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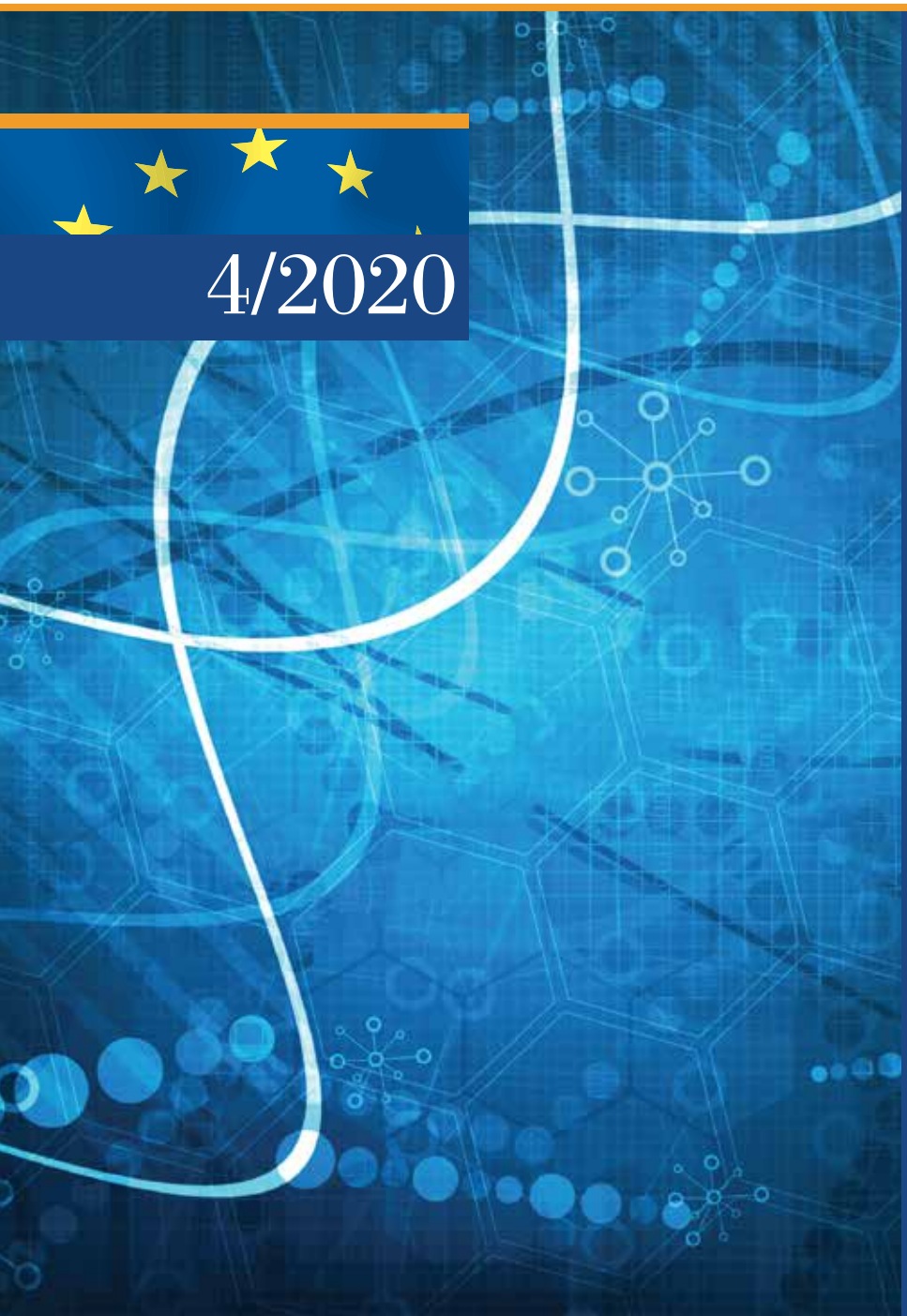

4/2020

Asthma and COPD "overlap": a treatable trait or common several treatable-traits?

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Asthma and COPD "overlap": a treatable trait or common several treatable-traits?

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Summary
In the last years, disease classification of chronic respiratory diseases (CRD) has been vivaciously discussed and new concepts have been introduced, namely asthma-chronic obstructive pulmonary disease (COPD) overlap (ACO). Controversially the GOLD consensus document of 2020 considered that we should no longer refer to ACO, as they constitute two different diseases that may share some common traits and clinical features.
The treatable traits approach has numerous strengths that are applicable to several levels of health care. In this paper we review the application of the treatable traits to CRD and describe in detail the ones already identified in patients with asthma and COPD. Treatable traits in CRD can be divided in pulmonary, extra-pulmonary and behavior/lifestyle risk factors. Patients with both asthma and COPD have clearly recognized treatable traits in all these subtopics but it is notorious the severe and frequent exacerbations, the associated cardiovascular disease and the low health related quality of life and productivity of these patients.

Introduction

In recent years the disease classification of chronic respiratory diseases has been put into debate (1,2). Classical classification used pre-20th century descriptions and concepts, based on symptoms, signs and functional abnormalities instead of pathophysiological mechanisms. Diagnostic terms such as asthma, chronic obstructive pulmonary disease (COPD) and chronic bronchitis were originally proposed to define a clinical presentation that appeared to encompass a more or less distinct disease process (1). These labels were defined in a subjective manner taking into account non-specific symptoms and signs such as cough, wheeze and breathlessness. As our knowledge of disease mechanisms progressed to a detailed molecular level the general approach to chronic respiratory diseases has been to try and match these abnormalities to the original disease classification rather than taking into consideration the pathophysiologic mechanisms themselves as the basis for classification (1,2).
In the last years there has been a renewed debate about how asthma and COPD may likely represent a continuum of different diseases that may share biological mechanisms (i.e. endotypes) and present similar clinical, functional, imaging and/or biological features that can be observed, (i.e. phenotypes) which require precision medicine treatment (2). Current consensus documents about asthma and COPD assume that "heterogeneity" and "complexity" are part of both diseases and even went further including these characteristics in disease definition (3,4). Our inability to understand the complexity of airway diseases is clinically relevant as it may conduct to suboptimal management due to the potentially need of different therapeutic strategies and may constraint our pathophysiologic investigation about patients whose clinical phenotype is not easily classified. Additionally, this "Oslerian diagnostic label" may jeopardize drug devel-

opment for specific endotypes and it limits the generalizability of the results of most randomized controlled trials (2). Despite the identified limitations, the diagnostic labels approach has many advantages: they are useful to discriminate grossly defined groups of patients, are an "easy basis" for teaching students, as well as explaining to patients and to use in interventional studies to convince authorities to fund medications. In clinical practice, they are also useful to identify a syndrome but this will probably lead to empirical management. With nowadays knowledge the labels should represent the start of the assessment process, not the end (5).

The concept of "treatable trait" has been proposed by A. Agusti in 2016, encompassing a label-free, precision medicine approach to the diagnosis and management of chronic airway diseases (2). This strategy has the purpose of "treatable traits" identification in each patient and these traits can be "treatable" based on "phenotypic" recognition or on deep understanding of the critical causal pathways (2). From a patient perspective it is important to recognize that a given patient may have more than one treatable trait and actually this is often the case. A treatable-trait should fulfil the following three characteristics: 1. *Clinical relevant* (requires to be clinically important, associated with specific disease outcomes); 2. *Identifiable and measurable* (should have a marker to objectively be identified, typically would be a biomarker) and 3. *Treatable* (should be effectively treated and this effect should ideally be measured in randomized controlled trials; traits not currently treatable or partly treatable are research opportunities)(6). One of the main potential strengths of this approach is that it does not start on the assumption that the diagnosis (asthma or COPD) is well established and clear, a situation which is not the case in many instances in clinical practice, particularly in primary care. In the "treatable traits" approach specific diagnostic criteria are defined for these "traits" and this may have a considerable impact in patient treatment assuming an expected larger therapeutic response. Another relevant aspect is that this approach may stimulate best translational research by identifying knowledge gaps that can be in future addressed (2). In **table I** we present the potential advantages and disadvantages (2,5) of traditionally "Oslerian approach" versus a "Treatable-traits approach" in chronic respiratory diseases.

Another interesting point is that in the last years a new phenotypic entity has re-emerged, the asthma-COPD overlap (ACO) (7). This entity has been put into debate due to the evidence that in clinical practice there is a significant number of patients who exhibit features of both diseases (7). Taking into consideration the well-known heterogeneity of both diseases our question is why should patients with overlap of both diseases be "homogeneous" and not also rather "heterogeneous"?

The purpose of our review is to discuss nowadays criteria of asthma-COPD overlap and possible treatable traits among this group of patients.

Asthma-COPD overlap definition

The possible first description of ACO is thought to be proposed by Orie and Sluiter in 1961, the well-know "Dutch hypothesis". This hypothesis theorized that asthma and COPD may have a mutual origin and subsequent expression of each disease is explained by individual variables encompassing genetic factors and environmental exposures (7). On the other hand, an opposite theory has been called the "British hypothesis", which postulates separate origins for asthma and COPD driven by its own individual genetic traits, inflammatory profile and treatment (7). In 2015 a consensus document elaborated together by GINA (Global Initiative for Asthma) and GOLD (Global Initiative for Chronic Obstructive Lung Disease) proposed a clinical de-

Table I *- Potential advantages and disadvantages of traditionally "Oslerian approach" versus a "Treatable-traits approach" in chronic respiratory diseases.*

	"Oslerian approach"	**"Treatable-traits approach"**
Advantages	• Simpler definition patients' groups (convenient for students teaching, patient education) • Easy to use in interventional studies • Useful syndrome identification	• No diagnostic assumptions • Precise definition of the "traits" • Disease mechanisms based • Expected larger therapeutic response • Stimulates translational research • Helps the identification of key criteria for future RCT
Disadvantages	• Fails to provide optimal care (no consideration to endotypes) • No appreciation of common patterns of disease • Increases clinical practice variability • Inhibits research progress	• Nowadays only partially based on evidence • Requires comparison about efficacy, safety and cost-effectiveness with "classic strategies" • Uncertainty about patients, physicians and stakeholder acceptance

RCT – Randomized clinical trials.

scription of patients who exhibited features of both asthma and COPD and called this entity the asthma-COPD overlap syndrome (ACOS)(8). In this document it was already emphasized that ACOS was not a single disease entity, including different forms of airways diseases. This constitutes one of the first and main misleading concepts associated with this subject as a syndrome by definition is "a recognizable complex of symptoms and physical findings which indicate a specific condition for which a direct cause is not necessarily understood" (9). In the context of the overlap between asthma and COPD we could argue that the causes are not necessarily understood but by no means the symptoms and physical findings indicate a specific condition.

Recent reviews also concluded that the ACOS does not represent a unique form of disease, and that the inclusion of patients with different endotypes and phenotypes under this umbrella term may not facilitate treatment decisions (10). Although the conceptual interest about ACOS is totally understandable it is now recognized that another syndrome is not needed in the already complex matrix of airways disease and that its use in clinical practice is likely to require a treatable-traits approach (10). Current GINA consensus document also discourages the use of previously described term ACOS given the propensity to consider this entity as a single disease, emphasizing the heterogeneity of patients with ACO (3).

One clear and paradigmatic example of how ACO cannot be considered one single disease is the vast number of published definitions. In the last recent years, we can find several examples of an "Oslerian approach" in "consensus" or "guidelines" to define ACO. In **table II** are detailed the several proposed criteria for asthma-COPD overlap.

Apart from the above cited definitions several other definitions have been used for clinical research purpose and this is by sure one of the main limitations about ACO clinical research (16-22). In 2018, using a Delphi structured survey of Portuguese specialists in respiratory diseases, it was published other ACO diagnostic criteria. This group of physicians established a consensus for these criteria: a fixed airflow obstruction (FEV1/FVC<0.7) associated with 2 major criteria (previous history of asthma; presence of a previous history of smoking exposure and/or exposure to biomass combustion; positive bronchodilation test (increase in FEV_1 of at least 200mL and 12%) on more than 1 occasion) plus 1 minor criteria (history of atopy; age ≥40 years; peripheral eosinophilia (>300 eosinophils/μL or >5% of leukocytes); elevation of specific IgEs or positive skin tests for common allergens (23). The proposed criteria, as compared to the above definitions, are quite similar to what had been proposed by guidelines/consensus (11). This study, as others published using only expert opinion clearly use an "Oslearian approach" but have the interest of setting the stage about the national general agreement about ACO definition adapted to our local context.

Table II *- Proposed criteria for asthma-COPD overlap (11).*

Consensus	Diagnostic Criteria for ACO	Ref.
GesEPOC-GEMA (2017)	Age≥35 years old Tobacco exposure ≥10 pack-years Post BDT FEV1/FVC < 0.70 Diagnosis of asthma In absence of asthma diagnosis: very positive BDT (≥400 mL and 15%) and/or blood eosinophilia≥300 cells/mL	(12)
Sin et al. (2016)	ACO is confirmed by the presence of three major and at least one minor criteria **Major criteria** Post BDT FEV1/FVC <0.70 in individuals>40 years of age At least 10 pack-years of tobacco smoking Documented history of asthma before 40 years of age BDT of >400 mL in FEV1 **Minor criteria** Documented history of atopy or allergic rhinitis BDR of FEV1≥200 mL and 12% from baseline values on 2 or more visits Peripheral blood eosinophil count of ≥300 cells/lug	(13)
Finnish guidelines (2015)	ACO is confirmed by presenting two main criteria or one main criteria and two additional criteria: **Main criteria** Very positive BDT FEV1 >15 % and >400 mL Sputum eosinophilia or elevated FENO (>50 ppb) Previous asthma symptoms (starting age at < 40 y) **Additional criteria** Elevated total IgE Atopy Repeated significant positive BDT (FEV1 >12% and >200ml) Peak expiratory flow follow-up typical of asthma	(14)
Czech guidelines (2013)	ACO is confirmed by the presence of two major criteria or one major plus two minor criteria: Definitive diagnosis of COPD **Major criteria** Very positive BDT FEV1 >15% and >400 mL FENO≥45–50 ppb and/or sputum eosinophils≥3% history of asthma **Minor criteria** Positive BDT (FEV1 >12% and >200 mL) Elevated total IgE History of atopy	(15)

ACO: asthma-COPD overlap; GesEPOC-GEMA: Spanish COPD guidelines-Spanish Asthma Guidelines; BDT: Bronchodilator test; Ref.: reference.

The clinical research (including also clinical trials) about asthma and COPD always used useful approaches to define both conditions in order to exclude patients that did not fulfil the "pure form" of both diseases (24). Though is an easy going approach it limits available evidence and does not fully depict the spectrum of obstructive airway disease that is seen in clinical practice (24). If in the recent years several studies have tried to answer this knowledge gap, the methodologic issue associated with ACO definition may prejudice achieved conclusions.

Treatable traits among asthma-COPD overlap

Currently available data about asthma-COPD overlap has increased in the last years and several conclusions can be discussed. From our conceptual point of view and assuming that the "treatable-traits approach" of diseases is the update method to define patient characteristics we will now discuss the treatable traits identified among ACO patients. In this paper we review the available data published until 2018 about treatable traits identified in patients with ACO. We performed a PubMed search of all the papers published from 2010 to 2018 that included simultaneously all the search terms "COPD", "Asthma" and "Overlap". A total of 436 papers were selected for a first analysis. All the guidelines, consensus, reviews and editorials were excluded from our review. After exclusion of the previous mentioned papers a total of 304 papers were included in our review and we here discuss the papers that describe treatable traits in ACO.
As previously described by Agusti et al. treatable traits of chronic airway diseases can be divided in pulmonary, extrapulmonary and behaviour/lifestyle risk factors treatable traits (2).

Pulmonary treatable traits in ACO

In **table III** are listed the pulmonary treatable traits described in patients with asthma-COPD overlap.
Revising the pulmonary treatable traits described in patients with ACO we can clearly notice that airflow limitation, airway smooth muscle contraction and eosinophilic airway inflammation are constantly found in the literature. This makes perfect sense and is expectable as nowadays available ACO definitions continually include them as diagnostic criteria (12-15). This evidence underlines the interest about bronchodilators treatment in patients with ACO as recommended by nowadays documents of GINA and GOLD (3,4).
Evidence about eosinophilic airway inflammation in ACO patients has emerged from several studies, not only studies considering it a diagnostic criterion but also an evaluated outcome. Evidence about eosinophilic airway inflammation has emerged using different methodologies: eosinophils in induced sputum but also with eosinophils in peripheral blood and fractional exhaled nitric oxide (FeNO) as surrogate markers (19,20,29,31,41, 42,44,47,48,58,61-64,67,71,73,74,77-79,85-87,90). The presence of eosinophilic airways inflammation in patients with COPD/ACO is a nowaday matter of debate and may have a therapeutic consequence with the recommendation for the need of inhaled corticosteroids treatment (3,4). The interest about FeNO evaluation in ACO patient's management is also a trending topic and more studies are needed. A study from Chen et al. in 2016 found a FeNO optimal diagnostic cut-off of 22.5 ppb in differentiating patients with ACO from COPD patients, with 70% sensitivity and 75% specificity (38). More recently

Table III *- Pulmonary treatable traits described in asthma-COPD overlap.*

	References
Airflow limitation	(16-20, 25-88)
Airway smooth muscle contraction	(16-20, 26, 27, 29-32, 36, 41-45, 47, 50-58, 61-64, 66, 67, 69, 70, 73-79, 81, 85-90)
Hyperinflation	(44, 47, 57)
Emphysema	(16, 19, 20, 30, 40, 41, 53, 54, 67, 71, 73, 77, 83, 88, 91)
Eosinophilic airway inflammation	(19, 20, 29, 31, 41, 42, 44, 47, 48, 58, 61-64, 67, 71, 73, 74, 77-79, 85-87, 90)
Severe and frequent exacerbations	(16-20, 30, 36, 40, 49, 50, 52, 57, 63, 64, 69, 75, 76, 78, 82-84, 92-100)
Chronic bronchitis	(19, 20, 41, 57, 65, 75-77, 80, 83, 88, 91, 101)
Bronchiectasias	(81, 88)
Chronic respiratory failure	(60)
Pulmonary embolism	(102)
Pulmonary hypertension	(75)
Pneumonia	(99)
Incident tuberculosis	(103)

in 2018 Takayama et al. described that a composite cut-off of FeNO ≥ 25 ppb combined with blood eosinophils counts≥250 cels./μL showed a 96.1% specificity for differentiating ACO from COPD.

