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Table of Contents

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Metabolic interactions in asthma

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Key words

asthma; biomarkers; metabolic changes; metabolic pathways; obesity

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Summary

Metabolomics can be used to explore altered metabolic pathways in asthma, giving insights into its pathophysiology. We aimed to review how metabolomics has been used to understand asthma by describing metabolic pathways under research and discussing clinical implications.

The search was performed in PubMed, and studies published since 2000 using a metabolomics approach, were included.

A total of 32 studies were analysed. Pathways related with cellular energy homeostasis, lipid metabolism and oxidative stress, immune and inflammatory processes and others were altered. Initial studies focused on biomarker discovery. But metabolomics can be used to evaluate drug effects on specific pathways, to highlight pathways that can further develop in new targeted treatments, and to identify differences according to asthma severity and phenotypes.

Abbreviations

BALF, bronchoalveolar lavage fluid; CS, corticosteroid; EBC, exhaled breath condensate; GCxGC-TOF/MS, two-dimensional gas chromatography with time-of-flight mass spectrometry; GC-MS: gas chromatography-mass spectrometry; GSH, glutathione; HETE, hydroxyeicosatetraenoic acids; HPETE: hydroperoxyeicosatetraenoic acids; LC-MS: liquid chromatography-mass spectrometry; LC-Q-TOF/MS: liquid chromatography quadrupole / time-of-flight-mass spectrometry; LT, leukotrienes; MS, mass spectrometry; NMR, nuclear magnetic resonance; NO, nitric oxide; PG, prostaglandin; PUFAs, polyunsaturated fatty acids; ROS, reactive oxygen species; TCA, tricarboxylic acid cycle; VLDL, very low-density lipoprotein.

Introduction

Asthma is a heterogeneous condition characterized by variable respiratory symptoms and airflow limitation driven by underlying pathophysiology mechanisms, namely, airway inflammation and remodelling (1). As a complex disease, with genetic and environmental influences, the role of molecular determinants and related pathways are not fully elucidated yet. Additionally, regarding the management and burden of the disease, severe asthma remains a significant clinical problem, and search for biomarkers to improve the target of new treatments is believed to be crucial (2,3). Nowadays, the physician goal in chronic disorders is to offer the best personalized treatment and man-

agement. Precision medicine aims to identify which approaches will be effective for each patient according to genetic, environmental and lifestyle factors (4). However, the application of precision medicine in day-to-day healthcare is still limited, and current research provided by several approaches aims to discover and give insights into biomarkers and key pathophysiology determinants. Metabolomics is an important tool in medical research, being able to manage complex diseases by giving insights over metabolic changes and pathophysiology (5). Studies can be designed to provide metabolic signatures of asthma severity and corticosteroid (CS) resistance, and to help in defining phenotypes or to evaluate treatment effects.

Metabolomics is a comprehensive analysis of metabolites in biological specimens. Metabolites are small molecules, including peptides, amino acids, nucleic acids, carbohydrates, organic acids, vitamins and others small molecules that drive cellular functions, such as energy production, representing the functional phenotype of a cell, tissue or organism (6). Since metabolomics aims to profile a large number of molecules than the standard clinical laboratory techniques, and to cover biological processes and metabolic pathways, it holds promise in biomarker discovery and precision medicine. The most used techniques are nuclear magnetic resonance (NMR) and mass spectrometry (MS) and the main methodologies used for identification can be targeted or untargeted. The untargeted methodology measures the wide range of metabolites extracted in a sample without a priori knowledge of the expected metabolome. The targeted analysis yields higher sensitivity and specificity, since metabolites are analysed based on a priori information, allowing to measure concentrations in the extracted sample. Moreover, targeted analysis is important to validate results from untargeted analysis. The major challenge related with metabolomics is the identification of meaningful metabolites and its validation (6).

This review focus on how metabolomics has been used to understand asthma. Metabolic pathways altered in asthma will be described, considering studies performed in humans. Research and clinical implications will be discussed, as well future perspectives.

Methods

The scientific literature used in this review covered studies published from 2000 to November 2018 in PubMed and was focused on metabolomics applied to asthma. Only full-text in English and trials performed in humans were assessed for eligibility, independently of the type of document (original article, review, comment, conference paper, letters and book chapters). The selected search keywords were “metabolomics” or “metabolic profile” and “asthma”. The adopted inclusion criteria were: a) diagnosis, monitoring or phenotyping of asthma using metabolomics; and b) clinical trials. The exclusion criteria consisted in: a) trials not related with asthma; b) trials not related with metabolomics; and c) trials not related with diagnosis and/or monitoring of asthma. Additionally, some studies were found by cross-referencing.

Results

The systematic search using the aforementioned methodology yielded 89 studies. However, this number was increased to 102 after the inclusion of studies found by reference list searching. During the screening of titles and abstracts using the pre-specified inclusion criteria, 44 studies were rejected (studies not related with asthma n = 23; studies not related with metabolomics n = 14; and studies not related with diagnosis and/or monitoring of asthma n = 4), yielding 58 studies for full revision. After, each of these studies was entirely reviewed. In the end, 32 original articles were found to meet the inclusion criteria. Additionally, 26 reviews, comments, letters and book chapters met inclusion criteria and were used for reference list searching. **Figure 1** illustrates the flow diagram of search and selection process.

Urine (n = 9), serum (n = 6), plasma (n = 4), exhaled breath condensate (EBC) or exhaled breath (EB) (n = 13), and bronchoalveolar lavage fluid (BALF) (n = 1) were used to identify the metabolic profile of patients with asthma. Abnormal metabolic activity is primarily localized in the lung and respiratory tract; however, asthma can lead to systemic metabolic alterations as several circulating metabolites have been found to differ in asthmatics in regard to healthy individuals. MS and NMR were the main techniques used to achieve these discoveries.

Table I summarizes the main altered pathways in asthma found in studies using metabolomics - pathways related with 1) cellular energy homeostasis and hypoxia, 2) lipid metabolism and oxidative stress, 3) immune and inflammatory processes and 4) other pathways were described in several studies. Main metabolic changes reported are related with cellular energy homeostasis since inflammation, bronchoconstriction and airways hyperresponsiveness lead to a higher energetic burden. In response to these events, metabolites involved in tricarboxylic acid (TCA) cycle are increased, especially succinate, fumarate, oxaloacetate, cis-aconitate and 2-oxoglutarate. Poor oxygenation and hypoxic stress can also cause changes in TCA cycle, as well as in lactic fermentation, which is enhanced by inosine, to facilitate metabolism under these conditions. Finally, energetic demand obligates to lipids activation and mobilization. High levels of carnitine and acetyl-carnitine reinforce the oxidative burden, being essential to transport fatty acids into mitochondria for oxidation. Inflammatory status leads to oxidative stress, which triggers lipid peroxidation of polyunsaturated fatty acids (PUFAs) resulting in the release of hydrocarbons and other volatile compounds in the airways and urine. Additionally, some inflammatory markers were found increased, such as nicotinamide, adenosine mo-

nophosphate, arachidonate, arachidonic acid, leukotrienes and prostaglandins, contributing to pathophysiology. Furthermore, metabolites with anti-inflammatory properties were found decreased (urocanic acid). Amino acids metabolism was found deregulated, leading to changes in bile acids production and in urea cycle to eliminate end reaction products.

