.070	21,459€
PSA CEAC probability (10,000 WTP)	-	-	0%
SCIT			
Base case	1,060€	0.088	11,997€
PSA (average)	1,060€	0.087	12,103€
PSA CEAC probability (10,000 WTP)	--	-	25%
#4.2 – Longer time-horizon (15 years)			
SLIT			
Base case	1,302€	0.146	8,928€
PSA (average)	1,306€	0.145	9,010€
PSA CEAC probability (10,000 WTP)	-	-	62.5%
SCIT			
Base case	789€	0.196	4,032€
PSA (average)	797€	0.196	4,059€
PSA CEAC probability (10,000 WTP)	--	-	97%
#5 – Long-term effect of AIT on asthma prevention (10 years)			
SLIT			
Base case	1,400€	0.113	12,380€
PSA (average)	1,402€	0.114	12,341€
PSA CEAC probability (10,000 WTP)	-	-	62.5%
SCIT			
Base case	915€	0.151	6,074€
PSA (average)	914€	0.148	6,169€
PSA CEAC probability (10,000 WTP)	-	-	90%
#6 – 100% adherence to AIT			
SLIT			
Base case	2,190€	0.282	7,778€
PSA (average)	2,190€	0.283	7,725€
PSA CEAC probability (10,000 WTP)	-	-	77%

Scenarios	Cost difference	Effect difference	ICER
SCIT			
Base case	1,279€	0.284	4,506€
PSA (average)	1,278€	0.281	4,549€
PSA CEAC probability (10,000 WTP)	--	-	97%
#7 – Discount of 50% in AIT acquisition costs			
SLIT			
Base case	637€	0.112	5,704€
PSA (average)	638€	0.110	5,778€
PSA CEAC probability (10,000 WTP)	-	-	92%
SCIT			
Base case	629€	0.148	4,257€
PSA (average)	631€	0.144	4,380€
PSA CEAC probability (10,000 WTP)	-	-	98%
#8 – Different utilities, adjusted for SLIT and SCIT according to Di Bona et al. (20)			
SLIT			
Base case	1,408€	0.189	7,463€
PSA (average)	1,408€	0.188	7,505€
PSA CEAC probability (10,000 WTP)	-	-	88%
SCIT			
Base case	933€	0.345	2,708€
PSA (average)	936€	0.342	2,738€
PSA CEAC probability (10,000 WTP)	-	-	100%

AIT: allergen immunotherapy; AR: allergic rhinitis; CEAC: cost-effectiveness acceptability curve; WTP: willingness-to-pay threshold; PSA: probabilistic sensitivity analysis; SCIT: subcutaneous immunotherapy; SLIT: sublingual immunotherapy.

Mariana Farraia <mariana.farraia@gmail.com> sábado, 19/11, 12:03

para Jessica

Dear Jessica,
What is the preferred contact of the journal/editors? I want to request a formal authorization to use this manuscript in my PhD thesis. Can you help me with this? I send an email a few days ago to p.ficicchia@lswr.it , but I'm not sure if it is the most correct. The content of the email is below:

"Dear Dr L. Cecchi and Dr P. Carreiro Martins,
Editors-in-Chief of the European Annals of Allergy and Clinical Immunology,

I am finishing my PhD thesis, which aims to evaluate the longitudinal patterns of allergic sensitization and its impact on allergic multimorbidities management, thus I would like to include the results of the manuscript "Cost-effectiveness analysis of grass pollen specific therapy in children with allergic rhinitis compared to the standard of care symptomatic treatment in Portugal" accepted for publication in European Annals of Allergy and Clinical Immunology in 2021 (doi: 10.23822/EurAnnACI.1764-1489.240). To include the results and also the manuscript in my thesis, the faculty requires the journal's authorization to integrate the manuscript into the thesis.
Therefore, I would like to ask for permission to include the manuscript in the thesis.

Thank you.

Best regards,
Mariana Farraia"

Thank you for you support,
Mariana Farraia

Jessica Guenzi 21/11/2022, 13:29

para mim

Dear Dr. Farraia,
The articles are free and citable. The only requirement is to properly mention the article on your thesis when you use the data from this article. In case of other necessity, such as the use of the article for business purpose, it is not admitted.

I hope it can help,
Let me know,
Sincerely,
Jessica Guenzi

Da: Mariana Farraia <mariana.farraia@gmail.com>
Inviato: lunedì 21 novembre 2022 13:14

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[Mensagem reduzida] Ver toda a mensagem