Another interesting point of debate about ACO patients is their rate and severity of exacerbations. Although evidence is not consistent in all the published papers there is clearly a trend for considering that ACO patients have not only more frequent exacerbations, as more severe, with increased mortality and associated costs (19,20,29,31,41,42,44,47,48,58,61-64,67,71,73,74,77-79,85-87,90). The study from Gerhardsson de Verdier et al. showed that patients with asthma and COPD had nearly double health care costs compared to patients with asthma without COPD, and this large difference was mainly driven by the rates of hospitalizations and emergency department visits (95). A fascinating study from Lange et al. published in 2016 concludes that regarding long-term prognosis of individuals with ACO, we can even identify a more susceptible and severe subgroup with late-onset asthma with an extraordinarily poor prognosis according to FEV_1 decline, exacerbations, pneumonias, and survival (49). All this information together highlights that ACO patients should be carefully managed as an inappropriate management will have a high individual burden but also for the healthcare system.

Emphysema is another pulmonary treatable trait found in patients with ACO but with a frequency apparently lower than in patients with COPD (16,19,20,30,40,41,53,54,67,71, 73,77,83,88,91). Two different papers published by Yeh et al. in 2016 analyzing the National Health Insurance Research Database of Taiwan found attention-grabbing associations of ACO with pulmonary embolism and incident tuberculosis (102,103). The association of ACO with increased risk of pulmonary embolism was independent of age, sex, comorbidities and corticosteroids use. The proposed explanation for this risk is that the eosinophilic and neutrophilic inflammation of the airways with pulmonary artery inflammation might be the predisposing factors of pulmonary embolism (102). ACO was also found to be associated with incident tuberculosis in another paper published by Yeh analyzing the same database. The authors found an adjusted hazard ratio for tuberculosis of 2.41 (95% confidence interval: 2.19–2.66) in the ACOS cohort and the tuberculosis risk was significantly higher in the ACOS cohort than in the non-ACOS cohort when stratified by age, sex, comorbidities, and atopy. The authors proposed as probable explanations for this association the possible high doses of corticosteroid and likely frequent intensive care unit hospitalizations due to recurrent and severe exacerbations of ACOS (103).

There has been extensive investigation about the possibility of a biomarker or composite biomarkers to diagnose ACO, apart from what has been discussed for FeNO and blood eosinophils. In 2016, Gao et al. described sputum neutrophil gelatinase-associated lipocalin (NGAL) levels as potentially differentiators of ACO from asthma and COPD, as ACO patients had increased values of this biomarker compared to the other groups of patients. Although NGAL is also a COPD-related biomarker, NGAL is not only attributed to activated neutrophils but could also be secreted by the respiratory epithelial cells in response to inflammatory stimuli and by myeloid and epithelial cells in response to toll-like receptor activation during bacterial infections. Therefore, the high sputum NGAL levels found in ACO might be related to airway inflammation and low-grade microbial colonization, which predispose these patients to acute viral infections and exacerbations (42). Although this result seems promising induced sputum unfortunately is not settled as desired in routine clinical practice and this is a challenge to the implementation of this biomarker and others using this technique. More recently, in 2018, Wang et al. described that plasma YKL-40 also referred to as chitinase-3-like-1 protein (CHI3L1) is a promising candidate for distinguishing between patients with features of ACO and COPD patients, while plasma NGAL may be a valuable biomarker for differentiating between patients with features of ACO and asthma patients (87).

Extra-pulmonary treatable traits in ACO

In **table IV** are listed the extra-pulmonary treatable traits described in patients with asthma-COPD overlap.

The association of ACO with allergic rhinoconjunctivitis (16,30,48,58,70,71,73,80,88,103), atopic dermatitis (57,70,88,103) and atopy (20,27,31,33,41,42,44,48,52,57, 58,62,63,66,71,73,74,76-79,81,85,87,90,96) is also intuitive and in some papers were considered diagnostic criteria for ACO. There is some heterogenous data about the frequency of these treatable traits in ACO patients in comparison with asthma patients, some pointing out less frequency and others similar results. More consistent is the evidence that these treatable traits occur with superior frequency compared to patients with "pure" COPD and are even a factor to consider when comparing both diseases. Considering the other extra-pulmonary treatable traits associated with ACO and their relative frequency compared to "pure" asthma and COPD patient's data is somewhat discordant. The rates of obesity (25,39,57, 59,65,73,78,80,81,84,96,97,104), diabetes (22,25,39,40,57-59,70,73,76,77,86,94,95,98,99,102,103), dyslipidemia (40,59, 66,70,73,95,102,103), cataracts (99), gastroesophageal reflux disease (39,58,59,66,71,95), cerebrovascular disease (22,25,91,98-100,103), osteoarthritis (20,22,59), osteoporosis/fractures (20,58,59,66,68,71,85,94,102), depression (19,20,40,57,91,94,106), anxiety (19,20,40,57,99,100), autoimmune diseases (20) and malignancies (35,98) are in general superior to "pure" asthma and COPD patients although some papers have conflicting results. The noteworthy association of

Table IV *- Extra-Pulmonary treatable traits described in asthma-COPD overlap.*

	References
Allergic Rhinoconjunctivitis	(16, 30, 48, 58, 70, 71, 73, 80, 88, 103)
Atopic Dermatitis	(57, 70, 88, 103)
Atopy	(20, 27, 31, 33, 41, 42, 44, 48, 52, 57, 58, 62, 63, 66, 71, 73, 74, 76-79, 81, 85, 87, 90, 96)
Obstructive Sleep Apnoea Syndrome	(39, 40, 57, 77)
Obesity	(25, 39, 57, 59, 65, 73, 78, 80, 81, 84, 96, 97, 104)
Cardiovascular disease	(20, 22, 25, 28, 29, 39, 40, 57-59, 66, 70, 71, 73, 76, 77, 84, 86, 91, 94, 95, 98-100, 102, 103, 105)
Diabetes	(22, 25, 39, 40, 57-59, 70, 73, 76, 77, 86, 94, 95, 98, 99, 102, 103)
Dyslipidemia	(40, 59, 66, 70, 73, 95, 102, 103)
Cataracts	(99)
Gastroesophageal reflux disease	(39, 58, 59, 66, 71, 95)
Cerebrovascular disease	(22, 25, 91, 98-100, 103)
Osteoarthritis	(20, 22, 59)
Osteoporosis/Fractures	(20, 58, 59, 66, 68, 71, 85, 94, 102)
Depression	(19, 20, 40, 57, 91, 94, 106)
Anxiety	(19, 20, 40, 57, 99, 100)
Dementia	(100)
Autoimmune diseases	(20)
Malignancies	(35, 98)
Persistent systemic inflammation	(20, 29, 77)

ACO with malignancies has been shown not only for lung cancer but also to other malignancies (35,98). Cardiovascular disease is an exception to what was previously mentioned as in this case the frequency of disease is consistently superior in ACO patients compared to patients with only asthma or COPD. This evidence has been shown in several forms of cardiovascular disease, including hypertension, ischemic heart disease, angina, acute myocardial infarction and congestive heart failure (20,22,25,28,29,39,40,57-59,66,70,71,73,76,77,84,86,91,94, 95,98-100,102,103,105). The pathophysiology of this association may be related to the persistent systemic inflammation found in patients with ACO (20,29,77). In the paper of Fu et al. published in 2014 it was suggested that systemic inflammation is commonly present in ACO, and ACO resembled COPD in terms of systemic inflammation. The evidence about systemic inflammation in COPD is for long known and matter of debate sincere early 2000s (107,108). In the same study it was shown that IL-6 is a pivotal inflammatory mediator that may be involved in airflow obstruction and cardiovascular disease and may be an independent treatment target for ACO. The systemic inflammation that occurs in ACO has also been proposed as a possible mechanism involved in osteoporosis associated with ACO. In the study of Oh et al. patients with ACO had a significantly lower bone mineral density than did those with asthma, after adjusting for age, sex, body mass index, smoking and corticosteroid use (85).

Behaviour/lifestyle risk factors treatable traits in ACO

In **table V** are listed the behavior/lifestyle risk factors treatable traits described in patients with asthma-COPD overlap.
The role of smoking exposure in ACO´s pathophysiology is clear and is emphasized in several publications about ACO. Although the data is somewhat contradictory if patients with ACO have different smoking exposure than patients with "pure" COPD, compared with patients with only asthma it is convincing that ACO patients have an increased smoking exposure (16,17,19,20,25-27,30,31,33,35,38-41,44,45,47,48,54-58,60,62,63,65-67,69,71-78, 80,81,84,86-89,93,96,97,101,104,109,110). This evidence is so clear that smoking exposure is included as a diagnostic criteria for ACO in some of the proposed guidelines (12, 13). From a clinical and management perspective this associa-

Table V *- Behaviour/lifestyle risk factors treatable traits described in asthma-COPD overlap.*

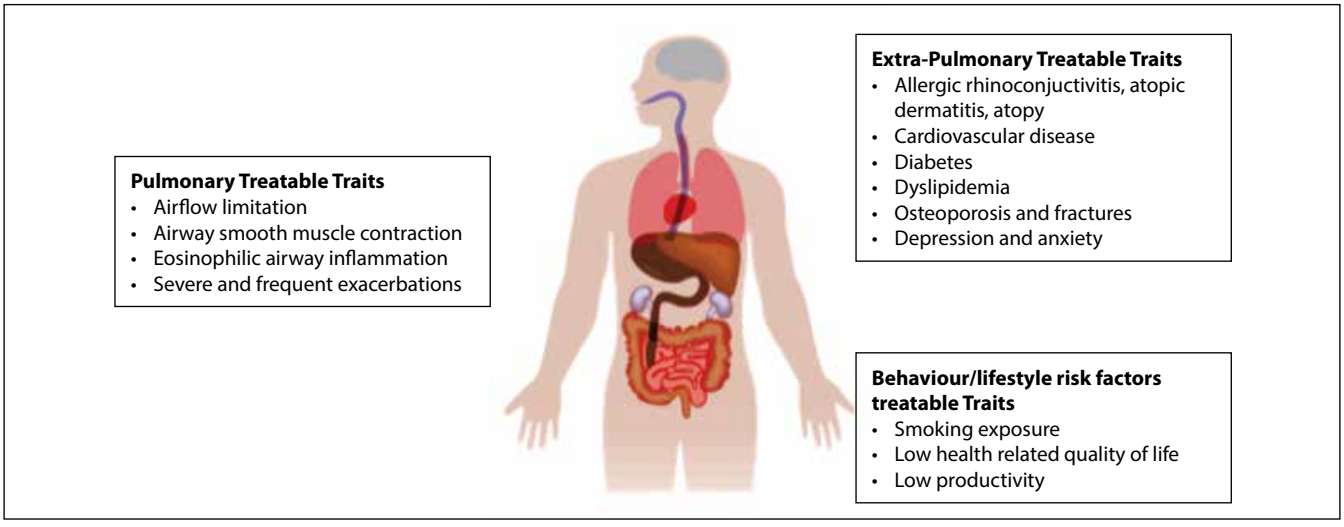
	References
Smoking exposure	(16, 17, 19, 20, 25-27, 30, 31, 33, 35, 38-41, 44, 45, 47, 48, 54-58, 60, 62, 63, 65-67, 69, 71-78, 80, 81, 84, 86-89, 93, 96, 97, 101, 104, 109, 110)
Exposure to pollution	(98)
Childhood respiratory infections	(33)
Familiar history of asthma	(30, 77, 80, 88)
Low health related quality of life	(17, 18, 25, 30, 37, 52, 82, 83)
Sedentarism	(17, 80, 97)
Low education level	(22, 78, 82, 97, 101, 104, 110)
Low productivity	(83)
Low household income / unemployment	(65, 104, 110)

tion should highlight the need for effective smoking cessation strategies in patients with ACO, as a truly disease modifying approach. Nonetheless other exposures have been associated with ACO and constitute identified treatable traits, including exposure to pollution (98) and childhood respiratory infections (33). The familiar history of asthma is an identified treatable trait (30,77,80,88) although the potential to be "treated" nowadays is still somewhat debatable.

Another remarkable discussion is the low health related quality of life found in patients with ACO, that has been described in general health related quality of life as well as in respiratory related quality of life (17,18,25,30,37,52,82,83). This evidence may be probably related to the high number and to the severity of the exacerbations but also to the several comorbidities described in patients with ACO.

At last but not least, there are some behavioral/lifestyle risk factors treatable traits that current evidence cannot clearly describe as cause or consequence of ACO although their association is clear. Sedentarism (17,80,97), low education level (22,78,82,97,101,104,110), low productivity (83), low household income and unemployment (65,104,110) are consistently described in ACO. From a commonsense perspective, we can speculate that the low education level can act as a risk factor for ACO development probably related to an unhealthy

Figure 1 *- Main treatable traits described in patients with asthma-COPD overlap.*



lifestyle, smoking and sedentarism. On the other hand, taking into consideration the high disease burden of ACO, it is perfectly conceivable that it will cause low productivity and consequently low household income and unemployment.
In **figure 1** is a summary of the main treatable traits described in patients with asthma-COPD overlap.

Conclusions

The recently proposed concept of "treatable traits" will definitely substitute our traditional "Oslerian approach" of diseases. The identification of "treatable traits" in each patient should be based on deep understanding of the critical causal pathways. There are several potential advantages of this approach, valuable for all levels of care and different areas of medicine. Chronic respiratory diseases management are always included among this approach, particularly the asthma-COPD overlap due to the lack of definitive diagnostic criteria in an "Oslerian approach". There are several pulmonary, extra-pulmonary and behavioral treatable traits associated with asthma-COPD overlap. The deep knowledge of these treatable traits will possible permit a better disease management in order to diminish the high disease burden described in patients with asthma-COPD overlap.

Conflict of interests

J. Gaspar-Marques declares to have received a research grant for chronic respiratory diasease in elders by Astrazeneca - Projeto OLDER (CEDOC/2015/59). The other authors declare they have no conflict of interests.

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Blood or skin: what is best in predicting cow's milk allergy diagnosis?

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Summary
Cut-off values for both skin prick tests (SPT) and specific IgE (sIgE) levels for predicting cow´s milk allergy (CMA) diagnosis are not universally defined. This study is a retrospective analysis of consecutive children (0-18 years-old) with suspected CMA tested with SPT and sIgE for cow's milk (CM) and its fractions between 2016-2017. CMA diagnosis was defined by a positive oral food challenge or a highly suggestive clinical history of CMA and SPT and/or sIgE positive to CM and/or its fractions. One hundred and five patients were included, 58% males with a median age of 2.5 (P25-P75:1-6) years and the diagnosis was confirmed in 83 patients (79%). The variables associated with CMA diagnosis were SPT with CM (p<0,05) and casein (p<0,05) and all sIgE to CM and its fractions (α-Lactalbumin, β-Lactoglobulin and casein; p<0,05 for all). Optimal cut-off points (Youden's index) for CMA diagnosis were 4.5mm for the mean wheal diameter to Cow's milk and 3mm to casein. For sIgE levels the optimal cut-off points were: were: CM 4.36 KUA/L , alfa-lactalmunin 1.6 KUA/L, b-lactoglobulin 1.7 KUA/L and casein 2.6KUA/L. The role of SPT and sIgE levels to cow's milk and its fractions is unequivocal in CMA follow-up. Moreover, sIgE levels seem to be more discriminatory than SPT.

Introduction

Cow's milk allergy (CMA) affects 1 to 3% of children and is one of the most common food allergies in pediatric age (1,2). CMA is classified according to the immunological reaction to milk proteins as IgE mediated or non-IgE-mediated, although a combination of both reactions may occur (1,3). The most common reactions are IgE-mediated, which are immediate, appearing within minutes to up to two hours after ingestion of cow's milk, and may affect one or more organs including systemic reactions as anaphylaxis (1,3). The delayed reactions are typically non-IgE mediated, occurring several hours after cow's milk ingestion and affect mainly the gastrointestinal system (3-5). Cow's milk contains several potentially sensitizing proteins, which are found in the whey and casein fractions, including α-lactoalbumin, β-lactoglobulin and casein allergens (5). When a patient is diagnosed with CMA, dairy exclusion with replacement with dietary alternatives is indicated, for maintaining adequate nutrition, growth and development. Strict avoidance of cow's milk has a negative influence on the quality of life of these patients and families (6-8). The diagnosis of IgE-mediated CMA is based on a detailed clinical history, sIgE levels and/or SPT to whole milk and the main sensitizing proteins, α-lactalbumin, β-lactoglobulin and casein, followed by oral food challenge (OFC), which is the diagnostic gold standard. In the literature there are several studies that try to estimate the accuracy of SPT and sIgE levels for predicting the result of the OFC, in order to decrease the risk of a positive OFC (9,10). At this moment, there are no universally defined cut-off values, due to a lack of reproducibility in different populations (9,11-15).
The aim of this study was to estimate the accuracy of sIgE levels and SPT to cow's milk and its fractions in CMA diagnosis.