Most studies were designed to discover biomarkers able to differentiate asthmatics and healthy controls, although severity was also studied (n = 3), as well as corticosteroid resistance (n = 2), asthma control (n = 2) and treatment effects of inhaled therapy (n = 1).

Discussion

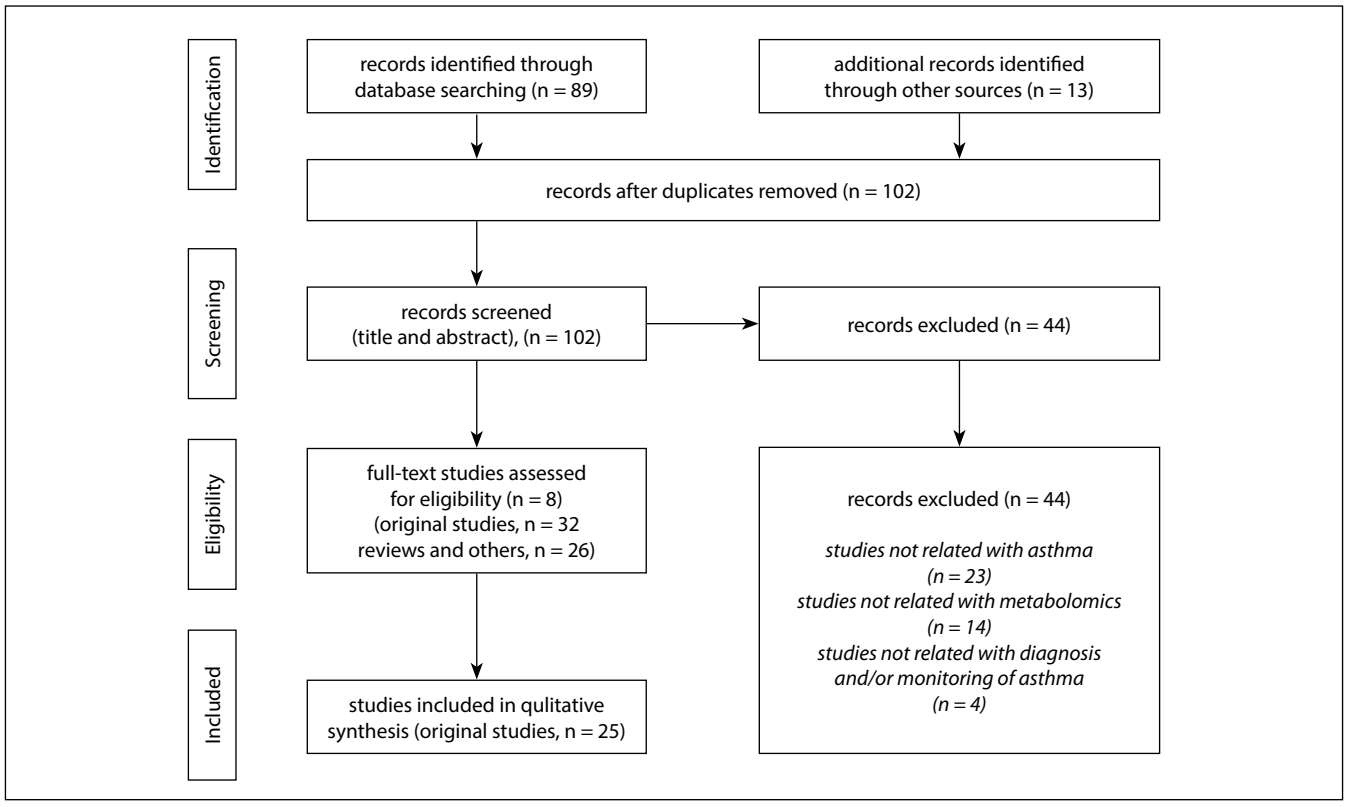
Metabolomics findings

Metabolomics studies revealed several altered pathways associated with asthma. The main findings in human studies, conducted in asthmatics and healthy controls, included not only changes in cellular energy and hypoxia, lipid metabolism and oxidative stress, immune / inflammatory pathways, but also amino acid, steroid, nitrogen and glutamate-glutamine metabolism, as well as bile acids production and vitamins metabolism. Most of these studies were targeted to identify diagnostic biomarkers for asthma or to improve its pathophysiology understanding.

Cellular energy homeostasis and hypoxia. Metabolites involved in tricarboxylic acid (TCA) cycle are increased in asthmatics, which possibly reflects the energetic burden due inflammation and bronchoconstriction. These metabolites were found in urine and serum of asthmatics, and succinate was the most consistent between studies (7-9). Fumarate, oxaloacetate, cis-aconitate and 2-oxoglutarate were also found higher in asthmatics who had recently suffered an exacerbation (9). TCA cycle changes can also be resultant of hypoxic stress due to reduced oxygenation, especially during an exacerbation (8). These changes are supported by the presence of high levels of lactate and low levels of glucose. Additionally, inosine, a breakdown product of adenosine, was increased in asthmatics and is capable of penetrating in cells and enhancing activity of pyruvate oxidase and other enzymes, facilitating cell metabolism under hypoxic stress during poor oxygenation (7).

Lipid metabolism and oxidative stress. Lipid metabolism is enriched in asthmatics since lipids drive inflammatory responses, promote release of histamine and are essential to cellular energy

Figure 1 *- Flow diagram of the search and selection process.*



metabolism (8,9,11-15,22,24). The presence of high levels of LDL, VLDL and its hydrolysis products have been found to activate the release of histamine, which promotes constriction of airways smooth muscle (8,12,26). The energetic demand causes a decrease in glucose levels and lipids can be activated to provide acetyl-CoA (8). Lipids breakdown, under insufficient glucose, leads to production of acetone which was found in high levels in serum of patients (8). However, low levels of acetone were found in a different study conducted in children with asthma which, until now, is a contradictory finding between studies (13). Also, increased levels of carnitine and acetyl-carnitine were found in urine and plasma of asthmatics during exacerbation, which highlights the oxidative burden, since these metabolites are essential to transport fatty acids into mitochondria for oxidation (9,11,12). The increased phosphocholine levels, an important component of the endothelial cell barrier, in the serum of patients, indicates a lack of airways protection (8,9). Moreover, the release of reactive oxygen species (ROS) by inflammatory cells and the decrease in glutathione levels, leads to oxidative stress which triggers lipid peroxidation of the polyunsaturated fatty acids (PUFAs) of cells, reducing the ability of the epithelium for damage repair (8,11,17). The resultant metabolites are compiled in a systematic review (27). End products of lipid peroxidation are mainly hydrocarbons including hexane, heptane, pentanal, heptanal, decanal, octane, nonadecane, 4-methylheptane, 2,4-dimethylheptane 2,4-dimethylpentane, 2-methylpentane and other alkanes and aldehydes (13-17,27-29). Interestingly, in elite swimmers, both with or without asthma, swimming was associated with a decrease in oxidative stress markers (30).