Materials and methods

Study population and design

Retrospective analysis of the clinical records of children (0-18 years-old) with suspected CMA, followed at the Immunoallergy Department, who were tested to cow's milk proteins (whole milk protein, α-lactoalbumin, β-lactoglobulin and casein) between 2016-2017. In this analysis, we included consecutive patients with SPT and sIgEs performed within 6 months previous to the OFC. Patients that did not perform OFC but had a highly suggestive clinical history of CMA (more than one allergic episode upon cow's milk protein ingestion in the previous 6 months with at least one positive SPT and/or positive sIgE) were also included. Patients with non-IgE mediated CMA were excluded. Demographic data, clinical manifestations and diagnostic procedures were evaluated. The diagnosis of CMA was considered when OFC was positive or there was a suggestive clinical history with the criteria defined above.

Skin prick tests and specific IgE assessment

Skin prick tests were performed in all patients with whole cow's milk extract (5 mg/mL), α-lactoalbumin (5 mg/mL,) β-lactoglobulin (1 mg/mL), and casein (10 mg/mL), from Bial Aristegui®. Histamine (10 mg/mL, ALK-Abelló®) was used as a positive control and glycerosaline was used as a negative control. SPT were evaluated 15 minutes after testing. The appearance of a wheal with a mean diameter ≥ 3 mm was considered positive (16). The levels of specific IgE antibodies to whole cow's milk, α-lactoalbumin, β-lactoglobulin and casein were determined using the ImmunoCAP® method (Thermo Fisher Scientific®, Uppsala, Sweden). A result of ≥ 0.35 kUA/L was considered positive (16).

Oral food challenges

Oral food challenges (OFC) were performed in the Allergy Unit of the Hospital with increasing doses of cow's milk, given at regular intervals according to the standard protocol of the Unit (236 mL of milk divided in 7 incremental doses every 15-30 minutes). All the OFC were performed with an open protocol just as routine clinical practice. Informed consent was previously obtained from the parents. All patients remained for, at least, 2 hours under observation after the last milk dose intake, before being discharged. If a clinical reaction appeared, the challenge was discontinued and treatment was provided and the test was considered positive.

Statistical analysis

Statistical analyses were conducted using IBM SPSS for Mac version 20.0 (SPSS, Chicago, IL, USA) and MedCalc 14.10.2 (MedCalc Software bvba, Ostend, Belgium). Continuous variables were expressed as mean ± standard deviation, median (25th to 75th percentiles) and categorical variables were expressed as counts (percentages). The relationship between sensitivity and specificity and the optimal decision points for sIgE and SPTs were determined by analysis with the receiver-operating characteristic (ROC) curve. Correlation between the SPT wheal diameters and sIgE levels was evaluated with Pearson's correlation coefficient. The Yates and Fisher chi-squared test was used for comparison between groups. The Mann-Whitney non-parametric test was used to compare continuous variables of the two groups. The relationship between SPT or sIgE and food challenge outcome was analysed using logistic regression. Youden's index (17) was used to calculate the optimal cut-offs for the considered variables associated with CMA diagnosis. An alternative cut-off was also calculated for maximization of the specificity (specificity=100%). The level of significance $\alpha = 0.05$ was considered.

Results

In this analysis, we included 105 patients, 61 (58%) males. The median age of the children evaluated was 2.5 years (P25-P75: 1-6 years). The group with confirmed allergy consisted of 83 patients (79%) and the control group (allergy excluded) of 22 (21%). In the group of patients with confirmed allergy, 37 (45%) were included for having had positive oral food challenge and the remaining (46, 55%) due to a strongly suggestive clinical history of CMA. Among the patients with confirmed allergy, 57% had a previous history of anaphylaxis, 87% had cutaneous manifestations (hyperemia, urticaria and angioedema), 54% gastrointestinal (vomiting, diarrhea) and 13% respiratory symptoms (rhinorrhea, sneezing, laryngeal stridor, hoarseness, coughing, dyspnea).

The main characteristics of the patients with confirmed allergy and the patients with excluded allergy regarding age, gender, SPT and sIgE are shown in **table I**.

The associations between the evaluated variables and the CMA diagnosis are shown in **table II**.

The associations between the SPTs and the sIgE to milk and milk fractions were: cow's milk rho=0.234 (p=0.023); α-lactalbumin rho=0.372 (p<0.0001); β-lactoglobulin rho=0.349 (p=0.001); Casein rho=0.489 (p<0.0001).

The ROC curves constructed from the ratio between sensitivity and specificity of SPTs and sIgE levels are shown in **figure 1**.

Using Youden's index, only taking into consideration the variables with a statistically significant association to CMA diagnosis, the optimal cut-off points, description of the sensitivity (S), specificity (Sp), positive predictive value (PPV) and negative predictive values (NPV) for mean wheal diameters in SPTs and sIgE levels are presented in **table III**.

Table I *- Main characteristics of the patients with excluded and confirmed cow's milk allergy.*

	Excluded allergy (n=22)	**Confirmed allergy (n=83)**	**p-value**
Male gender (%)	54.50	59.00	0.809
Age (Median; P25-P75)	2.00 (0.90-3.00)	3.00 (0.92-3.00)	0.126
Skin prick tests (mean wheal diameter in millimetres); median (P25:P75)			
Cow's milk extract	4.00 (0.00-6.00)	7.00 (5.00-10.00)	0.013
α-Lactalbumin	9.50 (6.00-11.00)	8.80 (6.50-12.10)	0.537
β-Lactoglobulin	6.00 (0.00-7.50)	7.00 (4.50-10.00)	0.136
Casein	3.00 (0.00-6.00)	7.30 (5.00-10.30)	0.001
Cow's milk extract	0.66 (0.28-3.24)	11.40 (2.90-25.60)	<0.0001
α-Lactalbumin	0.56 (0.16-1.31)	1.86 (0.69-11.80)	0.002
β-Lactoglobulin	0.23 (0.06-1.06)	1.89 (0.43-9.02)	<0.0001
Casein	0.30 (0.03-1.34)	6.01 (0.99-14.00)	<0.0001

Specific IgE levels (kUA/L); median (P25:P75).

Table II *- Associations between the evaluated variables and cow's milk allergy diagnosis.*

	Odds-ratio (CI 95%)	**p-value**
Male gender	1.201 (0.466-3.094)	0.704
Age	1.127 (0.974-1.304)	0.114
Skin prick tests		
Cow's milk	1.242 (1.041-1.481)	0.017
α-Lactalbumin	1.034 (0.935-1.144)	0.513
β-Lactoglobulin	1.133 (0.979-1.311)	0.090
Casein	1.280 (1.085-1.510)	0.003
Specific IgE levels		
Cow's milk	1.393 (1.137-1.707)	<0.001
α-Lactalbumin	1.264 (0.995-1.606)	0.049
β-Lactoglobulin	2.142 (1.176-3.898)	0.012
Casein	1.712 (1.166-2.514)	0.006

CI 95%: Confidence-interval.

Table III *- Sensitivity (S), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) for mean wheal diameters in SPT and sIgE levels.*

SPT cut-offs by Youden's index	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
Cow's milk extract (>4.50 mm)	77%	60%	93%	38%
Casein (>3.00mm)	92%	60%	31%	13%
sIgE cut-offs by Youden's index				
Cow's milk extract (>4.36 kUA/L)	71%	95%	100%	30%
α-Lactalbumin (>1.60 kUA/L)	55%	86%	100%	52%
β-Lactoglobulin (>1.70 kUA/L)	58%	95%	100%	44%
Casein (>2.60 kUA/L)	64%	95%	100%	37%

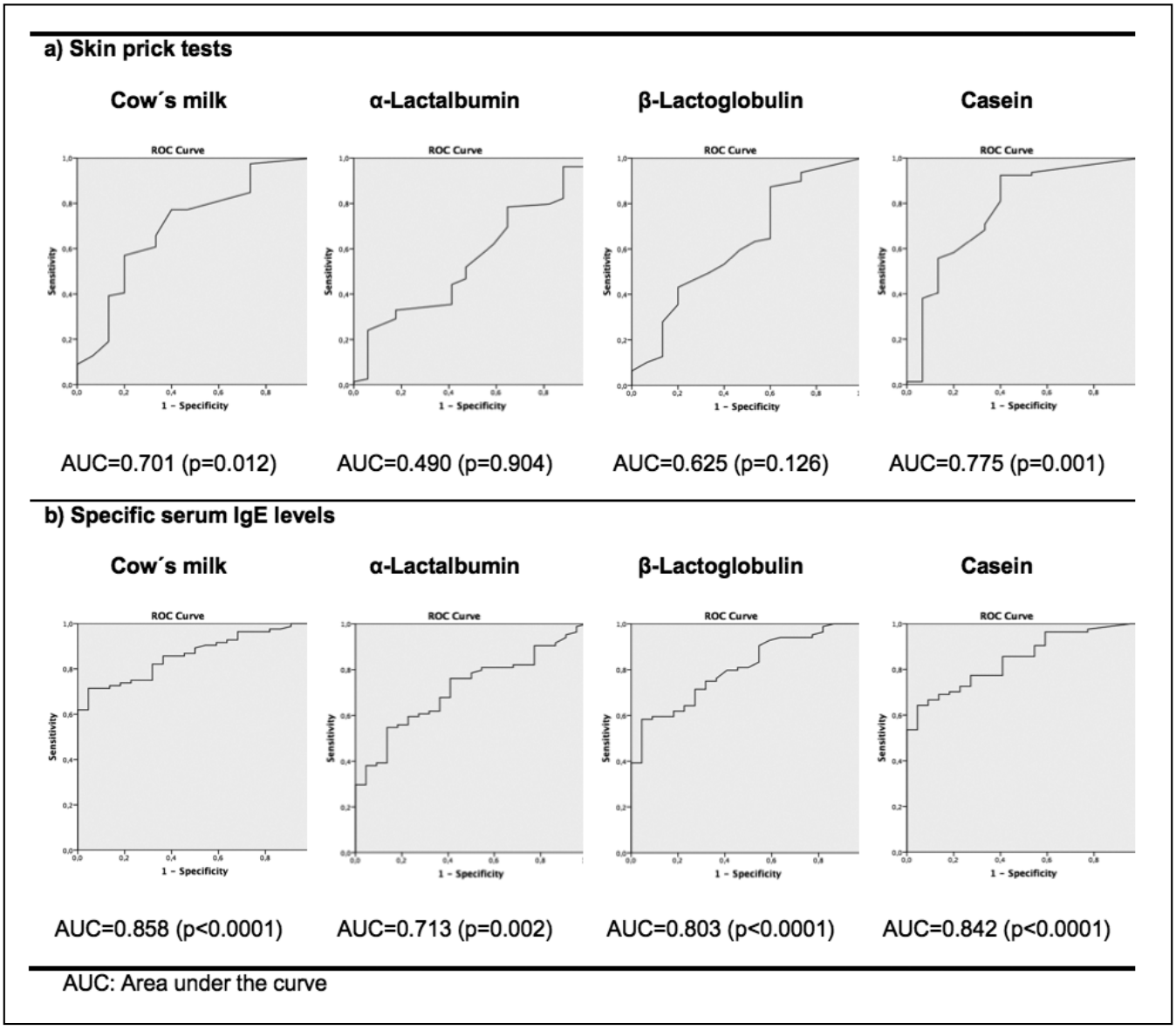
sIgE specific IgE; SPT: skin prick tests.

Using alternative cut-offs for maximization of the specificity, only taking into consideration the variables with a statistically significant association to CMA diagnosis, the optimal cut-off points for the mean wheal diameter to cow's milk was 12.5 mm and casein 20mm. For sIgE, also using maximum specificity criteria, the optimal cut-off points for cow's milk extract was of 8.2 kUA/L, α-Lactalbumin of 8.6 kUA/L, β-Lactoglobulin of 3.1 kUA/L and casein of 4.2 kUA/L.

Discussion

We investigated the accuracy of sIgE levels and SPT mean wheal diameters in the management of children with CMA diagnosis. In our sample, children's age distribution was similar to the published data about CMA, suggesting that most of patients achieve tolerance within 3 to 4 years (5). Age was not associated with CMA diagnosis and this may be influenced by the fact that

Figure 1 *- ROC curves to obtain optimum levels of mean wheal diameter of SPT (a) and sIgE levels (b).*



patients followed in our Department are high-risk patients with decreased chances of spontaneous CMA outgrow.

From the evaluated variables analysed, the ones that had a statistically significant association with CMA diagnosis were the SPT with cow's milk and casein and all the sIgE to cow's milk and its fractions. In the evaluated patients, and consistently with available literature, allergic patients had higher levels of sIgE to cow's milk and its fractions although the differences where less notorious on SPT (10).

Comparing the optimal cut-offs for sIgE found in our study with available literature they were similar when using Youden's index criteria (Franco et al. (10); milk: 5.17 kUA/L; α-Lactalbumin: 0.95 kUA/L; β-Lactoglobulin: 0.82 kUA/L; casein: 0.72 kUA/L) but inferior when considering the maximum specificity (Franco et al. (10); milk: 77.7 kUA/L; α-Lactalbumin: 20.7 kUA/L; β-Lactoglobulin: 50.8 kUA/L; casein: 15.9 kUA/L). Recent systematic reviews have reported the heterogeneity in these cut-offs (9) and our study adds more data to

clarify his question. A previous study conducted in our Hospital (18) also found different cut-offs for sIgE levels to cow's milk extract using Youden's index (sIgE milk cut-off: 2.15 kUA/L) and maximum specificity criteria (sIgE milk cut-off: 25 kUA/L) but a different methodology was used in patient inclusion as only were included patients that performed OFC (18).

The optimal cut-offs for SPT found in our study compared with available literature were similar when using Youden's index criteria (Franco et al. (10); milk: 3.5 mm; casein: 3.0 mm) but superior when considering the maximum specificity (Franco et al. (10); milk: 5.0 mm; casein: 10.0 mm).

Our study has some limitations, as not all patients performed OFC to confirm CMA. Nevertheless, with the methodology used, this analysis reflects routine clinical practice, with OFC protocols and postponement of OFC when clinical history is highly suggestive of active CMA. Another potentially limitation is that the accuracy found for SPT and sIgE levels may only apply to patients in a tertiary allergy unit and with a higher risk of having severe manifestations. We may speculate that these results may not be applicable to other clinical settings. Nevertheless, it was used a large sample of consecutive patients with CMA suspicion with different ages what strengthens our conclusions.

From a clinical practice perspective, we must highlight that the definition of optimal cut-offs for sIgE and SPT to cow's milk and correspondent fractions is extremely important. This may avoid stressful and hard to implement cow's milk eviction and, on the other hand, may avoid unnecessary and potentially dangerous oral food challenges (3).

Conclusions

The role of SPT and sIgE to cow's milk and its fractions is unequivocal in CMA follow-up. Moreover, sIgE levels seem to be more discriminatory than wheal diameters of SPT in CMA confirmation. Optimal cut-offs for confirmed CMA are still not universally defined and our study adds data to clarify this question.

Conflict of interests

The authors declare that they have no conflict of interests.

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Healthcare costs associated with allergic rhinitis, asthma and allergy immunotherapy

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Key words

Allergic rhinitis; allergy treatment; asthma treatment; cost analysis; immunotherapy.

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Summary

Allergic rhinitis (AR) and asthma are chronic diseases in which the airways become inflamed in response to allergens. Allergy immunotherapy (AIT) is recommended for those unable to manage symptoms using pharmacotherapy. This study estimated healthcare costs and utilisation for patients with AR and asthma. Mean annual outpatient visits, pharmaceutical costs and inpatient hospitalisations were calculated for 2010 and 2014, with pharmaceutical and inpatient costs stratified by AIT use. AR and asthma patients had a 35% higher mean number of physician visits and up to 90% higher mean pharmaceutical costs compared to controls. The cost of pharmaceuticals and inpatient hospitalisations were 54% lower in those prescribed AIT. Further research is recommended to understand the reasons for these cost differences.

List of abbreviations

ADHD – Attention deficit hyperactivity disorder.
AIT - Allergy immunotherapy.
AR – Allergic rhinitis.
ENT – Ear nose and throat.
ICD - International Classification of Diseases.
NPP – Named patient product.
OTC – Over the counter.
TAO - Therapy Allergen Ordinance.

Introduction

Allergic rhinitis (AR) affects 17 to 26% of the population in Europe, with prevalence expected to rise (1). Although symptoms such as blocked and itchy nasal passages, frequent sneezing, and inflamed eyes, are sometimes seen as trivial, evidence indicates that they have a detrimental impact on quality of life and work productivity (2,3). Moreover, accumulating evidence indicates that asthma, with more 'serious' symptoms including restricted airways (4), can develop as a consequence of AR. As one of the strongest independent risk factors for asthma development (5), evidence has shown that AR increases the risk of adult-onset asthma 3-fold (6). Researchers have hypothesised that AR and asthma are different expressions of the same disease, with chronic inflammation of a 'united airway', occurring in the upper airway in AR, and in the lower airway in asthma (7). Indeed, more than 80% of asthma patients have comorbid AR, while 20 to 60% of AR patients have comorbid asthma (5).

Both AR and asthma present cost burdens to the healthcare sectors, with costs for both usually incurred through a combination of pharmaceutical treatments and outpatient visits to assess disease management. In addition, asthma may also require inpatient hospitalisation for serious asthma exacerbations. Recommended first-line treatments such as anti-histamines and nasal steroids in AR, and inhaled steroids in asthma, aim only to alleviate the symptoms of the diseases, rather than treating the underlying immune response. Depending on the pattern of exposure and allergen sensitivity, an individual may require daily

treatment during particular seasons, or all year round, and once developed remission is rare, meaning both diseases commonly require lifelong treatment (4,8).