Immune and inflammatory processes. Urocanic acid, which is an intermediate of histidine catabolism and a potent immune-suppressor, was decreased in asthmatics urine and EBC, contributing to a poor resolution of the inflammatory process. Nicotinamide, adenosine monophosphate and arachidonate are inflammatory markers, and were increased in plasma of asthmatics (19). In addition, leukotrienes B4, D4 and E4 were found higher in EBC. Leukotrienes are potent inflammatory lipid mediators and chemoattractant of granulocytes, contributing to the pathophysiology of asthma and being synthetized from arachidonic acid via 5-lipoxygenase. Seventeen PUFAs were found in high levels in the urine of asthmatics, specifically hydroxyeicosatetraenoic acids (HETE), hydroperoxyeicosatetraenoic acids (HPETE), prostaglandins and arachidonic acid. These compounds are biological mediators linked to inflammatory and immune responses. Arachidonate, an inflammatory biomarker and precursor of leukotrienes, was found high in plasma and was positively correlated with taurine levels, highlighting the relation between its oxidation and the release of taurine.

Other metabolic pathways. Amino acid metabolism is also altered in asthmatics. Some amino acids appeared to be found in higher levels and others in lower levels. Glutamine, asparagine, leucine, valine, alanine and arginine were found in low levels in serum and phenylalanine, methionine, histidine and taurine were upregulated (7,8,19). Histamine and its downstream product, 1-methylhistamine, were higher in asthmatics urine, being involved in inflammation and bronchoconstriction (8). The precursor of histamine, histidine, was increased in plasma (8). High taurine levels were found in plasma and its release by cells is associated to taurine-releasing pathways that are activated by arachidonic acid oxidation via 5-lipoxygenase to leukotrienes (19). Taurine levels were also associated with bile acids production (cholate, glycocholate, glycodeoxycholate, taurocholate and lathosterol, an intermediate) which is the major pathway for its elimination (19). Nitrogen metabolism was also changed in the serum of asthmatics, showing low levels of ornithine, citrulline, arginine and formate, suggesting alterations in the urea cycle (important pathway in the excretion of ammonia resultant from amino acids catabolism) (7,8). Sinha et al. detected low levels of ammonia in the EBC and connected the finding to low levels of glutaminase activity, possibly indicating alterations in the glutamate-glutamine cycle (25). Glutaminase is responsible for the generation of glutamate and ammonia from glutamine. This hypothesis is reinforced by Jung et al., that found increased levels of glutamine and glutamate in the serum of asthmatics (8).

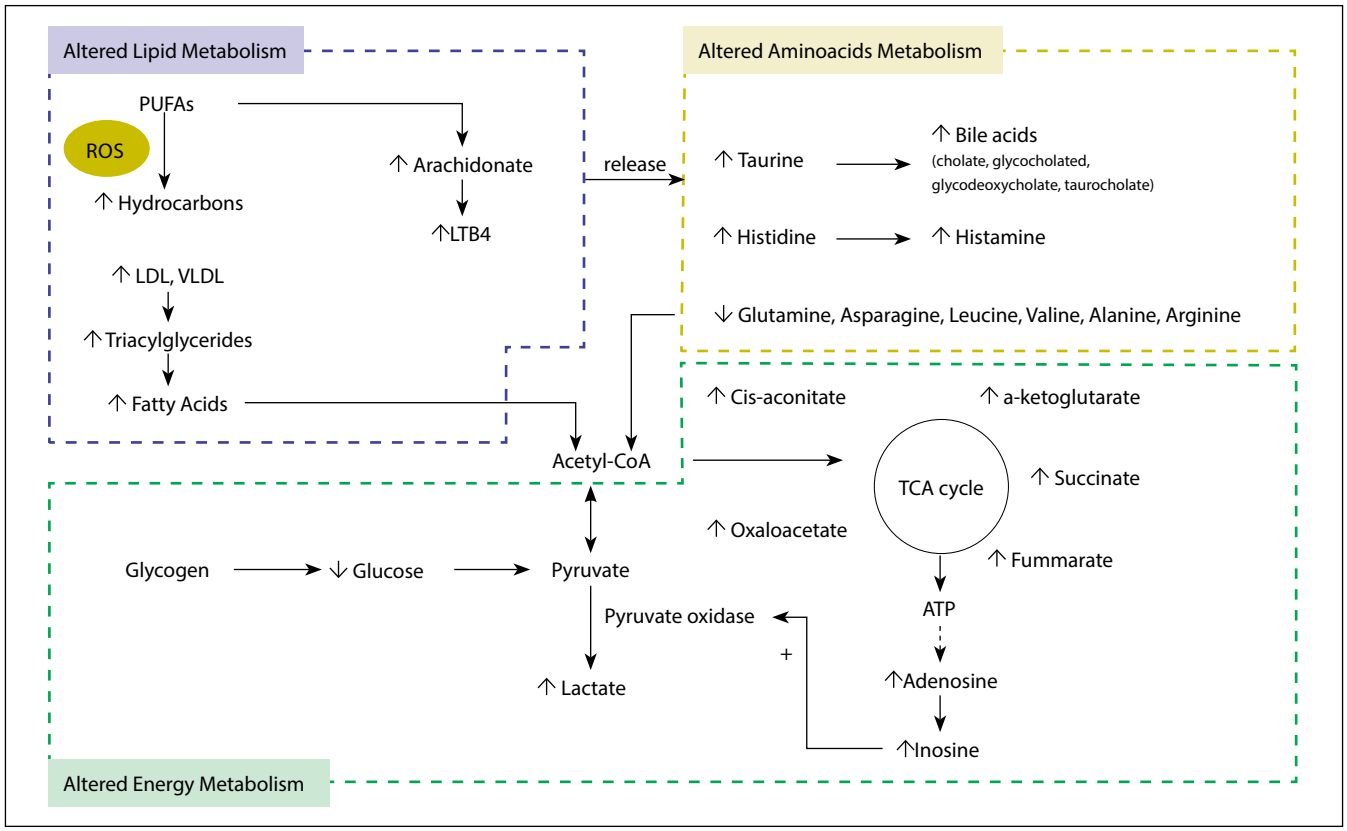
In summary, asthma is associated with abnormalities in energy metabolism such as TCA cycle, lipid and amino acids metabolism, possibly relating to increased respiratory muscles activity and to reduced oxygenation leading to hypoxic stress. Immune and inflammatory markers are also amplified in asthmatics. Some of these altered pathways are schematized in **figure 2**.

Research and Clinical value

Metabolomics studies comparing asthmatics and healthy controls are useful to identify altered metabolic pathways and to expand our knowledge about the disease pathophysiology. Metabolomics can be also useful for clinical practice, such as evaluating consequences and the effect of a specific treatment or giving insights about altered pathways in treatment-resistant subjects and encourage the development of new target therapies (18,19,22,31-34). Metabolomics can also be used to differentiate asthma among other airway diseases, such as chronic obstructive pulmonary disease (35).

Severe asthmatics, usually including those taking high doses of corticosteroid (CS), exhibited pronounced metabolic effects on steroid metabolism when compared to other asthmatics not taking CS therapy or under low doses. This group of patients is characterized by low levels of steroids in plasma (1-stearoylglycerol, dehydroisoandrosterone sulphate, androsterone sulphate and epi-androsterone sulphate) and in urine (dehydroepiandrosterone, cortisone, cortisol, urocortisol and urocortisone)

Figure 2 *- Representation of some of the altered metabolic pathways in asthma linked to cellular energy, lipid and amino acid metabolism. ATP, adenosine triphosphate; LDL, low-density lipoprotein; LTB4, leukotriene B4; PUFA's, polyunsaturated fatty acids; ROS, reactive oxygen species; TCA cycle, tricarboxylic acid cycle; VLDL, very-low-density lipoprotein.*



(18,19,31). Metabolomics revealed a hypothalamus-pituitary-adrenal axis suppression, which is now a well-documented consequence of this treatment. Additionally, decreased levels of prolyl-hydroxyproline (ProHyp) and pipecolic acid were found in urine and were associated to an increased risk of osteoporosis and bone injury due to CS treatment (31). Metabolomics was also used to evaluate the effect of a combined treatment of budesonide and salbutamol in children during asthma acute exacerbation (36). Arginine and proline metabolism, as well TCA cycle, were the most impacted pathways. This combined treatment, improving asthmatic symptoms, interacts also with arginine metabolism, since arginine and its downstream products, such as proline, are involved in collagen synthesis and cell proliferation during tissue remodelling. These findings suggest a potential metabolic reprogramming due to this combined treatment, and contribute to understand metabolic regulation of budesonide and salbutamol in asthmatic children at the molecular level.

Regarding CS resistance, metabolomics can also provide some valuable results to further developing new therapy targets. CS-resistant asthmatic children presented statistically significant differences for some metabolites, such as γ-glutamylcysteine and cysteine-glycine, suggesting a decrease in glutathione (GSH) synthesis (32). GSH, as an antioxidant, plays an important role to prevent oxidative stress and its pathway can be a target for some cases of CS resistance. Moreover, lower levels of ascorbic acid were reported in the serum of children with asthma (17). Ascorbic acid has an important role in protecting lung tissues, especially the alveoli, against oxidative stress, and decreased levels were associated with pulmonary dysfunction (37). On the other hand, retinoic acid was found to be increased in asthmatic children, especially in severe cases, and its levels have been associated to inflammation and airway remodelling in asthma (22). Controlled and uncontrolled asthmatics were also studied, and differences were found in their exhaled breath and plasma metabolomics (33,34). Loss of asthma control was evaluated in a longitudinal study using two breath analysis methods, mass spectrometry and electronic nose technology (33). Participants enrolled in this study had a previous history of medical diagnosis of

mild to moderate persistent asthma, and presented a good control of the disease according to the parameters established in the study. The samples were collected in three phases in time: baseline, loss of control after cessation of inhaled CS, and recovery. GC-MS distinguished the samples with an accuracy of 68-77% and electronic nose achieved an accuracy of 86-95%. Previously, McGeachie et al. studied biochemical predictors of asthma control in the plasma of children with controlled and uncontrolled asthma, using liquid chromatography tandem mass spectrometry (LC-MS) (34). Metabolites related with linoleic acids metabolism (linoleic acid and γ-linoleic acid) and arachidonic acid metabolism (arachidonic acid, 5-HETE, PGE2, 12(S)-HPETE, 15(S)-HETE and LTB4) were different between the two groups, showing a high pathway impact score despite no statistical significant differences, probably due to the small sample size.

These studies provide useful clues that can lead to improvements in diagnosis and inspire further studies to discover new pathological pathways and possible therapeutic targets and biomarkers. Metabolic changes related with asthma severity and control of the disease may be suitable as specific biomarkers for diagnosis and management or to identify targets to develop new specific treatments.

The obesity-related asthma phenotype

There is evidence that obesity increases the risk of developing asthma (38-41). Generally, obese-asthma patients present decreased levels of airway eosinophilic inflammation, increased symptoms, risk of hospitalization, healthcare-associated costs and poor response to CS (39,40). Several proposed mechanisms suggest the pro-inflammatory role of adipocytes, that can lead to the development of airway inflammation and asthma (38). Differences in obese and non-obese asthmatics were detected for some metabolites, methane (energy metabolism) and pyruvate, glyoxylate and dicarboxylate (carbohydrate metabolism) in the EBC (42). Additionally, other mechanisms independent of the inflammatory status can be present, such as hyperglycaemia, hyperinsulinemia and dyslipidaemia in the context of metabolic syndrome. Metabolic syndrome was associated with asthma in a prospective study and an odds ratio of 1.57 (95% CI 1.31-1.87) was achieved after adjustments, being considered a risk factor to develop asthma (43). Other studies also support a relationship between metabolic syndrome and asthma (44-46).

The arginine-NO pathway is altered in asthmatics and in patients with metabolic syndrome, being a potential involved pathway in obese-asthma patients (47,48). Arginine is a substrate for enzymes, such as NO synthases (NOS) and arginases, which are induced by inflammation and arginine availability (reduced in these patients). Arginine can be converted to citrulline in a reaction catalysed by endothelial NOS in the airways, releasing NO. In the aforementioned studies (**table I**), citrulline and arginine were found decreased in asthmatics serum (7,8). Additionally, supplementation with arginine in experimental asthma resulted in a proper arginine balance and a decrease of inflammation and airway hyperreactivity (49). Nevertheless, a clinical trial (NCT00280683) evaluated arginine supplementation in moderate to severe persistent asthmatics, and no significant differences were found in the number of exacerbations, exhaled nitric oxide levels or lung function (50). The effect of arginine supplementation in severe asthmatics, grouped by nitric oxide levels, and citrulline supplementation in overweight late onset asthmatics, are also being studied, but results are not available yet (NCT01841281 and NCT01715844, respectively). Supplementation in subjects with metabolic syndrome also achieved good results on glucose levels, insulin sensitivity, endothelial function and oxidative stress (51).

Dyslipidaemia is also a characteristic of obese subjects, and asthma patients also experienced changes in cholesterol levels. LDL and VLDL were increased in asthmatics plasma and HDL was diminished (8,52). Thus, the use of statins in obese-asthma patients can be convenient. The use of statins added to inhaled CS and bronchodilators in severe obese asthmatics resulted in better asthma control, through ACQ questionnaire evaluation, and improvement of lung function, when compared to non-statin users (53,54). However, a systematic review showed statins seem not to have additional benefits in asthma control, regardless the decrease of airway inflammation and slight improvement of lung function in individuals with mild allergic asthma (55). Still, more research is needed to verify the benefits of statins in certain subpopulations, such as the obese-asthma patient.