Over 90% of individuals with seasonal AR use symptom-relieving pharmacotherapy, with the majority using more than one form of medication, most commonly to evoke a more effective nasal response (9). Even so, dissatisfaction with symptom management is commonly reported (9), and evidence has shown that the resource use and costs associated with treating those sensitive to perennial allergens is higher than for seasonal AR, due to increased requirements for pharmaceuticals (10,11).

Despite the relatively low costs of the available symptom-relieving pharmacotherapy, the high prevalence and chronic nature of AR corresponds to a high economic burden of disease at the population level. For example, in Europe, a Swedish questionnaire study analysed the direct and indirect costs related to self-reported AR in a random population of residents between the ages of 18-65 (12). The study found that pharmacological treatment (most commonly oral antihistamines, nasal steroids and nasal sprays), along with health care consultations for AR cost €210 per individual annually, while productivity loss due to absenteeism and presenteeism at work cost €751 per individual annually. In total, this gave an average cost per year for an individual with AR of €961. Costs also varied depending on the severity of AR, with the cost of those suffering from moderate to severe persistent AR being four times higher than those with mild persistent disease (€1757 per year and €464 per year respectively). Due to the prevalence of AR being reported at 24% in the survey, it was estimated that the total cost of AR in Sweden is €1.3 billion euros (12).

Further studies have also assessed costs of AR in the US, for instance, a 2001 review of cost-of-illness studies for AR showed direct costs (those associated directly with disease management) in the US estimated to be between $1.2 billion in a study conducted in 1990, to $4.5 billion in a 1997 study. Overall costs of treating allergic rhinitis in 2005 were estimated at $11.2 billion, nearly double the $6.1 billion spent in 2000 (in 2005 dollars); more than half was spent on prescription medications (13). On top of these costs, indirect costs associated in particular with lost productivity at work have been estimated to be between $86 million and $7.7 billion (14). Asthma is more expensive still, with a more recent study estimating a total annual cost of $82 billion in the USA in 2013, of which $50 billion were medical costs and a further $32 billion costs were due to productivity losses resulting from missed work and school days and asthma-related mortality (15). Per person annual medical costs were estimated at $3,266; 56% incurred for pharmaceuticals, 25% for hospitalisations and emergency room admissions, and 19% for outpatient assessments in primary and secondary care. Moreover, another study demonstrated how in asthmatic children who had been hospitalised, co-morbid AR increased the hazard of asthma-related readmissions by 1.72 times, and predicted significantly more days in hospital (16).

Allergy immunotherapy (AIT) is a treatment option that is recommended for patients with moderate-to-severe persistent AR who are unable to manage their disease using symptom-relieving pharmacotherapy (17). AIT can be administered both subcutaneously (via injection) as well as sublingually (in both tablet and drop formulations). Uniquely, AIT aims to desensitise the immune response to trigger allergens. Not only has evidence shown AIT to effectively reduce AR symptoms, but when administered in childhood, it can reduce the risk of experiencing asthma symptoms and using asthma medication 5 years after initiation of a 3-year AIT treatment programme (i.e. 2 years after treatment completion) (18). In addition to health benefits, treatment of the underlying disease is likely to reduce resource use, a perspective supported by a Cochrane systematic review published in 2010, which reported that subcutaneous AIT effectively reduced not only symptoms, but also medication use (19). One study demonstrated that among children, pharmacy, outpatient and inpatient costs and resource use were significantly reduced for AR patients compared with pre-AIT levels (20).

It is generally accepted worldwide that AIT can reduce the long term economic burden of allergic disease, particularly following the first six years following the start of treatment (21). However, certain regulatory issues have come into fruition with regards to immunotherapy in the last ten years (21). It is now necessary, as a medicinal product, for this kind of treatment to obtain a marketing authorization in Europe by proving its safety, efficacy and quality (22). This has led to withdrawals of many Named Patient Products (NPPs), including allergen immunotherapy. For instance, in Germany more than 6400 NPPs have been removed from the market due to these regulations (known as Therapy Allergen Ordinance or TAO) (23).

To illustrate the actual economic impact born by health insurance payers, this project aimed to show the resource use and costs associated with allergic rhinitis, asthma and immunotherapy treatment in adults and children, but without considering causality links such as comorbidities and socioeconomic status.

Methods and materials

Data source

An anonymised dataset of all German National Health Insurance beneficiaries insured by the AOK PLUS healthcare fund in Saxony between January 1st 2005 and December 31st 2014 was accessed at the Centre for Evidence-based Healthcare, TU Dresden. The database was used previously for several analyses in the field of allergy and other disease areas (24,25). This dataset included, for each patient: demographic characteristics, ICD-10 (International Classification of Diseases – 10) diagno-

ses, prescription data (ATC-code, volume, price, pack size and defined daily doses), outpatient physicians assessments and inpatient hospitalisation costs (diagnoses, DRG-codes and any other broad costs covered by the hospital budget). Data for inpatient hospitalisations were only available from 2008 onwards.

Population

The total cohort comprised all individuals insured consecutively with AOK PLUS Saxony between 2005 and 2014, or until death if death occurred within this time period. ICD-10 codes for AR (ICD-10 J30) and asthma (ICD-10 J45) were used to identify six subgroups:
1. prevalent AR;
2. prevalent asthma;
3. prevalent AR and asthma;
4. controls - no AR;
5. controls - no asthma;
6. controls - no AR or asthma.

Prevalent groups were defined, in accordance with good practice guidelines (26) as including all cases where ICD codes (J30 for AR; J45 for asthma) were recorded at least twice in four consecutive quarters between 2005 and 2006. For prevalent asthma, cases were only categorised as prevalent if they had also filled two prescriptions of inhaled corticosteroids in the same time period, alongside the ICD reference. Control cohorts were defined as all individuals from the overall cohort who were not diagnosed with the relevant ICD codes (and, for asthma, had not filled two prescriptions of inhaled corticosteroids) twice in any four consecutive quarters across the full observation period of 2005 to 2014.
AR and asthma cohorts are not mutually exclusive, thus individual patients may be included in more than one cohort.

Analysis methods

Analyses were completed using Stata V13.1. Sample characteristics were calculated for the total cohort and each subgroup. Frequency statistics for annual outpatient healthcare visits to all physicians, ear nose and throat (ENT) specialists, and pulmonologists were calculated (total and stratified by age group in 2005; <12 years, 12 to 17 years, 18 to 50 years, 50 years and over).
Mean (standard deviation) annual direct costs were calculated for pharmaceutical and inpatient hospitalisations (total and stratified by subgroup and age group). For each prevalent group, costs were further stratified into those patients who had filled at least one prescription for AIT, and those who had not filled any prescriptions for AIT. These statistics are reported for 2014, the most recent year available, as well as for 2010 to give an indication of the consistency of the findings.

Sensitivity analyses (see Supplementary Material) were performed where appropriate to exclude outlying data (top 1%) that may have skewed the pattern of results.

Results

Population

The total cohort included a total of 1,739,440 individuals (54% female), with a mean age of 49 years (SD= 24; see **table I** for full sample characteristics of the study population). The sample comprised approximately 7% children (younger than 12 years), 5% adolescents (12 to 17 years), 37% younger adults (18 to 50 years) and 52% older adults (older than 50 years). Approximately 4% of the overall cohort (n = 74,642) were defined as having prevalent AR, approximately 2% (n = 34,362) were defined as having prevalent asthma, and approximately 0.6% (n = 9,832) were defined as having co-morbid prevalent AR and asthma. Of the prevalent subgroups, 23% (n = 17,289) of those with prevalent AR, 10% (n = 3,460) of those with prevalent asthma, and 25% (n = 2,488) of those with prevalent AR + asthma had filled at least one prescription for AI.

Outpatient care utilisation

Across both years, and all age groups and physician types, patients in prevalent AR and/or asthma subgroups had, on average, 5.1 (35%) more outpatient physician contacts than their respective control subgroups. Physician contacts were an average of 19% higher in the prevalent subgroups with asthma (17% higher for prevalent AR + asthma; 22% higher for prevalent asthma) than in the prevalent AR subgroup. All Mean (SD) outpatient physician contacts in prevalent and control subgroups for the whole cohort and stratified by physician type and age group are presented in **table II**.

Healthcare costs

Comparing prevalent and control subgroups

Mean costs for pharmaceuticals and inpatient hospitalisations are presented for each subgroup in **table III**. Patients in the prevalent asthma subgroup incurred the highest healthcare costs, averaging around €2500 per year per patient. For the prevalent asthma subgroup, both pharmaceutical and inpatient costs were higher than for the no asthma control subgroup, with pharmaceutical costs 90% higher, and inpatient costs 38% higher. For the prevalent AR and prevalent AR + asthma subgroups, although costs of pharmaceuticals were generally higher than their respective control groups (by 3% in the prevalent AR group, and 75% in the AR + asthma group), inpatient

Table I *- Sample characteristics of the study population.*

	Overall cohort	Prevalent AR	Prevalent asthma	Prevalent asthma + AR	Control AR	Control asthma	Control asthma + AR
Total, *n*	1,739,440	74,642	34,362	9832	1,477,433	1,656,319	1,433,533
Male, *n* (%)	792,605 (45.6)	31,065 (41.6)	14,727 (42.9)	4178 (42.5)	689,804 (46.5)	758,329 (45.8)	671,603 (46.9)
Female, *n* (%)	946,835 (54.4)	43,577 (58.4)	19,635 (57.1)	5654 (57.5)	787,629 (53.3)	897,990 (54.2)	761,930 (53.2)
Age, Mean/(SD)	49.1 (23.2)	39.4 (21.6)	48.47 (24.0)	39,25 (22.1)	50.76 (23.0)	49.30 (23.2)	50.71 (23.0)
Age groups							
<12 years, *n* (% of all)	122,714 (7.1)	7,137 (9.6)	3,527 (10.3)	1274 (13.0)	94,547 (6.4)	112,638 (6.8)	90,963 (6.4)
12-17 years, *n* (% of all)	79,785 (4.6)	8,456 (11.3)	2,077 (6.0)	1059 (10.8)	57,105 (3.9)	75,008 (4.5)	55,681 (3.9)
18-50 years, *n* (% of all)	637,411 (36.6)	34,302 (46.0)	10,157 (29.6)	4024 (40.9)	517,910 (35.1)	609,391 (36.8)	506,367 (35.3)
>50 years, *n* (% of all)	899,530 (51.7)	24,747 (33.2)	18,601 (54.1)	3475 (35.3)	807,871 (54.7)	859,282 (51.9)	780,522 (54.5)
Total by year							
2005, *n*	1,739,440	74,642	34,362	9,832	1,477,433	1,659,535	1,433,533
2006, *n*	1,739,440	74,642	34,362	9,832	1,477,433	1,659,535	1,433,533
2007, *n*	1,705,296	74,237	33,615	9,771	1,443,705	1,626,161	1,400,282
2008, *n*	1,671,594	73,786	32,875	9,704	1,410,628	1,593,316	1,367,824
2009, *n*	1,637,546	73,303	32,145	9,651	1,377,686	1,560,284	1,367,824
2010, *n*	1,603,667	72,770	31,446	9,580	1,345,432	1,527,508	1,335,667
2011, *n*	1,570,536	72,200	30,783	9,512	1,314,349	1,495,573	1,304,289
2012, *n*	1,537,416	71,633	30,108	9,421	1,283,602	1,463,771	1,274,167
2013, *n*	1,503,573	71,015	29,344	9,337	1,252,650	1,431,452	1,244,274
2014, *n*	1,471,716	70,400	28,700	9,247	1,223,879	1,401,076	1,214,749

costs did not follow such a consistent pattern, with higher costs more commonly observed in control subgroups than in prevalent subgroups.

Comparing costs for those prescribed AIT and not prescribed AIT in prevalent subgroups

Collapsing across all subgroups, total costs were on average €753 (54%) lower for those prescribed AIT than those not prescribed AIT. **Figure 1** demonstrates that in the prevalent AR subgroup, costs of both pharmaceuticals and inpatient hospitalisations were lower for patients prescribed AIT than those not prescribed AIT (€153 (29%) lower for pharmaceuticals; €510 (48%) lower for inpatient costs). A similar pattern was observed for both the prevalent asthma subgroup (see **figure 2**) and the prevalent asthma + AR subgroup (see **figure 3**). The pattern of results remained the same when the cost of the AIT pharmaceuticals themselves was both included and excluded from the analysis.

For the prevalent asthma subgroup, the cost difference was highest. Pharmaceutical costs were an average of €303 (34%) lower, and costs of inpatient hospitalisations an average of €777 (115%) lower for patients prescribed AIT, than those not prescribed AIT (see **figure 2**).

Finally, for the prevalent asthma + AR subgroup, pharmaceuticals were an average of €239 (28%) lower, and costs of inpatient hospitalisations an average of €430 (65%) lower for patients prescribed AIT, than those not prescribed AIT (see **figure 3**).

Table II *- Mean (SD) outpatient physician contacts in prevalent and control subgroups in 2010 and 2014, for the whole cohort and stratified by physician type and age group.*

	Prevalent AR		Control AR		Prevalent asthma		Control asthma		Prevalent AR + asthma		Control AR + asthma	
	2010	**2014**	**2010**	**2014**	**2010**	**2014**	**2010**	**2014**	**2010**	**2014**	**2010**	**2014**
All physicians	18.0 (15.4)	18.7 (16.0)	16.6 (16.2)	17.3 (16.6)	22.2 (17.9)	22.7 (18.1)	16.4 (15.9)	17.2 (16.4)	21.1 (17.5)	21.7 (17.3)	13.3 (15.0)	12.9 (15.4)
<12 years	11.0 (8.3)	10.4 (9.1)	8.0 (6.8)	8.1 (7.9)	12.2 (8.5)	10.8 (9.1)	8.2 (6.9)	8.3 (8.0)	13.5 (9.2)	11.6 (9.8)	7.1 (6.5)	7.1 (7.5)
12-17 years	11.0 (9.5)	11.3 (11.0)	9.4 (9.6)	9.9 (10.6)	12.6 (10.7)	12.4 (11.8)	9.6 (9.5)	10.1 (10.6)	12.7 (9.8)	12.5 (11.2)	7.7 (8.4)	8.1 (9.4)
18-50 years	15.6 (13.8)	17.0 (14.4)	11.7 (13.1)	13.1 (14.1)	18.1 (16.4)	19.7 (16.7)	11.9 (13.1)	13.3 (14.0)	19.5 (16.2)	20.5 (15.8)	9.0 (11.3)	10.5 (12.6)
>50 years	25.6 (17.5)	26.7 (17.9)	21.4 (17.5)	22.8 (17.9)	27.9 (18.7)	29.2 (18.8)	21.4 (17.4)	22.7 (17.8)	29.7 (19.6)	30.3 (18.9)	17.2 (16.9)	15.4 (17.4)
ENT	3.4 (3.6)	2.9 (3.0)	2.3 (2.2)	2.1 (1.9)	3.0 (3.2)	2.7 (2.7)	2.4 (2.4)	2.3 (2.1)	3.5 (3.7)	3.1 (3.1)	2.2 (2.1)	2.1 (1.8)
<12 years	3.6 (3.9)	3.0 (3.3)	2.4 (2.2)	1.9 (1.7)	3.2 (3.6)	2.8 (3.0)	2.6 (2.5)	2.3 (2.4)	3.9 (4.5)	3.0 (3.3)	2.4 (2.2)	1.9 (1.7)
12-17 years	3.4 (3.9)	3.1 (3.5)	2.0 (2.0)	1.9 (1.8)	2.9 (3.2)	2.7 (3.0)	2.4 (2.7)	2.3 (2.6)	3.2 (3.4)	2.9 (3.1)	2.0 (2.0)	1.9 (1.8)
18-50 years	3.7 (4.1)	3.2 (3.4)	2.2 (2.3)	2.1 (2.1)	3.5 (3.9)	3.1 (3.5)	2.5 (2.8)	2.4 (2.5)	3.8 (4.1)	3.2 (3.4)	2.2 (2.2)	2.1 (2.0)
>50 years	3.1 (3.0)	2.7 (2.4)	2.3 (2.2)	2.1 (1.7)	2.8 (2.8)	2.5 (2.2)	2.3 (2.2)	2.2 (1.8)	3.2 (3.2)	3.0 (2.6)	2.2 (2.05)	2.1 (1.7)
Pulmonology	3.2 (2.7)	3.0 (2.4)	2.7 (2.8)	2.7 (2.7)	3.4 (2.5)	3.2 (2.3)	2.6 (2.9)	2.5 (2.8)	3.4 (2.59)	3.2 (2.4)	2.5 (3.0)	2.5 (2.8)
<12 years	3.1 (2.8)	2.5 (2.3)	1.9 (1.3)	1.9 (1.5)	2.7 (2.5)	2.4 (2.1)	2.3 (2.3)	1.9 (2.1)	3.2 (2.6)	2.2 (1.5)	1.7 (1.3)	1.6 (1.1)
12-17 years	3.0 (2.9)	2.6 (2.5)	2.1 (1.7)	2.2 (1.9)	2.9 (2.7)	2.8 (2.7)	2.1 (2.3)	1.97 (2.0)	3.2 (2.9)	2.8 (2.8)	1.8 (1.6)	1.6 (1.2)
18-50 years	3.3 (3.0)	3.1 (2.7)	2.5 (2.3)	2.6 (2.7)	3.4 (2.8)	3.3 (2.6)	2.4 (2.5)	2.4 (2.9)	3.4 (2.9)	3.2 (2.7)	2.2 (2.2)	2.3 (2.9)
>50 years	3.2 (2.4)	3.0 (2.1)	2.8 (2.9)	2.8 (2.7)	3.5 (2.3)	3.3 (2.1)	2.6 (3.1)	2.6 (2.8)	3.2 (2.4)	3.3 (2.2)	2.6 (3.2)	2.6 (2.8)

Note: visits to ENTs and Pulmonologists were only reported for those patients who visited this specialty at least once in the respective year.