Mitochondrial dysfunction in various organs is known in metabolic syndrome and was recently discovered in airway epithelial injury and asthma (56). Mabalirajan et al. showed that 13-S-hydroxyoctadecadienoic acid (13-S-HODE), a lipid metabolite derived from linoleic acid, induces mitochondrial dysfunction in airway epithelia to drive severe asthma in experimental asthma, and demonstrated increased 13-S-HODE levels in human asthmatic airways (57). Moreover, the imbalance between oxidant and antioxidant species may also lead to mitochondrial changes (56). Many mitochondrial-targeted antioxidants have shown beneficial effects in metabolic syndrome and asthma in independent studies, such as coenzyme-Q10 and α-tocopherol (58-61). Coenzyme-Q10 showed beneficial effects by reducing CS dosage in asthmatics and, in experimental metabolic syndrome, prevented hyperinsulinemia, improved endothelial dysfunction and reduced hypertension and oxidative markers (59,60). α-tocopherol also demonstrated promising results in reducing mitochondrial dysfunction in experimental asthma and in individuals with metabolic syndrome (58,61). Mitochondrial dysfunction seems to be shared by both conditions.

Obesity-related asthma phenotype is characterized by a variable and non-eosinophilic inflammation and CS resistance. There-

Table I *- Summary of metabolomics analysis and identification of altered pathways in asthma found in studies conducted in humans (asthmatics vs healthy controls).*

Cellular pathway	Altered metabolites		Biofluid	Method
	high levels	low levels		
1. Cellular energy homeostasis and hypoxia				
cellular energy homeostasis and hypoxia	succinate (7,10), inosine (10), lactate (7)	glucose (7)	serum	GC-MS (10), NMR (7)
	fumarate, oxaloacetate, cis-aconitate and 2-oxoglutarate (11)	-	urine	NMR (11)
2. Lipid metabolism and oxidative stress				
lipid metabolism	VLDL and hydrolysis products, acetone (8)(7), phosphatidylcholines (10)(8)	phosphocholine, choline (8)	serum	NMR (8), MS (10)
	carnitine, acetyl-carnitine (9,11)	-	urine	NMR (9,11)
	carnitine (12), VLDL (12)	-	plasma	NMR (12)
lipid peroxidation and oxidative stress	2,4-dimethylpentane (13), 2,4-dimethylheptane (13), 2-undecenal, octane (13), 2-methylpentane (13), 2-methylhexane (13), 1-(methylsulfanyl)propane (14), ethylbenzene (14), 2-octenal (14), butanoic acid (15), benzoic acid (15), tridecane (15) and other VOC	acetone (13), 2,2,4-trimethylheptane (13), 2,3,6-trimethyloctane (13), 1-pent-2-one (15), undecane (15), p-xylene (15)	exhaled breath	GC-MS (13-15)
	hexane, heptane, pentanal, heptanal, decanal, octane, nonadecane, 4-methylheptane, 2,4-dimethylheptane and other alkanes and aldehydes (16)	-	urine	GCxGC-ToFMS (16)
	hypoxanthine (17)	glutathione (17)	serum	LC-MS (17)
3. Immune and inflammatory processes				
immune and inflammatory processes	histamine, 1-methylhistamine, nicotinamide (9)	urocanic acid (18)	urine	NMR (9), LC-MS (18)
	nicotinamide, adenosine monophosphate, arachidonate (19)	-	plasma	GC-MS (19)
	LTB4 (20,21), LTD4 (21), LTE4 (21), deoxyadenosine (22), thromboxane B2 (22)	urocanic acid, adenosine (23)	EBC	LC-MS (20,22), NMR (23), GC-MS (21)
arachidonic acid pathway	hydroxyeicosatetraenoic acids (15-HETE, 8-HETE, 11-HETE, 5-HETE, 12-HETE), hydroperoxyeicosatetraenoic acids (15-HPETE, 5-HPETE), prostaglandins (PGE1, PGF1a, PGJ2, PGF2a, PGA2, PGB2, 15-keto-PGF2a), arachidonic acid (24)	-	urine	LC-Q-TOF/MS (24)
	20-hydroxy-PGF2a, 6-keto-PGF1a (22)	-	EBC	LC-MS (22)
4. Other pathways				
amino acid metabolism	phenylalanine (7), histidine (8), methionine (8), glycine (8)	asparagine (7), arginine (8), leucine (8), valine (8), alanine (8), isoleucine (8)	serum	GC-MS (7), NMR (8)
	alanine, threonine (11)	-	urine	NMR (11)
	taurine (19)	tyrosine (12), isoleucine (12), leucine (12), valine (12), alanine (12)	plasma	GC-MS (19), NMR (12)
	alanine, proline, phenylalanine, arginine, isoleucine (23)	valine, tyrosine (23)	EBC	NMR (23)
nitrogen metabolism and urea cycle	-	ornithine (7), citrulline (7), formate (8), arginine (8)	serum	GC-MS (7), NMR (8)
	-	creatine (12), creatinine (12)	plasma	NMR (12)
glutamate-glutamine pathway	-	ammonia (25)	EBC	NMR (25)
	glutamate, glutamine (8)	-	serum	NMR (8)
bile acids production pathway	taurine, lathosterol, cholate, glycocholate, glycodeoxycholate, taurocholate (19)	-	plasma	GC-MS (19)
	ursodeoxycholic acid, isodeoxycholic acid (24)	-	urine	LC-Q-TOF/MS (24)
vitamins metabolism	retinoic acid (22)	ercalcitriol (22)	EBC	LC-MS (22)
	-	ascorbic acid (17)	serum	LC-MS (17)

EBC, exhaled breath condensate; GCxGC-TOF/MS, two-dimensional gas chromatography with time-of- flight mass spectrometry; GC-MS, gas chromatography-mass spectrometry; HETE, hydroxyeicosatetraenoic acids; HPETE, hydroperoxyeicosatetraenoic acids; LC-MS, liquid chromatography-mass spectrometry; LC-Q-TOF/MS, liquid chromatography quadrupole / time-of-flight-mass spectrometry; LT, leukotrienes; NMR, nuclear magnetic resonance; PG, prostaglandin; VLDL, very low-density lipoprotein.

fore, it is relevant to studying altered metabolic pathways in this population, and understanding the possible overlapping mechanisms between metabolic syndrome and asthma. Arginine-NO pathway, mitochondrial dysfunction and altered cholesterol levels seem to be common pathophysiological features in both conditions. Thus, exploring metabolic overlapping mechanisms between obesity and asthma could open new therapeutic hypothesis for the obese-asthma phenotype, such as supplementation with arginine, citrulline, statins and mitochondrial target antioxidants.

Conclusions and future perspective

The metabolome is highly dependent of several variables and confounders, such as sample type, sample collection, age, sex, circadian rhythm, exercise, diet, microbiome, medication and other xenobiotics. Other major limitations concern procedure standardization, from data collection to data processing and interpretation, and external validation of the results. However, the pathophysiology understandings described in this review and the recent nature of most studies encourage the design of new ones in this field. Initial studies were focused on biomarker discovery for asthma and performed in asthmatics and healthy controls. Several altered pathways were described and replicated in more than one study, such as cellular energy homeostasis and hypoxia by TCA cycle alterations, lipid metabolism, including induction of lipid peroxidation due to oxidative stress, and increased levels of carnitine and lipids breakdown metabolites; metabolites of immune and inflammatory processes with significant alterations in the arachidonic acid pathway, as well as in other pathways such as amino acid metabolism (up regulation of urea cycle and bile acids production), steroid metabolism, and vitamins metabolism. Still, metabolomics can be used with other purposes, such as to evaluate drug effects on different pathways, and even its adverse consequences, to highlight pathways that can be better studied to achieve new drug targets, and to identify differences according to severity or even phenotypes (obesity-related asthma phenotype). Additionally, these results, despite still limited to date, emphasize the need of longitudinal studies to evaluate predictive biomarkers or to monitor specific treatment approaches.

Conflict of interests

The authors declare that they have no conflict of interest.

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Pru p 3 sublingual immunotherapy ultra-rush protocol is safe and clinically effective

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Keywords
LTP syndrome; peach allergy; Pru p 3; sublingual immunotherapy; ultra-rush protocol

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Summary
Introduction. *Sublingual immunotherapy (SLIT) with Pru p 3 can prevent severe allergic reactions to LTP-containing foods but the standard initiation protocol is time-consuming.* **Aim.** *Establish the safety of a novel ultra-rush initiation protocol for SLIT with Pru p 3.* **Methods.** *Prospective study comparing the side effects of the standard vs novel ultra-rush initiation protocols of SLIT with Pru p 3 in patients with anaphylaxis to LTP.* **Results.** *Fifteen patients were included (standard initiation, 5; ultra-rush initiation, 10), 80% females. All patients had oropharyngeal pruritus during initiation, 80% with spontaneous recovery, but no other gastro-intestinal, respiratory, cutaneous or systemic side effects occurred in any patient of both groups.* **Conclusion.** *The novel ultra-rush protocol halved the build-up time without increasing side effects.*

Introduction

Lipid transfer protein (LTP) is a panallergen widespread throughout the plant kingdom and is a cause of allergic reactions to a large number of taxonomically unrelated plant foods (1, 2). LTP "syndrome" is typically described in adolescent and adult populations, and it is more frequent in the Mediterranean countries (14), with an increasing incidence in recent years (1). Co-factors, such as exercise, non-steroidal anti-inflammatory drugs, alcohol consumption and long fasting periods, can modulate clinical manifestations (5, 6, 7, 8). Strict avoidance of LTP-containing foods is difficult to maintain and has a great impact in quality of life (9). Furthermore, avoidance does not seem to modify the natural course of the disease.

Specific allergen immunotherapy elicits a wide range of immunological mechanisms that allow tolerance to some foods (10, 12, 13). However, side effects are frequently observed, including anaphylaxis, particularly during the build-up phase (11). SLIT with peach extracts has been developed and is a disease-modifying treatment for LTP syndrome (16).

A peach LTP (Pru p 3) extract is commercially available (ALK, Spain. The manufacturer's standard initiation protocol has a duration of 4 days (**Table I**) (15, 16, 17) and adverse reactions to this therapy largely occurred during this build-up phase. Most adverse events were mild oropharyngeal symptoms, easily controlled with antihistamines (15, 16). The 2009 Fernandez-Rivas et al. study was the first double-blind, placebo-controlled, randomized clinical trial assessing the safety and efficacy of SLIT with Pru p 3 (16). The treatment consisted of a build-up phase performed for five days (cumulative dose of 84.94 µg of Pru p 3) followed by six months of three days per week administration of a maintenance dose at home (cumulative dose of 948 µg of Pru p 3) (16). Eighty-two per-

cent of the participants in the active group experienced local adverse reactions, nearly all located to the oropharynx, both in build-up and maintenance phases (16). Systemic reactions were recorded in 13.5% of patients (87.5% of which occurred during the build-up phase) and included skin pruritus, skin erythema, urticaria, rhinoconjunctivitis, stomach pain and diarrhoea (16). No severe adverse events were observed (16). The 2015 Costa et al. study evaluated clinical and immunological parameters in patients that initiated SLIT with Pru p 3 (17). Initiation consisted in a 4-day build-up phase in Day Hospital followed by an outpatient maintenance phase of 3 years. Fifty percent of the patients had local reactions (itching) with spontaneous resolution during induction, and no other side effects on build-up or maintenance, confirming the safety of SLIT with Pru p 3 (17).

The manufacturer's standard protocol is time-consuming for both patient and medical staff. Pereira et al. (2009) described a novel ultra-rush protocol with a build-up phase completed in one day (18). The patient described had oral pruritus and paraesthesia of the tongue and lips during the first three doses but no treatment was required and maintenance dose was reached in one day (18). Daily SLIT treatment was safely completed during one year with no further symptoms and several immunological changes related to immunotherapy were observed (18).

The aim of our study was to compare the frequency and severity of side effects of the novel ultra-rush protocol versus the standard protocol, both during build-up and maintenance phases of Pru p 3 SLIT.

Methods

This retrospective study included patients with, at least, one episode of anaphylaxis after the ingestion of peach or other foods containing LTP and confirmed IgE-mediated sensitivity to Pru p 3, that initiated SLIT with Pru p 3 between the years 2012 and 2018. The inclusion criteria were: unequivocal clinical history of allergy to peach and/or other fruits containing LTP, one or more episodes of anaphylaxis following the ingestion of peach and/or other fruits containing LTP, positive skin prick tests (SPT) to peach extract and/or other fruits containing LTP, positive SPT to Pru p 3, positive specific IgE to peach and Pru p 3.

The skin prick and prick-to-prick tests were performed according to the standardized European protocols (19), using commercialized extracts from ALK and Roxall-Aristegui or foods in nature, respectively. Tests were considered positive when wheals were equal or larger than 3 millimetres compared to the negative control.

Specific IgE measurements were conducted by ImmunoCAP specifications according to manufacturer's recommendations (Thermo Fisher Scientific, Sweden). Results higher than 0.35 kU/L were considered positive.

Oral provocation tests (OPT) were not performed since all patients had anaphylactic episodes after the ingestion of food containing LTP in the previous two years and also declined a challenge that could induce a new anaphylaxis.

All patients or patient caregivers (in patients <18 years-old) signed an informed consent.

Table I. *SLIT with Pru p 3 – standard build-up phase protocol*

	Day	Concentration (µg/mL)	Number of drops	Dosage (µg)	Time between administrations (minutes)
Build-up phase (hospital)	1	0.05	1	0.002	15
			10	0.02	
		0.5	1	0.02	15
			10	0.2	
	2	5	1	0.2	15
			10	2	
	3	50	1	2	15
			2	4	
			5	10	
			10	20	
	4	50	20	40	Unique administration
Maintenance phase (home)	Everyday	50	5	10	Unique administration

Immunotherapy to LTP used the commercialized extract of enriched peach with Pru p 3 (50 µg/mL) from ALK, Spain. The extract drops were administered sublingually after a fasting period of six hours, and kept under the tongue for two minutes before expelling.