Discussion

Findings and implications

This study describes the healthcare utilisation and costs associated with allergic rhinitis and asthma in a large population-based cohort. Overall, the pattern of findings found higher costs and resource use in prevalent subgroups than controls, with the highest costs and resource use in the prevalent asthma subgroup. Of interest, within prevalent subgroups, mean costs were, on average, 54% lower for those prescribed AIT than for those not. This data is important as it shows the actual costs to the healthcare system for these different patient groups. The study did not aim to explore the reason for these cost differences, and this could be an area of future research.

Comparison of prevalent and control subgroups

Outpatient contacts and pharmaceutical costs were greater in prevalent subgroups than in control subgroups. This is in

Table III *- Mean (SD) direct costs (Euros) for pharmaceuticals and inpatient hospitalisations in all subgroups in 2010 and 2014, overall and stratified by patient age.*

	Prevalent AR		**Control AR**		**Prevalent asthma**		**Control asthma**		**Prevalent AR + asthma**		**Control AR + asthma**	
	2010	**2014**	**2010**	**2014**	**2010**	**2014**	**2010**	**2014**	**2010**	**2014**	**2010**	**2014**
Pharmaceutical treatments	625 (2615)	675 (3140)	621 (2686)	641 (3488)	1217 (2729)	1104 (3354)	597 (2672)	623 (3473)	1038 (2867)	997 (3505)	577 (2540)	588 (3137)
<12 years in 2005	348 (1596)	287 (2948)	136 (1426)	157 (2199)	486 (1954)	405 (4146)	144 (1392)	169 (2088)	636 (2155)	581 (6593)	121 (1323)	143 (2116)
12-17 years in 2005	229 (3684)	248 (3766)	130 (1392)	171 (3330)	373 (1737)	303 (1524)	141 (1938)	182 (3582)	350 (1224)	293 (974)	115 (1386)	155 (3490)
18-50 years in 2005	471 (2354)	581 (2941)	337 (2782)	455 (3861)	913 (2425)	1019 (3397)	332 (2773)	450 (3861)	881 (2482)	936 (2918)	303 (2728)	408 (3580)
>50 years in 2005	1055 (2697)	1063 (3182)	903 (2772)	856 (3343)	1616 (3015)	1373 (3275)	883 (2748)	843 (3336)	1576 (3667)	1434 (2877)	842 (2554)	787 (2876)
Inpatient hospitalisations	723 (3307)	871 (4186)	1005 (4677)	1063 (5702)	1328 (5148)	1419 (5987)	967 (4561)	1026 (5559)	899 (3655)	1064 (4990)	974 (4590)	1010 (5350)
<12 years in 2005	319 (2145)	512 (3990)	332 (2766)	414 (3087)	459 (2426)	483 (2797)	320 (2673)	410 (3096)	430 (2423)	486 (3129)	303 (2509)	387 (2993)
12-17 years in 2005	326 (1888)	430 (2049)	262 (2706)	440 (2599)	415 (2035)	520 (2275)	347 (2554)	431 (2496)	336 (1656)	491 (1927)	331 (2537)	405 (2523)
18-50 years in 2005	480 (2312)	651 (3528)	535 (3557)	668 (5883)	789 (4260)	943 (4362)	522 (3448)	654 (5690)	681 (2918)	848 (4328)	503 (3373)	629 (5199)
>50 years in 2005	1309 (4743)	1429 (5395)	1443 (5504)	1445 (5951)	1890 (6063)	1957 (7286)	1420 (5451)	1423 (5871)	1496 (4937)	1701 (6587)	1403 (5450)	1374 (5765)

Figure 1 *- Mean direct costs (Euros) for pharmaceuticals (all and non-AIT) and inpatient hospitalisations in prevalent AR, stratified by AIT use, in the years 2010 and 2014.*

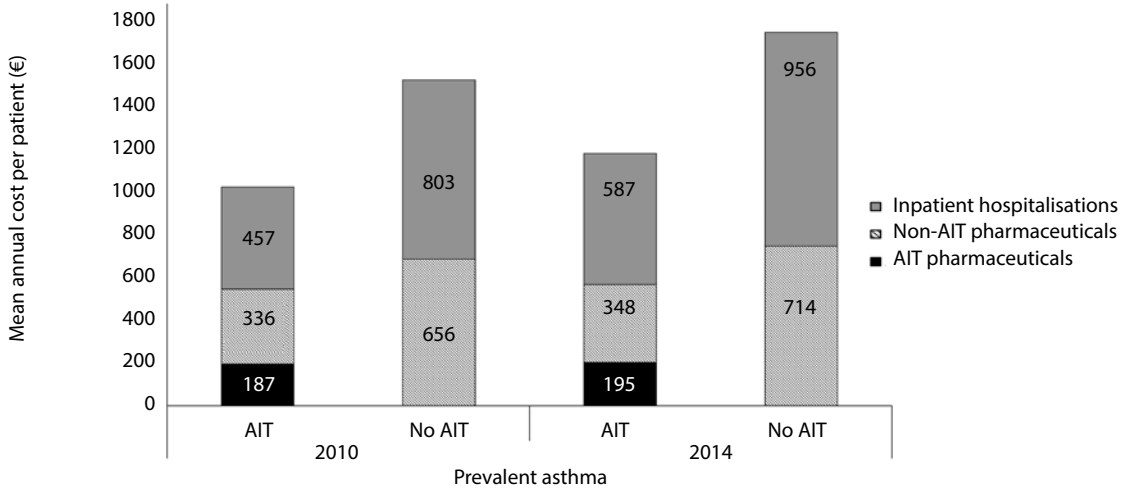


Figure 2 *- Mean direct costs (Euros) for pharmaceuticals (all and non-AIT) and inpatient hospitalisations in prevalent asthma, stratified by AIT use, in the years 2010 and 2014.*

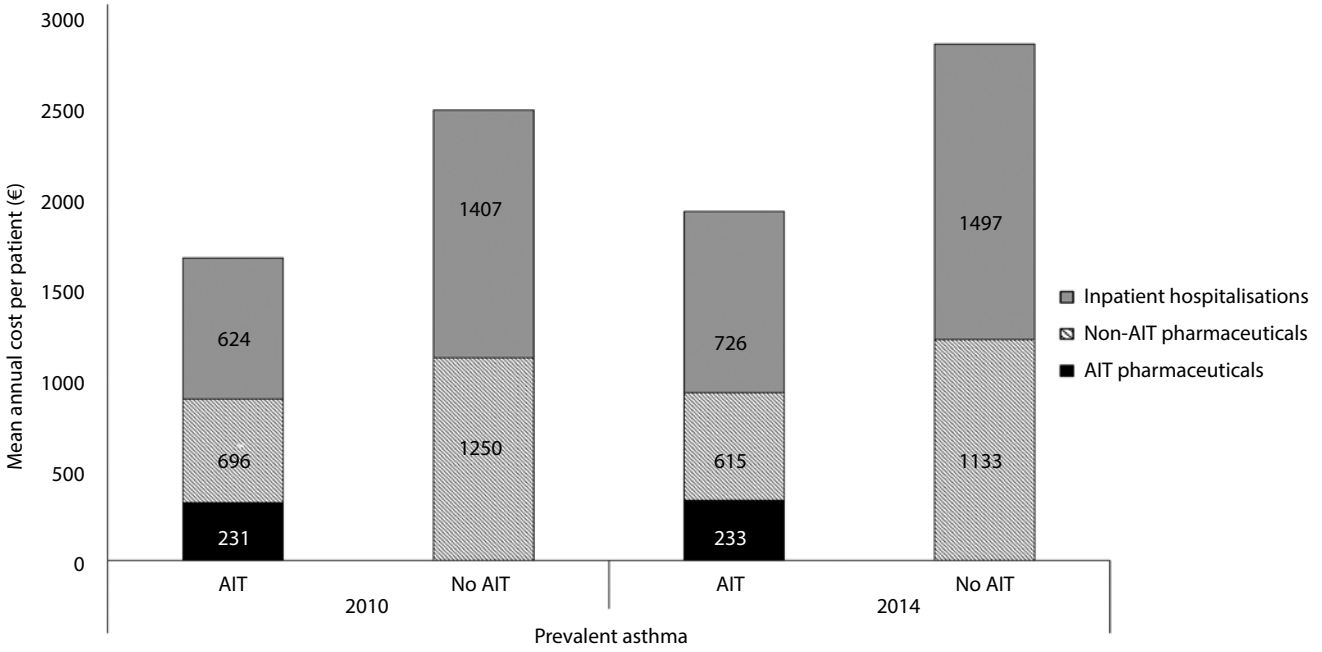
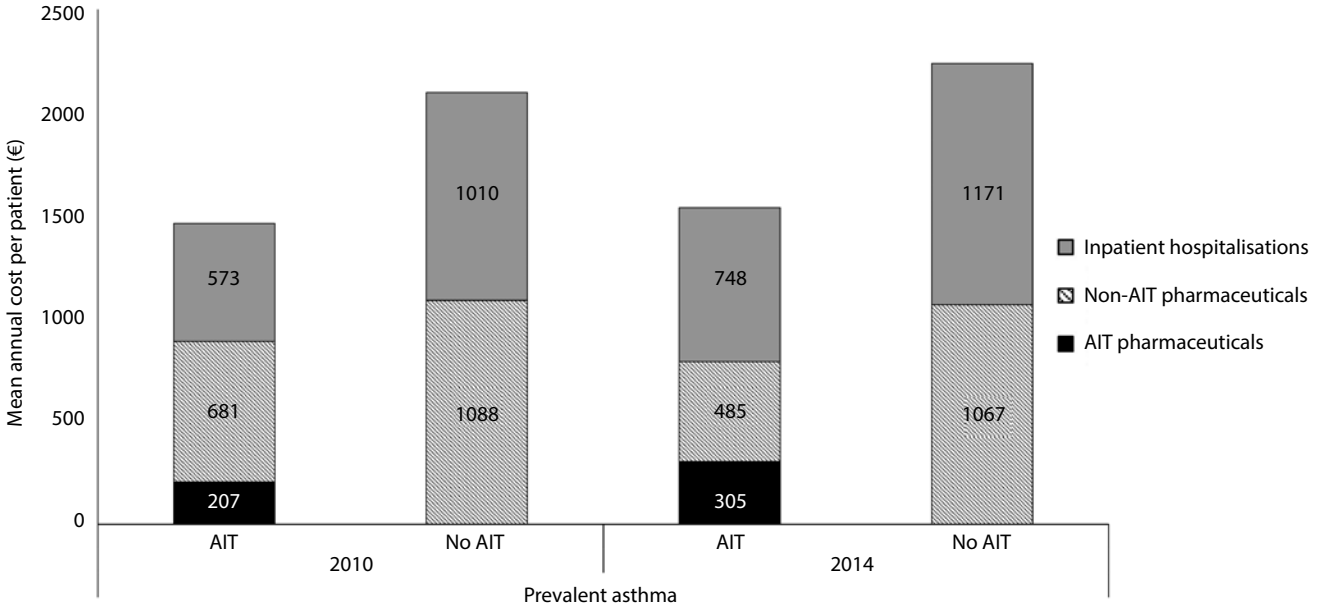


Figure 3 *- Mean direct costs (Euros) for pharmaceuticals (all and non-AIT) and inpatient hospitalisations in prevalent asthma + AR, stratified by AIT use, in the years 2010 and 2014.*



line with expectations, given that both diseases require ongoing pharmaceutical management, thus incurring costs for the pharmaceutical treatments themselves, as well as outpatient appointments for prescribing and monitoring purposes, that would not be incurred by people without these diseases. However, for inpatient costs, the pattern was less consistent. While inpatient costs for the prevalent asthma subgroup were consistently higher than those for the no asthma control subgroup, for the other two prevalent subgroups inpatient costs were, in many cases, lower than for the respective control subgroups. The reasons for this are unknown. Inpatient hospitalisations are rarely required for the management of AR, so inpatient costs in the prevalent AR subgroup are likely incurred due to co-morbid health problems. As such, we speculate that co-morbid health problems may be more easily identified and treated in prevalent cohorts due to regular contact with physicians, thus reducing the risk for costly inpatient visits. In contrast, inpatient hospitalisations are more common in the management of asthma (16), particularly in patients with uncontrolled asthma, thus a proportion of the inpatient costs in the prevalent asthma subgroup are likely directly related to asthma treatment. Accordingly, this subgroup is therefore likely to incur greater inpatient costs than those with no asthma.

One unexpected finding was that resource utilisation and costs were generally lower in the co-morbid prevalent AR with asthma subgroup than in the prevalent asthma subgroup. This may appear contrary to the logic of treating two diseases compared with treating one, but we consider it likely to be related to the nature of disease classification. In co-morbid cases where asthma is considered severe, or predates the onset of AR, the diagnostic code for AR may not be applied to patient notes (16), resulting in underreporting of AR in more severe (and more expensive) cases of asthma. In contrast, the prevalent AR + asthma subgroup may be more likely to include a large proportion of patients with AR who have only recently begun showing signs and symptoms of asthma, and therefore as a cohort, the asthma may be better controlled, requiring fewer outpatient contacts, less medication and being associated with a lower risk of asthma-related hospitalisation.

Comparison of costs for those prescribed AIT and not prescribed AIT in prevalent subgroups

Examination of healthcare costs demonstrated that the costs of both pharmaceutical treatments and inpatient hospitalisations were lower in patients prescribed AIT than those not prescribed AIT. One consideration when interpreting these findings, is that the overall healthcare utilisation and costs associated with pharmaceuticals and inpatient hospitalisations were not specific to those associated with the treatment of AR or asthma, and instead, refer to all-cause healthcare use. As such, the costs will reflect treatment for co-morbid health conditions in addition to that for the diseases of interest. If co-morbid health problems are caused, or mediated, by AR and/or asthma, better disease control in these disease areas may also reduce healthcare resource use in the co-morbid disease(s). A further consideration is that the data shows the actual cost to payers for different subpopulations patients, and there are many reasons why these costs may differ. It is possible that patients who receive AIT have different comorbidities and AR and AA disease severity to those who do not receive AIT. Further research would be needed to establish why the costs are different in each patient population.

Strengths and limitations

The study benefits from the use of a large insurance database as the data source. While this may somewhat under-represent high income patients who may be less likely to use statutory healthcare, we consider this sample relatively unselected and highly representative of the general population in Germany (28), thus the results are likely to be widely generalizable.

While use of an insurance database provides many strengths, it is limited in terms of the data available. As for all insurance claims databases, pharmaceutical costs only include those for which prescriptions were provided, and likely under-represent those incurred privately for over-the-counter (OTC) medications. This is likely to particularly affect the costs associated with AR pharmaceuticals, for which OTC medications are readily available at low cost. However, as OTC costs are paid by patients, the costs reported here are relevant when considering the burden for healthcare systems. Relatedly, it is possible that self-treating patients may not have consulted a physician and, without an ICD-10 diagnosis code, they would not have met criteria for inclusion in the AR prevalent group, thus resulting in misclassification. However, if these patients self-treat, the misclassification will not affect the resource use and costs incurred by statutory health services, thus it can be argued that such a limitation does not alter the conclusions of this study.

It was also discussed that the costs for inpatient hospitalisations and pharmaceutical costs in this study are not limited to the costs of AR and asthma only. It is therefore possible that non-related, co-morbid diseases could have influenced the cost calculations reported. However, this was equally biased for both AR and asthma patients and was therefore unlikely to have influenced the differences found in the costs reported. Additionally, co-morbid diseases found in asthma and AR patients in the study may also be found in the real-world population. Therefore, the costs reported reflect potential real-life circumstances that may be faced when treating these conditions.

It should be noted that a conservative approach to defining AIT use was implemented, with any patient who had filled at least

one prescription for AIT stratified to the AIT group. Given evidence-based recommendations that AIT should be used for at least 3 years (29), it is likely that the AIT group includes a proportion of patients who may not have received full clinical benefit, which may have resulted in an underestimation the cost differences between the AIT and no AIT groups.

The study design cannot provide evidence for a causal relationship between reduced cost and treatment with AIT. It is possible that higher costs for non-AIT subgroup may have been observed, for example, due to a high proportion of patients with complications that both contraindicate treatment with AIT and result in higher costs.

Conclusions

AR and asthma were associated with increased outpatient visits and pharmaceutical costs. Asthma also incurred greater inpatient costs compared with controls, reinforcing the importance of AR treatments that reduce the risk of developing asthma, such as AIT (30). Within prevalent populations, pharmaceutical and inpatient costs were lower for those prescribed AIT than for those not prescribed AIT.

Conflict of interests

AD, TSG, LE and EW were all employees of ALK Abelló while conducting this work; EW reports holding stock options for ALK Abelló; DK, FT and JS report an institutional grant from ALK Abelló for the submitted work; JS reports institutional grants from ALK Abelló, Novartis, Pfizer and Sanofi outside the submitted work.

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Evolution of Api m10 specific IgE and IgG4 after one year of bee venom immunotherapy

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Key words

Anaphylaxis; api m1; api m10; bee venom; venom immunotherapy.

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Summary

Background. *Bee-venom (BV) anaphylaxis can be life-threatening, requiring treatment with BV immunotherapy (bVIT). Different molecular profiles may be associated with different outcomes after bVIT. Methods. In 19 patients with BV anaphylaxis, sensitized both to Api m1 and Api m10, we evaluated sIgE and sIgG4 Api m1 and Api m10 levels before and after 1 year bVIT. Results 7 patients (37%) had higher baseline Api m10 than Api m1 sIgE levels (Api m10 predominant). bVIT reduced sIgE to both components but sIgG4 levels were increased only for Api m1. 5 patients (2 in the Api m10 predominant group) were re-stung without anaphylaxis. Conclusions. Although there was no increase in Api m10 sIgG4 levels after 1 year bVIT, we did not observe relevant differences in other outcomes between patients with predominant Api m1 or Api m10 sensitization.*

Introduction

Systemic reactions to bee stings are potentially fatal in bee venom (BV) allergic patients (1). Immunotherapy with bee venom (bVIT) is a well established therapy in patients with systemic reactions although doctors of other specialties, namely by emergency physicians are not always familiar (2,3). It has been shown to improve quality of life and to prevent life-threatening reactions following an accidental sting (4).