Patients were divided in two different initiation protocols, **Table I** and **Table II**:

- Group A (5 patients): standard protocol, according to manufacturer's instructions, with a total duration of 4 days under medical supervision until maintenance dose;
- Group B (10 patients): novel ultra-rush protocol, as detailed in **table II**, and with a total duration of 2 days under medical supervision. The maintenance dose was reached in the first day with the ultra-rush protocol and the total duration was two days (**table II**).

The assignment to each protocol was performed in two manners: (1) a chronological way, with the first patients initiating with the standard protocol; and (2) according to the preferred method of the prescribing doctor. Both the chronology of patients' appointment and doctor's distribution of the patients at the first appointment were random.

The enriched extract (50 µg/mL) was diluted into three concentrations in the standard protocol (5 µg/mL, 0.5 µg/mL and 0.05 µg/mL) and two concentrations in the ultra-rush protocol (5 µg/mL and 0.5 µg/mL) for immunotherapy initiation and build-up phase.

In both protocols, patients were under permanent medical supervision during the initiation and any suspected adverse effects were promptly evaluated and treated when necessary.

At 12 months after SLIT initiation, patients were re-evaluated: adherence to the SLIT was confirmed, new episodes of contact with LTP containing foods were reviewed, SPT were performed, and sIgE to Pru p 3 and other relevant foods were measured.

Normality test, two-tailed Mann-Whitney U-test and Fisher Exact Test were calculated where appropriate using STATA (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC). The level of significance considered was $\alpha = 0.05$.

All patients and/or guardians signed informed consent for the study.

Results

The study included fifteen patients with a confirmed diagnosis of IgE-mediated anaphylaxis to LTP that initiated SLIT to Pru p 3. Five patients underwent the standard SLIT initiation protocol (group A) and 10 patients initiated SLIT using the novel ultra-rush protocol (group B). The cumulative allergen doses in the build-up phase were different between the two groups, 47 µg in the ultra-rush protocol group and 78 µg in the standard group, up to the daily maintenance dose of 10 µg of Pru p 3. Detailed demographic data, clinical aspects and allergy workup are shown in **table III** for both groups.

Table II. *SLIT with Pru p 3 - ultra-rush build-up phase protocol*

	Day	Concentration (µg/mL)	Number of drops	Dosage (µg/mL)	Time between administrations (minutes)
Build-up phase (hospital)	1	0.5	1	0.02	30
			5	0.1	
		5	1	0.2	30
			3	0.6	
			5	1	
		50	1	2	30
			2	4	
			3	6	
			4	8	
			5	10	
	2	50	3	6	60
			5	10	
Maintenance phase (home)	Everyday	50	5	10	Unique administration

Most patients included in the study were females (80%), with a median age of 23.4 years-old at the beginning of the treatment (minimum of 17, maximum of 35). Atopy was present in 73% of the patients, and rhinitis was the most concomitant diagnosis (60%), followed by asthma (27%) and atopic dermatitis (7%).
All patients had at least one episode of anaphylaxis, and peach was the most frequently implicated fruit (5/15), followed by tomato (3/15) and pear (2/15). Other culprit foods were plum, cherry, hazelnut, rice and fig (one patient each). Co-factors were present in 3 patients, namely exercise, NSAIDs intake and alcohol ingestion.
All patients had positive SPT to commercial peach extract, with an average weal diameter of 7 mm (minimum of 3, maximum of 11). The 8 patients submitted to prick-to-prick tests with skin and pulp of peach had positive results, with an average weal of 8 and 7 mm, respectively. Prick-to-prick with peach was not performed in seven patients because of seasonal unavailability of the fruit. SPT with Pru p 3 extract was positive in eleven patients (average weal of 10 mm), negative in two and not performed in two other patients (extract was out of stock).
The initial measurement of sIgE to peach and Pru p 3 was positive in all patients, with an average concentration of 22.8 kU/L (minimum 1.13 kU/L, maximum >100 kU/L) for peach and 22.3 kU/L (minimum of 1.69 kU/L, maximum of >100 kU/L) for Pru p 3. Only one patient was monosensitized to peach, while the rest of the patients had polysensitization to other LTP-containing foods, as confirmed by sIgE.

Table III. *Clinical and demographic data of patients that initiated standard protocol of SLIT with Pru p 3 (group A) and ultra-rush protocol of SLIT with Pru p 3 (group B)*

Protocol	Patient	Sex	Atopy	Age when initiated SLIT (years)	Duration of SLIT (months)	Anti-histamine during initiation*	Culprit food	Co-factors	SPT peach (mm)	SPT Pru p 3 (mm)	Prick-to-prick peach skin (mm)	Prick-to-prick peach polp (mm)	sIgE peach before SLIT (kU/L)	sIgE peach 12 months after SLIT (kU/L)	sIgE Pru p 3 before SLIT (kU/L)	sIgE Pru p 3 12 months after SLIT (kU/L)	sIgE to culprit food before SLIT (kU/L)	Other food-containing LTP sensitizations
Group A Standard protocol of SLIT with Pru p 3	1	F	R A	26	40	no	peach	no	3	4	8	9	6.73	0.74	9.28	0.15	6.73	yes
	2	F	R AE	26	12	no	fig	no	8	ND	ND	ND	>100	50.1	>100	61.5	1.41	yes
	3	F	A	27	15	no	tomato	no	9	13	ND	ND	26.7	19.7	21.2	19.2	1.84	yes
	4	F	A	23	3	no	pear	no	7	9	ND	ND	10.2	ND	11	ND	6.61	yes
	5	F	R A	17	3	no	pear	no	6	12	ND	ND	1.75	ND	1.69	8.64	0.53	yes
Group B Ultra-rush protocol of SLIT with Pru p 3	1	F	R	26	14	no	peach	no	8	negative	8	4	4.6	5.8	2.09	8.73	4.6	yes
	2	F	no	26	54	no	plum	exercise alcohol	6	10	5	4	4.3	2.2	2.77	1.84	0.92	yes
	3	F	no	27	46	no	tomato	no	6	6	5	7	19.3	6.2	19.9	8.9	2.1	yes
	4	F	R A	23	17	no	cherry	exercise	5	14	7	8	2.83	13.9	2.99	15.3	1.61	yes
	5	F	no	17	17	no	hazelnut	no	9	6	7	6	30.7	19.8	39.9	28	3.39	yes
	6	M	no	35	15	yes	rice	no	4	negative	5	5	2.47	1.09	3.61	1.11	1.99	yes
	7	M	no	22	16	yes	tomato	alcohol NSAID	7	6	ND	ND	49.1	53.3	41.3	69.5	14.2	yes
	8	M	R A	19	10	no	peach	exercise	8	8	ND	ND	80.2	ND	84.7	ND	80.2	yes
	9	F	R	17	16	no	peach	no	8	8	ND	ND	1.13	ND	5.46	ND	1.13	no
	10	F	R	20	16	no*	peach	no	11	ND	12	12	3.96	1.36	3.84	ND	3.96	yes

Legend: SLIT, sublingual immunotherapy; SPT, skin prick tests; OPC, oral provocation challenge; F, female; M, male; NSAID, non-steroidal anti-inflammatory drug; R, allergic rhinitis; A, bronchial asthma; AE, atopic eczema; mm, millimeters; ND, not done. For statistical analysis, when sIgE values were measured above detection limit of the technique (>100 kU/L), the value 100 was considered for calculations.