Accepted criteria for bVIT include systemic reactions following a bee sting together with a certain degree of probability that the patient may be stung again, along with the unequivocal demonstration of a IgE-mediated reaction to bee venom, either by skin tests or serum specific IgE to whole BV extracts.

Component resolved diagnosis allows the identification of major species-specific allergens, which may contribute to a more accurate diagnosis in some patients (5). In recent years recombinant BV allergens, such as Api m4, have been associated with a higher frequency of adverse reaction to bVIT (6) or with lesser effectiveness of bVIT, and Api m10 (7).

Api m10 is a major BV allergen, that is recognized by more than 50% of BV allergic patients of different populations (8,9) and inclusively in some patients that are negative to Api m1. In an unselected population of BV allergic patients followed in our Hospital, positivity to Api m10 was present in 70%, being second only to Api m1 (positive in 86%) (9).

Since it has been reported that several bVIT extracts lack Api m10 or that is present in only very small quantities (10), the predominance of Api m10 sensitization has been proposed as a possible predictive marker of bVIT failure (7). Significant differences in Api m10 concentrations between different manufacturers and, in one case, significant differences between batches of the same manufacturer have been reported (7,11). These reports suggest differences in the quality of therapeutic BV ex-

tracts, which could also be related to different manufacturing processes, a fact that might be of major importance at least for patients with particular sensitization profiles (11).
Therefore, the aim of our work was to evaluate if BV allergic subjects, positive to Api m1 and Api m10 showed any changes in specific IgE and IgG4 to Api m10 after one year of bVIT, with a BV extract (Roxall®). According to the manufacturer, this extract contains Api m10 in an unknown quantity. As far as we know this particular BV extract was not evaluated in any of the previously published papers regarding this subject.

Material and methods

Population

Retrospective study of patients with BV anaphylaxis, grade III/IV according to Muller classification, with sIgE positivity both to Api m1 and Api m10. Patients should have completed at least one year of immunotherapy with the same commercial BV extract sera analysis before and after one year of bVIT. A total of 19 patients were evaluated, predominantly male (89%) with a mean age of 49.5 years (14-74 years).

Diagnosis of bee venom allergy

Diagnosis was based on a clinical history of recurrent anaphylaxis after a bee sting and positive skin tests and/or positive sIgE to BV whole extract. Furthermore, all patients have IgE-positive to both to Api m1 and Api m10.

Skin tests

Skin tests with BV extracts were performed according to EAACI guidelines (1) with Stallergenes® or Bial-Aristegui / Roxall® extracts, at least three weeks after the last sting reaction. The skin prick tests were performed using a 100 µg/ mL concentration and with 0.9% NaCl as the negative control and 10 mg/ml histamine as the positive control. Intradermal tests were performed with increasing concentrations from 0.001 to 1 µg/ml as well as a negative control.

Specific IgE/ IgG4 evaluation

Specific IgE antibody (sIgE) levels and specific IgG4 (sIgG4) to BV whole extract, and recombinants to Phospholipase A2 (Api m1) and Icarapin (Api m 10) were evaluated in all patients using ImmunoCAP® system according to the manufacturer's instructions (ThermoFisher Scientific, Uppsala, Sweden). Values of ≥ 0.35 kU/L for sIgE to BV and > 0.10 for sIgE to Api m1 or Api m10 were considered positive. These measurements were undertaken before and one year after start of bVIT

Venom immunotherapy ultra-rush (UR) protocol

The induction protocol used was the 210-minute UR proposed by Birnbaum (12), used by our group in the last years with a good safety profile (13). In this protocol a cumulative dose of 101.1 µg, divided in 6 injections, is administered as follows: an initial dose of 0.1 µg, followed by 1, 10 and 20 µg at 30-minutes intervals. Then 30 and 40 µg were given every 60 minutes. The maintenance dose of 100 µg was repeated 15 days after the UR and administered at 4-6-week intervals over a period of 3 to 5 years, as established by the EAACI guidelines (1). All patients received the BV extract from Bial-Aristegui / Roxall®.
All injections were given by trained medical staff in an Immunoallergology Day Hospital, equipped for the treatment of anaphylactic reactions. All patients had a venous access with saline during the procedure. Heart rate, blood pressure and peripheral oxygen saturation were continuously monitored. Patients received pretreatment with oral H1 antihistamine (cetirizine 10 mg, ebastine 10 mg or other equivalent 2nd generation non-sedating H1 antihistamine) in the 2 days prior to UR and in the morning of the UR.
Therapy with ACE inhibitors or with cardio-selective beta blockers in patients with stable cardiovascular disease was continued during UR and bVIT.
This study was approved by the Ethics Committee of the Hospital Santa Maria and was conducted according to ethical standards established in the Declaration of Helsinki. Informed consent was obtained from all participants before enrolment in the study.

Results

All individual measurements of sIgE and sIgG4 to BV, Api m1 and Api m10 are shown in **figure 1** and **table I** (before bVIT - T0) and in **table II** (after one year bVIT - T1). **Table III** shows mean, median and interquartile ranges of sIgE and sIgG4 values at T0 and T1.
In T0, the mean and the median Api m1 sIgE levels were higher than the Api m10 sIgE levels but the analysis of individual values shows that only 12 patients (63%) had higher Api m1 sIgE levels than Api m10 sIgE levels while in 7 patients (37%) the baseline Api m10 sIgE values were in fact higher. We found no differences in the age or in other characteristics between these two groups of patients. In T0 the sIgG4 values were low for both recombinants, and they were zero in the majority of patients and they did not have any correlation with sIgE values.
Figure 2 depicts individual variations of sIgE and sIgG4 values to Api m1 and Api m10, before and after one year of bVIT.
We observed reductions in Api m1 and Api m10 sIgE values, but these reductions were significant ($p<0.05$) only in the case of Api m10.

Table I *- Individual patients' values before bVIT.*

Patient no	Gender	Age	T0 (sIgE)			T0 (sIgG4)		
			BV	r Api m1	r Api m10	BV	r Api m1	r Api m10
1	M	30	92.90	61.10	8.84	0.00	0.00	0.77
2	M	60	25.60	0.94	4.71	1.66	0.10	0.00
3	F	43	6.05	5.78	0.59	0.01	0.00	0.00
4	M	55	30.00	7.34	2.75	1.37	0.53	0.00
5	M	72	24.70	0.87	0.49	0.32	0.01	0.00
6	M	56	0.39	0.39	0.12	3.08	0.00	0.00
7	M	31	4.12	1.46	1.50	0.00	0.00	0.00
8	M	64	11.20	0.16	0.73	0.00	0.00	0.00
9	M	74	3.55	0.28	2.75	3.27	3.57	0.00
10	M	66	8.99	7.17	0.14	0.00	0.00	0.00
11	M	14	100.00	90.70	11.10	6.00	0.09	0.00
12	M	43	2.71	0.60	2.64	1.03	0.74	0.00
13	M	66	11.40	7.85	0.19	0.00	0.00	0.00
14	M	39	11.10	0.65	2.82	11.20	5.18	0.00
15	M	43	16.70	18.80	2.45	2.88	2.58	0.00
16	F	48	33.00	20.90	0.34	0.00	0.00	0.00
17	M	35	23.30	31.60	6.99	0.00	0.00	0.00
18	M	60	8.99	5.42	12.60	0.00	0.00	0.00
19	M	43	1.39	0.47	0.36	0.00	0.00	0.00

BV- whole bee venom extract.

Figure 1*. – Individual sIgE and sIgG4 values to BV and recombinants before bVIT BV- whole bee venom extract.*

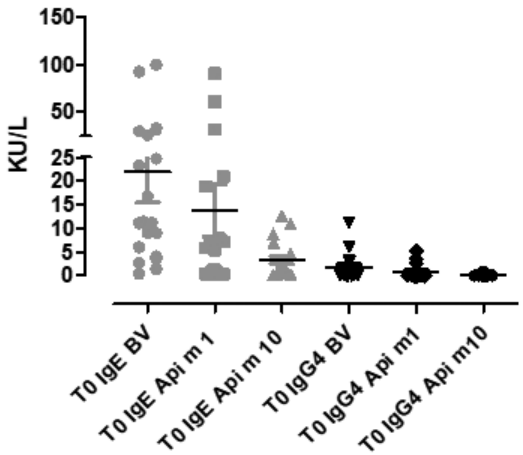


Table II *- Individual patients' values after one year of bVIT.*

Patient no	Field sting during bVIT (Yes/No)	T1 (sIgE)			T1 (sIgG4)		
		BV	r Api m1	r Api m10	BV	r Api m1	r Api m10
1	Yes	98.10	86.20	2.55	26.80	14.60	0.00
2	No	2.45	0.05	0.52	1.35	0.29	0.00
3	No	0.11	3.47	0.59	0.88	1.89	0.00
4	No	100.00	36.8	10.00	11.90	6.65	0.00
5	Yes	22.20	5.55	2.10	22.20	21.30	0.18
6	No	0.60	0.23	0.13	1.30	0.46	0.00
7	No	15.40	5.24	0.82	8.68	6.32	0.00
8	Yes	2.88	0.15	0.26	0.00	4.47	0.00
9	No	2.75	0.43	1.88	5.71	4.48	0.00
10	No	4.99	3.73	0.07	0.00	0.00	0.00
11	No	82.70	50.70	6.16	0.00	27.70	0.00
12	No	1.61	0.37	1.94	0.00	27.90	0.00
13	Yes	5.86	2.35	0.17	0.00	0.00	0.00
14	Yes	8.53	1.23	2.70	0.00	8.75	0.00
15	No	6.20	1.62	1.50	0.00	11.10	0.00
16	No	36.40	21.00	0.52	0.00	0.00	0.00
17	No	5.29	3.92	1.80	0.00	0.00	0.00
18	No	2.00	0.97	2.99	0.00	0.00	0.00
19	No	1.70	0.45	0.37	0.00	0.00	0.00

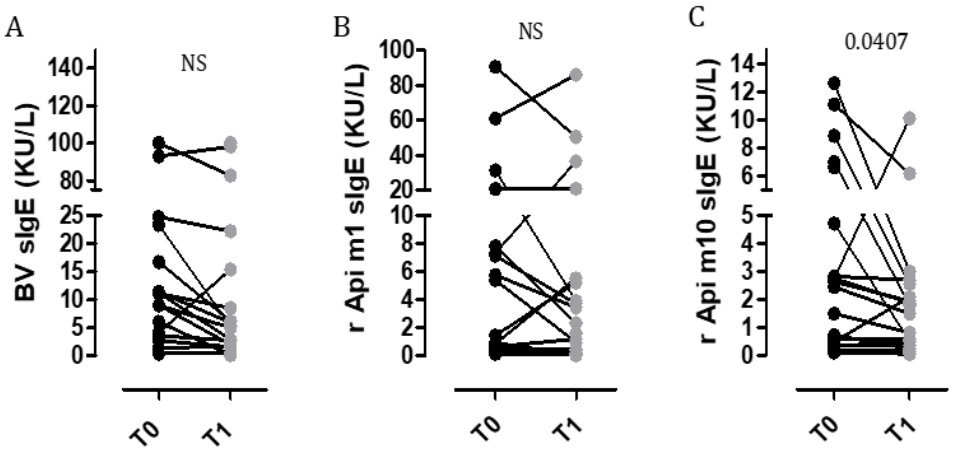
BV- whole bee venom extract.

Table III *- Mean, median and interquartile (IQR) specific immunoglobulin values before (T0) and after one year (T1) of bVIT.*

	sIgE T0			S IgE T1		
	Mean	Median	IQR25/75	Mean	Median	IQR25/75
BV	21.90	11.20	4.12/25.60	21.04	5.29	2.00/22.20
r Api m1	13.81	5.42	0.60/18.80	11.81	2.35	0.43/5.55
r Api m10	3.27	2.45	0.36/4.71	1.96	1.50	0.37/2.55
	sIgG4 T0			**S IgG4 T1**		
	Mean	Median	IQR25/75	Mean	Median	IQR25/75
Bv	2.20	1.20	0.00/3.13	7.57	1.62	0.00/14.48
r Api m1	0.91	0.05	0.00/1.20	9.72	6.49	1.53/16.28
r Api m10	0.06	0.00	0.00/0.00	0.01	0.00	0.00/0.00

BV- whole bee venom extract.

Figure 2 *– Whole bee-venom extract (A), Api m1 (B) and Api m10 (C) sIgE values of individual patients before (T0) and after one year bVIT (T1) NS – not significant BV- whole bee venom extract.*



Additionally we have also documented significant increases in Api m1 sIgG4 values but not in Api m10 sIgG4.
During this first year of bVIT, only 5 of our 19 patients were accidentally re-stung. We did not record any systemic reactions or use of adrenaline. Two of these 5 patients belonged to the group with baseline higher Api m10 sIgE than Api m1 sIgE values.

Discussion

Our results show that in these BV allergic patients with sensitization to both to Pai m1 and Api m10, one year of the bit, can induce immunologic responses to whole BV and to Apia m1 with mean singe reductions of more than 50% and significant increases (>300%) in sIgG4 to Pai m1. Besides these expected changes we have additionally shown that one year of bVIT that, according to the manufacturer, contains Api m10 in an unknown quantity, could also induce significant reductions in Api m10 specific IgE, but without any increases in Api m10 sIgG4. These results are in line with the reports by Kohler et al (14) that showed no increase in Api m10 sIgG4 levels in 20 BV-allergic patients receiving b-VIT for 12-48 months and by Frick et al (7) that showed that b-VIT with one of the three commercial extracts in which they did not detect significant amounts of Api m10 induced some significant reductions of sIgE to Api m10 but without any significant increase of sIgG4 to Api m10. On the other hand, patients treated with one of the two commercial extracts where they did detect amounts of Api m10 similar to crude venom preparations showed higher and very significant reductions in Api m10 sIgE levels as well as significant increases in sIgG4 to Api m10 (7).

Api m1 (phospholipase A2) comprises 12-15% of the dry weight of bee venom but it is the most relevant allergen present in crude venom and in venom extracts and it represents the sensitization most frequently found in bee-allergic patients (15). However, Api m1 sIgE is not always present in bee-venom allergic patients, ranging from 57 to 97% in previously published papers (14) with 86% positivity reported by our group in a prospective study of 30 portuguese bee-venom allergic patients (9). Api m1 sIgE negative patients with a clear history of bee-venom induced anaphylaxis can be a diagnostic challenge and it has been proposed that the inclusion of other bee-venom specific recombinant allergens, such as Api m3 or Api m10, in diagnostic panels could increase diagnostic sensitivity (16).
Api m10 (icarapin) comprises less than 1% of the dry weight of BV (15) but it is a major allergen. Api m10 positivity in populations of BV allergic patients has been reported to range between 49 and 62% in older studies (15), with more recent studies reporting frequencies around 70%, meaning that Api m10 is second only to Api m1 sensitisation (7,9). Furthermore, some of the Api m10 sIgE positive patients are negative to Api m1 sIgE, which raises not only diagnostic problems but also therapeutic concerns, since Api m1 is present in adequate quantities in all bVIT extracts but Api m10 is apparently underrepresented in many bVIT extracts (7,11,14), a fact that was proposed to explain treatment failures of bVIT in patients with a predominant Api m10 sensitisation (defined as a percentage of Api m10 sIgE in relation to honey bee venom sIgE > 50%) (7).
In our study we included only patients with double positivity to Api m1 and Api m10 and we observed that, in this group of patients, more than 1/3 had higher baseline sIgE values to Api

m10 than to Api m1. If we applied to our patients the definition of predominant sensitisation (sIgE to recombinant allergen>50% of sIgE to whole BV) used by Frick et al (7), we would have 9 patients with predominant Api m1 sensitisation and 3 patients with predominant Api m10 sensitisation, with the 7 remaining patients not showing any predominance with respect to Api m1 or Api m10. Independently of the way we look at it, it is a fact that patients in whom Api m10 constitutes the dominant sensitisation represent a non-negligible percentage of BV allergic patients.

All these data on the relevance of Api m10 sensitized patients have generated some debate whether particular BV sensitization profiles are related to better or worse outcomes of bVIT (7). It has also been suggested that, in a personalised medical approach, patients with a predominant Api m10 sensitisation should receive a bee-venom extract containing adequate amounts of Api m10 and that patients without Api m10 sensitisation should receive a bee-venom extract with low or absent Api m10 (11).

In Portugal we do not usually perform controlled sting challenges and the evaluation of the effectiveness relies mainly on patients reporting what happened when they were re-stung. In this study more than 25% of the patients were re-stung during bVIT and no one reported any systemic reaction or use of adrenaline following accidental stings, independently of the predominant sensitisation they had. This finding, that does not agree with the report by Frick et al (7), should be interpreted with caution because of the very small number of patients involved and the non-controlled nature of the observation.

The present study has clear limitations in that it used a retrospective study design with a limited number of patients. Also, the quantity of Api m10 in the BV extract used is unknown. But this study has the added interest of reporting individual immunological data obtained by the same BV extract, one that has not been addressed in previous studies focusing on the importance of Api m10 sensitisation profiles and Api m10 content in commercial BV extracts. Additionally, our data were obtained from a well-characterized population of BV allergic patients with anaphylaxis, showing double positivity to the two more prevalent recombinant allergens in our Portuguese BV allergic patients: Api m1 and Api m10.

We hope that our paper as well as other studies could stimulate a more in-depth and widespread knowledge of the full spectrum or recombinant allergens present in each of the different BV commercial extracts, since this knowledge could have potentially vital implications in therapeutic options for severe honey-bee venom allergic patients.

Conclusions

In our group of 19 BV-allergic patients with anaphylaxis to BV and with double positivity to Api m1 and Api m10, one year of bVIT induced reductions of Api m1 and Api m10 sIgE levels but only significant increases of Api m1 sIgG4 levels and not of Api m10. According to the manufacturer, this BV extract contains an unknown quantity of Api m10 allergen and it is possible that the Api m10 concentration present in the extract is not sufficient to induce sIgG4 responses. However, from a clinical point of view we did not observe any systemic reactions in re-stung patients, therefore suggesting clinical efficacy of this BV extract, even in Api m10 sensitised patients.

Further studies are needed to compare the relative Api m10 concentrations in all the different commercial BV extracts and to compare immunologic and clinical efficacy of bVIT with different extracts in patients with different sensitization profiles.

Conflict of interests

The authors declare that they have no conflict of interests.

Author contributions

MCPS, EP, MBF, MPB designed research; MCPS, TL, MBF, performed research and analyzed data; EP and MPB were involved in clinical investigation of patients; MCPS and MBF wrote the paper.

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Tree nuts anaphylaxis in preschool age children

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Summary
Introduction. *The incidence of food-induced anaphylaxis (FIA) is increasing in young children. Although the commonest culprits are cow's milk and egg, FIA to tree nuts (TNs) have been increasing.* **Objective**. *Characterization of children referred to our allergy department due to TNs-induced anaphylaxis (TNs-FIA) during preschool age.* **Materials and methods**. *We have retrospectively included 25 children with clinical history of preschool TNs-FIA, proven by allergological work-up. TNs sensitization was assessed by skin prick tests and/or specific IgE.* **Results.** *The mean age of the first anaphylactic episode was 3.1±1.2 years. The majority (92%) had an allergic disease (52% asthma). The implicated TNs were cashew (11 children), walnut (8), pine nut (5), hazelnut (2) and almond (1). The reaction occurred after the first known ingestion in 68%. In 92%, symptoms appeared within 30 minutes after exposure. The most frequent clinical symptoms were mucocutaneous (96%), respiratory (80%) and gastrointestinal (52%). Twenty-one children were admitted to the emergency department, although only 48% were treated with epinephrine. An underneath IgE-mediated mechanism was proven in all cases. Immunologic cross-reactivity with other TNs was identified in 84%, and with peanut in 36%. Overall, in our center, TNs-FIA represents 18% of all causes of FIA.* **Conclusions**. *In preschool age children with TNs-FIA, cashew and walnut were the commonest implicated nuts. Most reactions occurred briefly after exposure to minimal amounts of TNs, demonstrating the high potency of these allergens. About one-third also had peanut sensitization. Potentially life-threatening TNs allergy can occur early in childhood and adequate management should be undertaken.*

Introduction

Food allergy usually develops early in life and affects up to 10% of children (1-4). Its incidence and severity has been increasing worldwide, especially in preschool children (3-8). In this age group, the two most common food allergens are cow's milk and hen's egg (9-13). Nevertheless, severe allergic reactions to tree nuts (TNs) have been increasingly reported worldwide, both in raw form and within processed foods (7,8,14-16).
TNs are defined as a dry fruit composed of an inedible hard shell and a seed. The TNs that account for most allergic reactions are walnut, hazelnut, almond, pistachio, cashew, pecan nut, macadamia, brazil nut and pine nut (16,17). Although botanically unrelated, TNs and peanut (that belongs to the botanical family of *Leguminosae*) share many allergenic similarities (17).
Overall, TNs allergy affects 0.5 to 3% of the population (17,18), representing 11 to 40% of cases of food-induced anaphylaxis (FIA) (13,16,19). However, its prevalence differs according to the geographical region and dietary patterns (3,16-18,20). In the United States, walnut is the most commonly reported culprit of TNs allergy, followed by cashew and almond (17,20). In Europe, hazelnut allergy is the most prevalent (17). The exact TNs allergy prevalence in Portugal is unknown.

TNs allergy typically presents by the age of two to three years old, often after the first known ingestion (16-18). Symptoms are typically immunoglobulin E (IgE) mediated and arise from a massive mediator release from mast cells and basophils degranulation, usually appearing few seconds to two hours after the contact with the allergen (3,7,21). The pathogenesis of non-IgE-mediated tree nuts allergy is less clear. Despite their underlying mechanism, non-IgE-mediated reactions are clinically indistinguishable and have similar acute management (21).
The nature of the symptoms is often related to the age of the child, to the amount of the exposure (ingestion of large quantities is generally responsible for more rapid and pronounced reactions), and also to the ingested TN (cashew is associated with more severe reactions and airway narrowing) (18,20). In infants up to two years old, the most common symptoms are cutaneous and gastrointestinal, and in preschool children, cutaneous, gastrointestinal, and respiratory symptoms are the most prominent. Severe symptoms as throat tightness are more frequent in older children and adolescents. Cardiovascular symptoms are less frequent than in adults (7,9,12).
Risk factors for the development of TNs allergy include severe atopic eczema, egg allergy and the presence of family or personal history of allergic disease (20,21). The concomitant presence of asthma and eczema, a history of allergic reactions to extremely small amounts of food and a history of a previous food-induced anaphylactic reaction, are associated with more serious manifestations (16,18,20). TNs allergic reactions can be severe and account, together with peanut, for a high proportion of fatal FIA (up 70 to 90%) (15-17,20). The majority of fatal accidental reactions occur in adolescents and young adults (median age 27 years old) (18,20).
Children with TNs allergy have a significantly increased risk of co-sensitization or co-allergy to other nuts (16,18,20). The sensitization rate to TNs is high, reaching up to 86%.[16] Certain specific TN allergies appear to coexist more commonly, such as cashew with pistachio or walnut with pecan (16,18). Co-allergy between TNs and peanut ranges between 20% and 68% (16,18,20).
The aim of this study was to perform the characterization of children referred to our allergy department due to FIA related to TNs ingestion during preschool age.

Materials and methods

We have retrospectively evaluated the medical reports of a group of children with FIA after TNs ingestion during preschool age, proven by allergological work-up, followed at the Immunoallergy department of CUF Descobertas Hospital (Lisbon, Portugal). The evaluation of these children was included in a systematic reporting of anaphylaxis which was implemented in our allergy outpatient department over eight years (from January 2011 to December 2018). All allergists of the department were invited to participate and a meeting was organized in order to promote the voluntary notification of cases of FIA.
The diagnosis of TNs induced-anaphylaxis was assumed when "at least one episode of severe systemic reaction" (as defined by the international consensus) (7,13,22) occurred after the ingestion of TNs (described by the patient or caregiver) to which a confirmed sensitization has been proven. TNs were defined as walnut, almond, pistachio, cashew, pecan, hazelnut, macadamia, Brazil nut and pine nut.
The sensitization to TNs was assessed by *in vivo* skin prick tests (commercial extracts, *Roxall-Aristegui®,* Bilbao, Spain) and/or *in vitro* assays of serum specific IgE (*ImmunoCAP, Thermo Fisher Scientific®*, Waltham, Massachusetts, USA). Skin prick tests were performed by an allergist using standard methodology; the result was considered positive if the mean wheal diameter was 3 mm or greater, with negative control (0.9% saline) and positive control (histamine 10mg/mL). Regarding *in vitro* tests, results were considered positive if specific IgE was 0.35 kU/L or greater. The sensitization to peanut and aeroallergens was also assessed.
In addition to this allergological diagnostic work-up, the authors collected demographic and clinical data: age, gender, family history of allergic disease, personal history of allergic disease and atopy (defined as positive skin prick test for at least one aeroallergen), date of the first anaphylactic reaction and a detailed description of the clinical manifestations (mucocutaneous, respiratory, gastrointestinal and cardiovascular). Moreover, the following information has also been collected: implicated TNs, elapsed time between exposure and the onset of symptoms, estimated amount of ingested TNs, place of anaphylaxis occurrence, attendance to the emergency department, description of the performed treatment (including information about the use of epinephrine) and management after the anaphylactic event (including information about prescription of epinephrine auto-injectors).
A descriptive statistical analysis was performed using SPSS for Windows version 20.0®.

Results

Twenty-five children with a confirmed diagnosis of TNs-induced anaphylaxis during preschool age were included. The current mean age was 8.5 years (SD ± 3.8 years) and 17 (68%) were male.
Almost all (92%) children had a personal history of other allergic disease: 20 (80%) allergic rhinitis or rhinoconjunctivitis, 14 (56%) atopic dermatitis, 13 (52%) asthma, 4 (16%) other food allergy (3 with cow's milk allergy, 2 with egg allergy, 1 with lamb allergy and 1 with peach allergy) and 1 (4%) eosinophilic esophagitis. Eighteen children (72%) were atopic (14 sensitized to house dust-mites, 7 to pollens and 4 to pets). A family history of allergy was identified in 21 children (84%); four had family

history of food allergy (2 with allergy to crustaceans, 1 to walnut and 1 to cow's milk).

The mean age of the first anaphylactic reaction to TNs was 3.1 years (SD ± 1.2 years; minimum age of 14 months, maximum age of five years). In 16 children (64%) the first episode occurred in the first three years of life.

The TNs involved in the allergic reaction were: cashew in 11 children, walnut in 8, pine nut in 5, hazelnut in 2 and almond in 1 child. There was one case of walnut and hazelnut-induced anaphylaxis and one case with cashew and almond.

In 19 children (76%), the anaphylactic reaction was triggered by the ingestion of extremely small amounts of the TN involved. Ten children ingested a vestigial content, nine children ingested fragments or a single TN, five children ingested two or more tree TNs (maximum of three units) and one child ingested an unknown quantity.

The anaphylactic reaction occurred after the first known TN ingestion in 17 cases (68%). Only one child had a known allergy to walnut, and the anaphylactic episode occurred after an accidental exposure during holidays.

Regarding the place where the allergic reaction occurred, in 18 children (72%) the anaphylactic episode occurred at home, in 4 (16%) at a restaurant, in 1 (4%) at the beach and in 2 (8%) on vacation at recreational sites.

Concerning the reported symptoms, 24 patients (96%) had mucocutaneous symptoms (urticaria, angioedema, pruritus), 20 (80%) respiratory symptoms (cough, wheezing, dyspnea, stridor), 13 (52%) gastrointestinal symptoms (vomiting, diarrhea, abdominal pain) and three (12%) cardiovascular symptoms (prostration). Four children (16%) presented life-threatening glottis edema. There were no fatal events.

Considering the infant subgroup (children who had two or less than two years old at the time of the first anaphylactic episode), corresponding to ten children (40% of the studied sample), all (100%) had mucocutaneous symptoms, eight (80%) respiratory (including two cases of glottis edema), four (40%) gastrointestinal and one (10%) cardiovascular manifestations. In the older children subgroup (60% of the studied sample), 14 (93%) had mucocutaneous symptoms, 12 (80%) respiratory (including two cases of glottis edema), 9 (60%) gastrointestinal and 2 (13%) cardiovascular manifestations. Due to the small sample size, the authors did not carry out a comparative analysis between the two subgroups.

In 23 children (92%), symptoms appeared within the first 30 minutes after contact with the implicated allergen. In seventeen children (68%) the reaction occurred in the first five minutes, in six (24%) between five and thirty minutes and in two (8%) more than 30 minutes after the TN ingestion. The maximum time elapsed between the TN intake and the anaphylactic reaction was two hours (in one girl, after the ingestion of cereals with walnut and hazelnut).

Twenty-one out of the 25 children (84%), were admitted to the emergency department during this first anaphylactic episode. However, among patients who have been observed in the emergency department, only 10 (48%) were treated with intramuscular epinephrine. We must emphasize that an auto-injector epinephrine was prescribed to all children in our allergy department.

In all cases, an underlying IgE mediated mechanism was proven, by positive skin prick test and/or positive specific IgE to the culprit TN. Immunologic cross-reactivity (positive skin testing and/or *in vitro* immunoassays for specific IgE) with other TNs was confirmed in 21 children (84%) and with peanut in 9 (36%).

Overall, TNs induced-anaphylaxis represented 18% of all causes of FIA in our department, from a total of 277 cases of anaphylaxis related to food ingestion. Moreover, regarding the triggers of FIA in the pediatric age group (<18 years), from a total of 158 patients, although some patients reacted to more than one food allergen, the most frequent implicated foods were: cow´s milk in 51 (32%); TNs in 34 (22%); egg in 20 (13%); fresh fruits in 16 (10%), 9 to *Rosacea* fruits; peanut in 13 (8%); shellfish in 10 (6%), 7 to shrimp; fish in 7 (4%); seeds in 4 (3%), 3 to sesame seed and 1 to sunflower seed.

Discussion

FIA is an important health problem, with an increasing incidence in preschool age (5,6,8,12). TNs allergy is becoming more frequent worldwide, particularly in young children (14-16,20). In our center, we found TNs as an important trigger of FIA (18% of all FIA reports). We stress out that among the causes of FIA in our pediatric patients, it represents the second cause of FIA (22%), after cow's milk (32%).

In our pediatric sample, cashew and walnut were the most implicated TNs in preschool anaphylaxis. These are also the most allergenic TNs in the United States (17, 21). However, hazelnut is the most commonly reported TN allergy in Europe (17, 21), although allergy to hazelnut is often seen in the context of pollen-fruit syndrome due to PR-10 proteins sensitization, which causes mainly oral allergy syndrome and usually no anaphylactic reactions.

In a recent study also performed in our country, TNs were found to be the main cause of FIA (19%). In fact, in 62 patients observed in a food allergy outpatient department at Coimbra, walnut and hazelnut have been spotted has the most implicated TNs (19). It seems that the reported prevalence can vary significantly, according to the age range of the studied population and the geographical region.

Most of the anaphylactic reactions occurred with the first known ingestion of TNs, and briefly after exposure to minimal amounts of the implicated food. This clearly demonstrates the

high potency of these allergens. Therefore, successful elimination diets should include systematic education of the patient and their caregivers about food allergen labelling, proper food preparation and the risk of cross-contamination and hidden food ingredients (3). We highlight that the caregivers of these children may not be aware of the eventual accidental exposures that may have occurred and may have contributed to the occurrence of sensitization. Therefore, the diagnosis of food allergy cannot be excluded even if the caregivers deny previous contact of the child with the implicated allergen.

Mucocutaneous symptoms were the most frequent manifestations in these children. Respiratory and gastrointestinal symptoms were also very frequent, as reported by other authors in preschool age (7,23), being cardiovascular symptoms more uncommon than in adulthood. There were no fatalities, but four children presented life-threatening glottis edema. Indeed, TNs can cause severe allergic reactions.

In all studied children, an underlying IgE mediated mechanism was proven (by *in vivo* or *in vitro* tests). In fact, the most common form of FIA is IgE-mediated. Non-IgE-mediated TNs allergy is less frequent, especially in children (21). A high rate of co-sensitization and co-allergy to other TNs and to peanut is observed among different populations (16,18,20,21). In this sample, about one-third of children had peanut sensitization and more than four fifths were sensitized to other TNs. The high homology between their proteins can explain this strong association. We stress out that these children were considered to be included in the high-risk group since they had personal or family history of allergy.

The diagnosis of TNs allergy is made by the combination of a typical clinical presentation and evidence of TNs sensitization, assessed by *in vivo* tests (skin prick tests) or *in vitro* tests (identification of specific IgE antibodies in the serum) (7,16,18). Skin prick test equal to or greater than 8 mm or specific IgE test equal to or greater than 15 kU/L is highly predictive of clinical allergy, although do not predict its severity (16,18). Double-blind, placebo-controlled oral food challenges are the gold standard for the diagnosis of food allergy (7,16). They are usually not necessary in TNs allergy but may be used to confirm or refute the diagnosis, when history and test results are conflicting (7,16,18). In our study no oral challenge test has been performed since all included children had a severe systemic reaction clearly related to the TN ingestion and had a proven sensitization to the culprit TN.

As reported by other authors (24,25), less than half of children admitted to the emergency department during the anaphylactic episode were treated with epinephrine. Intramuscular epinephrine injection in the anterolateral thigh is the first-line treatment for anaphylaxis and should be administered as soon as possible (7,12,26). It is the only effective drug to prevent progression of the anaphylactic reaction and its delayed administration is considered a risk factor for a fatal outcome (3,7,16). The underuse of epinephrine by healthcare professionals can be explained by the complexity involved in establishing the diagnosis of anaphylaxis, lack of knowledge of how to administer epinephrine and use epinephrine auto-injectors, and misconceptions about epinephrine safety (15,25). To help improving the appropriate use of epinephrine in patients diagnosed with anaphylaxis within the emergency setting, physician training programs should be implemented.

Anaphylaxis may be even more difficult to recognize and diagnose in younger children, mainly in infants, due to inherent differences in their ability to communicate their symptoms (12,23,27,28). This difficulty may also be explained by the subjectivity of the clinical symptoms such as abdominal cramps, sudden cry and irritability, common in this age group, where frequently occurs the first clinical manifestation of the sensitization to the allergen (12,23,27).

An epinephrine auto-injector was prescribed to all children at the allergy outpatient department after the first TN induced anaphylactic event. As found in the literature, the prescription of epinephrine auto-injectors is strongly recommended for the proper management of future occurrence of anaphylaxis (3,16,18). Furthermore, encouraging these patients and caregivers to carry the auto-injectors all the time is an essential part of training (18).

TNs allergy can be severe, and usually persists over time, although resolution has been documented in some children (18). Although it was initially believed that TNs allergy rarely resolves, subsequent studies have shown that tolerance can develop in a minority of patients over time (up to 9%) (3,18,20). The predictors of outgrowing TNs allergies are a low or undetectable specific IgE levels, absence of other food/TNs allergy and a history of outgrowing peanut allergy (3).

The pillars of food allergy management are strict avoidance of the culprit allergens, prompt treatment of symptoms upon accidental exposure, patient and caregiver education (including food allergen labeling, food preparation and the risks of occult exposure), and management of allergic comorbidities (3,16,18). Allergen avoidance diets should be specific and limited to the relevant foods to minimize both risks of an allergic reaction and over-restriction (9,20). Complete nut avoidance is the safest approach, reducing the risk of an accidental reaction. This recommendation aims to simplify the message and improve avoidance while eating in schools and restaurants. However, it is difficult to achieve and can result in a significant restriction of certain food products. There are also nutritional, cultural and immunological arguments for the allowance consumption of other nuts (18). If a patient is already consuming a nut that he is not allergic to, it is reasonable to continue consuming it on a regular basis (18,20). The decision to avoid all nuts (all nut exclusion) or only the culprit nut (single nut exclusion) should ultimately involve the

patient and his family (16). Although in adults it is safe to avoid specifically the culprit nut in the patient´s diet, at pediatric age, the decision to avoid all nuts is more frequent, mainly at school. Furthermore, within a restaurant environment, all nut exclusion diet is always the safest approach, due to the risk of misidentification or inadvertent substitution with other nut types.

To our knowledge, this is the first Portuguese study about TNs-induced anaphylactic reaction in a preschool age population. However, the authors findings might not be directly applicable to other populations due to geographical and cultural differences.

In conclusion, potentially life-threatening TNs allergy may occur early in childhood and adequate management should be undertaken. There is a need for further studies to better characterize TNs-induced anaphylaxis prevalence and clinical manifestations in the young children population, particularly in Europe.

Conflict of interests

The authors declare that they have no conflict of interests.

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Omalizumab re-treatment rates in chronic spontaneous urticaria

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Key words

Omalizumab; chronic spontaneous urticaria; urticaria activity score; resistance.

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The review paper by Tonacci A and colleagues highlights the fact that re-treatment with omalizumab for patients with chronic spontaneous urticaria (CSU) is not unusual, which adds significantly to costs of treatment (1). Our experience with use of omalizumab in CSU has been similar and treatment responses with re-treatment rates are presented. The study was part of an outcome reporting audit aimed to document (1) efficacy; (2) safety profile; (3) failure rates; and (4) identification of factors relating to efficacy or resistance to omalizumab, and was approved by the Clinical Audit and Effectiveness team of the Hull University Teaching Hospitals NHS Trust.

The health records of patients with resistant CSU who received XolairÒ (Omalizumab, Novartis) between the years 2017-2019 were reviewed. Omalizumab 300mg was administered subcutaneously with antihistamines every 4 weeks for 6 months, followed by an 8-week treatment interruption. In case of recurrence, further doses were approved after clinic review. Patient demographics, laboratory features (autoantibody status, IgE level, tryptase), weekly urticaria activity score (UAS7) during treatment were analysed. UAS7 at zero was considered complete remission (CR), UAS7 1-28 as partial remission (PR), UAS7>28 as non-responder (NR). Descriptive statistics including parametric and non-parametric tests were done using GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA.

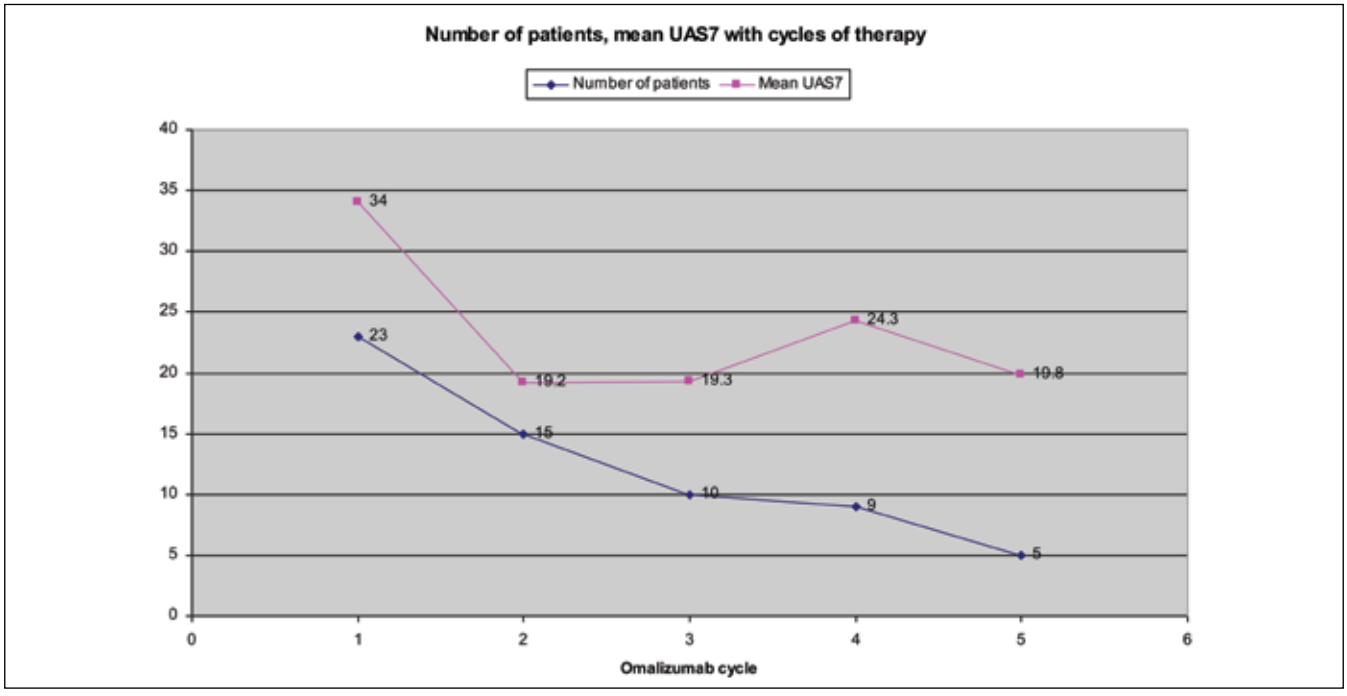
Twenty three patients (18 women, mean age 39.6 years, range 18-76 years) were reviewed. Mean UAS7 at baseline was 34(±SD 5.2) with range 20-42. A total of 396 doses of omalizumab was used (mean of 17 doses). Mean UAS7 post 1st cycle was 19 (±SD 15.1) with range 0-42 (difference in means extremely significant, $p<0.0001$). 6 patients achieved CR after 1st cycle (26%) and 4 in sustained remission (7 months follow up). 13 patients had PR (48%), 6 classed NR (26%). 15 patients required 2nd cycle, with good responses after each dose. 13% patients had sustained effect after 2nd cycle for 4-5 months, while 53% relapsed in 3-4 weeks. 10 patients required 3rd cycle again with excellent responses after each dose, but 9 patients required 4th cycle and 5 patients are on 5th cycle. Overall, 14 patients remain indefinitely on therapy (**figure 1**).

Thirteen of 23 patients had significant angioedema, only 15% attained CR after 1st cycle. Median baseline IgE was 250 U/ml (n=13, IQR25-75 25-470), tryptase 4.9 ng/ml (n=16, IQR25-75 range 3.8-6.5). Antinuclear antibody was negative in all patients tested and two patients were positive for anti-thyroid peroxidase antibodies. There was no difference between baseline IgE level and tryptase with response to omalizumab. A total of 396 doses of omalizumab were given and no serious adverse events such as anaphylaxis were seen. The commonest side effects were injection-site reactions (pain, erythema and itching), headache, slightly raised body temperature and fatigue after a median of 2 weeks of receiving the dose. Omalizumab appeared to be resistant in a third of patients and relapses of urticaria were common following interruption of therapy. No patient-specific factors to predict response to omalizumab were identified, apart from the presence of angioedema that appeared to have a negative outcome.

Our study had limitations with the retrospective nature and with low patient numbers we were unable to use log-transformed IgE to account for atopic status and perhaps why we were unable to find any relationship with total IgE level and response to omalizumab. This contradicts previous published studies where IgE level was a predictor of response. Marzano et al study (n=470) showed a lower mean IgE level (42 kU/L) was associated with resistance (2), similar to Asero et al (n=76) where they showed fast omalizumab responders had higher mean total IgE levels (404 kU/L) than slow responders (112 kU/L) (3), but the authors concluded that much higher numbers are required to make any meaningful comparison. Most studies show a wide range of IgE values between omalizumab responders versus non-responders and as suggested, it is therefore possible that those CSU patients who have a kind of 'auto-allergy' or self-reactive IgE to thyroid antigens or IL-24 have an excellent response to omalizumab (4, 5).

Since omalizumab has been approved by National Institute for Health and Care Excellence (NICE) in the United Kingdom for use in patients with CSU unresponsive to standard treatments, it has proved to be a game-changer in the treatment pathway. It is undoubtedly extremely safe when compared to ciclosporin or dapsone, with no requirement for routine monitoring of bloods. However, a significant number of patients relapse after the first cycle of omalizumab, but respond very well to continuous therapy. Achieving complete remission in CSU with anti-IgE therapy seems a difficult goal, and therefore combining immunosuppressive agents such as ciclosporin or dapsone in lower doses with omalizumab may be the way forward in some patients resistant after the first few doses of omalizumab therapy (6-8). This combined strategy may also reveal additional mechanisms that are at play in CSU and how we can explore further therapeutic options.

Figure 1 - *Patient numbers with cycle of treatment (each with 6 doses) with mean UAS7 scores.*



Note: All patients were selected from initiation of therapy, but were at various phases of treatment in the time period mentioned. UAS7 score was taken for the last week before the injection was due and not an overall mean for 4 weeks between doses.

Conflict of interests

The author declare that they have no conflict of interests.

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Anxiety/depression and impaired asthma control in adolescents. Is an increased basal cholinergic tone a possible link?

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To the Editor

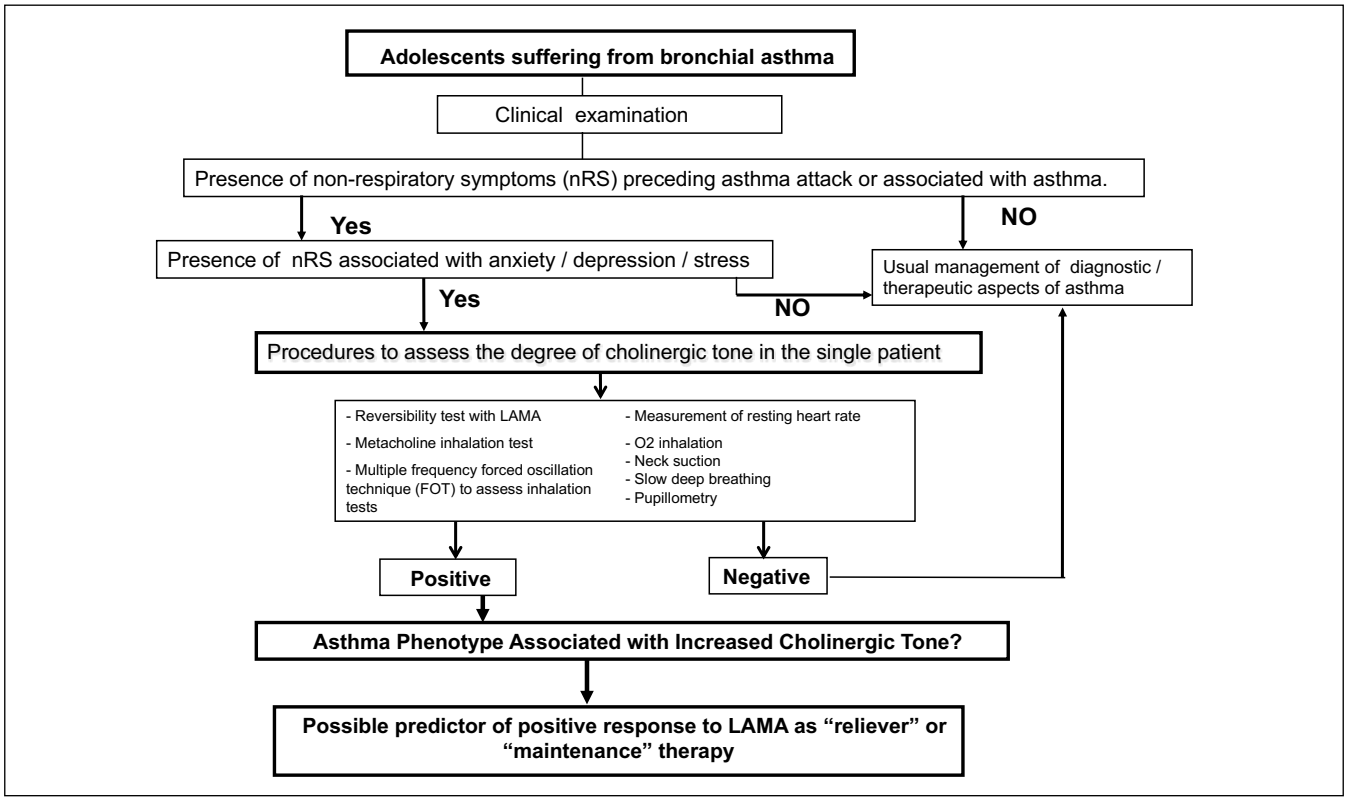
We read with interest the excellent article of Licari et al. (1) reporting that anxiety and depression are regulated with the perception of asthma control in adolescents. Since asthma control grade is significantly correlated with emotional scoring, the authors show that optimal asthma management improves both asthma control and anxiety/depression. However, they did not provide comments on the causal and temporal association between anxiety and asthma outcomes. From a general point of view, it is not clear if a poor asthma control worsens patient's psychological status or vice-versa. Otherwise, both hypotheses are possible. A fundamental premise is that asthmatic adolescents may experience a period of physical and psychosocial changes that affect their health and well-being. Overall, adolescents with asthma are at increased risk for asthma morbidity, asthma death and even suicidal behaviour. Increased rates of depression and anxiety, in adolescents and their caregivers, can lead to non-ad-

herence to their medical regimens, poor symptom control and poor treatment outcomes. Asthma during adolescence impairs health-related quality of life, especially in case of uncontrolled symptoms (2). It has been demonstrated that parental stress and air pollution were synergistically associated with increased childhood asthma, indicating a common biological effect of parental stress and air pollution during both prenatal and postnatal periods. Therefore, we would like to suggest some potential mechanisms to explain the correlation between stress/anxiety and asthma in adolescents. We have previously shown that about 63% of asthmatic patients reported the usual appearance of at least one non-respiratory symptom (n-RS) before an asthma attack (3). Anxiety and, to a lesser extent, depression, represented the most common n-RSs in our study, suggesting that both anxiety and depression may have a possible role in the development and trigger of an asthma attack. Several studies have shown that psychological stress may enhance bronchial hyperreactivity through different mechanisms, such as mast cell activation, mediator release, inflammation, impairment of respiratory tolerance. Moreover, Ritz. et al. (4) reported a significant correlation between psycho-social stress and stimulation of the cholinergic system, resulting in an increased airway resistance. Visual stimulations (i.e. scenes from educational surgery) can rapidly induce (after 1-2 minutes) vagal-mediated responses associated with airway resistance increase. Therefore, we suggest that attention should be focused on the potential role of the parasympathetic system as a trigger of bronchial obstruction in asthmatic adolescents reporting the usual onset of cholinergic-related n-RSs (i.e. stress and/or anxiety) before an asthma attack. Indirectly, the results of our study (5) confirm a significant role of the cholinergic pathway in the enrolled asthmatic subject.

The vagal hyperactivity induced by anxiety and stress in asthmatics also represents the basis of important considerations by a therapeutic point of view, such as the use of anticholinergic agents. Considering this background, we suggest the need of an adequate phenotyping of asthmatic adolescents who could exhibit an increased basal cholinergic tone (6,7). The effect of oxygen and methacholine inhalation, neck suction, slow deep breathing assessed by multiple frequency forced oscillation technique (FOT), as well as measurement of resting heart rate and pupillometry, represent the most effective methods for evaluating the level of vagal tone (8) (**figure 1**).

According to our previous study (5), a simple question exploring the presence of vagal-related n-RSs during the collection of an-

Figure 1 *- Suggested flow-chart for a better phenotyping of asthmatic adolescents suffering from anxiety/depression.*



amnestic data could help to identify asthmatics with imbalance between sympathetic and parasympathetic systems who could benefit of further diagnostic evaluation of vagal tone. Since the degree of cholinergic tone is likely to be different among asthmatics, we believe it is not possible to rule out that the effectiveness of anticholinergic agents such as tiotropium could be greater in patients with an increased degree of cholinergic tone (9). This possible increased responsiveness to tiotropium may be usefully exploited also in the event of poor efficacy or occurrence of adverse events with the use of long-acting β_2 agonists (LABAs) (**figure 1**).

In conclusion, the currently available literature indicates that anxiety and related psychological disorders should be considered as mechanisms that might trigger the airway inflammation, the onset of asthma attacks, and the severity of respiratory symptoms. We concur with Licari et al (1) and others (10) that adequate educational programs should be planned for those asthmatic patients suffering from psychological disorders (both in adults and minors). We believe that this approach requires a peculiar attention in adolescents, in order to obtain a better control of respiratory symptoms in the short term, a delay in asthma progression, and a reduced airway remodeling in the long term.

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Conflict of interests

The authors declare that they have no conflict of interests.

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