* Patient that initiated Pru p 3 SLIT with the standard protocol and switched to ultra-rush protocol after refractory oropharyngeal pruritus during standard build-up.

Twelve months after SLIT initiation, sIgE concentrations to peach averaged 15.8 kU/L, an average reduction of 7.0 kU/L (non-significant, p=0.1744), having increased in three patients, all from the ultra-rush group, and decreased in eight patients. The average sIgE to Pru p 3 after 12 months of treatment was 20.3 kU/L, an average decrease of 2 kU/L (non-significant, p=0.70) with increased concentrations observed in four patients, three of which from the ultra-rush group.

During the build-up phase, all patients reported mild symptoms attributable to therapy (**table IV**). The symptoms were oropharyngeal pruritus and tongue paraesthesia.

One patient started the immunotherapy with the standard protocol but was switched to the ultra-rush protocol (and for analysis she was included in this group). On the first day of the induction using the standard protocol, she developed oropharyngeal pruritus refractory to anti-histamine treatment and required additional doses of treatment. Two hours after complete resolution of the symptoms, SLIT treatment was reinitiated using the ultra-rush protocol without any symptoms during the rest of the build-up phase. Anti-histamine was administered in two patients from the ultra-rush group, both with rapid resolution of symptoms and both continued the induction with no further symptoms.

All the remaining patients from both groups had mild oropharyngeal pruritus during the first three doses of the administration of the extract without requiring any relieve medication, and proceeded with good tolerance to the following doses, having continued and completed the protocol with no further symp-

Table IV. *Description of duration of Pru p 3 SLIT and side effects*

	Total population	**Standard protocol group**	**Ultra-rush protocol group**	**Difference between treatment groups**
Number of patients	15	5	10	-
Age (median, mean, min., max.) (years)	22, 23, 17, 35	22, 23, 17, 33	20, 23, 17, 35	Mean: NS p=0.667
Female (n, %)	12, 80%	5, 100%	7, 70%	
Duration of the initiation phase (days)	-	4	2	-
Duration of treatment (median, mean, min., max.) (months)	16, 20, 3, 54	12, 15, 3, 40	16, 22, 10, 54	Mean: NS p=0. 112
Number of patients with side effects during initiation (n, %)	15, 100%	6, 100%*	10, 100%	-
Number of patients requiring medication for side effects during initiation (n, %)	3, 20%	1, 16.7%*	2, 20%	NS p=1.000
Number of patients with side effects during maintenance	0	0	0	-
Number of patients requiring medication for side effects during maintenance	0	0	0	-
sIgE peach before SLIT (average) (kU/L)	22.9	29	19.9	NS, P=0.582
sIgE peach after 1 year of treatment (average) (kU/L)	15.8	23.5	13	NA**
Average difference in sIgE peach before vs after 1 year of treatment (kU/L)	-7.1 NS, p=0.603	-5.5 NA**	-6.9 NS, p=0.757	NA**
sIgE Pru p 3 before SLIT (average) (kU/L)	23.3	28.7	20.7	NS, p=0.667
sIgE Pru p 3 after 1 year of treatment (average) (kU/L)	20.5	22.4	19.1	NA**
Average difference in sIgE Pru p 3 before vs after 1 year of treatment (kU/L)	-2.8 NS, p=0.795	-6.3 NA**	-1.6 NS, p=0.960	NA**

Legend: NA, non-applicable

* One patient initiated Pru p 3 SLIT with the standard protocol and shifted to ultra-rush protocol after manifestations of refractory oropharyngeal pruritus. **NA, not applicable (when groups had less than 5 samples, continuous variable statistics were not calculated).

toms. No systemic side effects were observed during the build-up phase in any patient. No patients reported additional side effects during the maintenance doses administrated at home (duration of treatment described below).

At the moment, 8 patients are currently on SLIT treatment, having completed a median of 24 months (minimum of 10, maximum of 54), all from the ultra-rush protocol group (**table IV**). On the standard protocol group all patients interrupted SLIT: one patient decided to interrupt the treatment herself after completing 40 months of immunotherapy; another patient also decided herself to stop the SLIT when she found she was pregnant; a third patient had to interrupt because SLIT was temporarily unavailable in the market; the other two patients interrupted due to the high costs of the treatment. In total, 4 patients interrupted the treatment for economic reasons, two from each group, with an average treatment duration of 10 months. No additional side effects were reported during the maintenance doses taken at home.

One of the patients that completed 40 months of immunotherapy (with the standard protocol initiation) reported an episode of urticaria during exercise after the ingestion of apple with skin occurring one year after stopping treatment. There were no new episodes of anaphylaxis reported by any patient.

Discussion

In our case series, both the standard and the novel ultra-rush protocols for the initiation of SLIT with Pru p 3 were safe and well-tolerated. In both groups, the symptoms reported during the build-up phase were mild and localized to the oropharynx, with spontaneous resolution in the majority of cases. The patient with the most severe secondary effects started the SLIT according to the standard protocol, but had complete relieve after the administration of two rounds of anti-histamine treatment. The medical staff decided to change the build-up protocol to the ultra-rush, which was better tolerated and completed without further symptoms. The decision to change for the ultra-rush protocol was based on previous experience with the ultra-rush protocol patients and also taking into consideration well-known mechanisms of sub-lingual immunotherapy (19). Only two other patients needed anti-histamine for mild pruritus of the mouth and lips, but also continued the build-up protocol and reached maintenance dose with success after recovery. In addition, no systemic side effects were observed during the build-up and maintenance phases, emphasizing the good tolerance to this treatment. The novel ultra-rush protocol here proposed corresponds to a reduction to half the time when compared to the standard protocol, abbreviating the inconvenience for both patient and medical staff.

Specific IgE to peach and Pru p 3 was reduced (albeit not statistically significant, possibly due to small group size) and there were no clinical reactions to LTP containing foods (no new episodes of anaphylaxis after the ingestion of LTP-containing foods were reported by any patient after SLIT initiation). Taking into consideration the short length of the build-up phase (2 or 4 days) in the total duration of SLIT treatment, we do not predict any differences in efficacy between groups.

The most common culprit food in our cohort was peach, in accordance to what has been described for the Mediterranean area. Nevertheless, eight other LTP containing foods were responsible for the anaphylaxis in the two groups, once again demonstrating the variability of foods implicated in LTP syndrome.

In Portugal, specific immunotherapy with allergens is not subsidized by the national health system. SLIT with Pru p 3 is an expensive treatment, with an approximate cost of 1000 euros per year, and it is entirely supported by the patients. This is a limitation in terms of adherence that we could observe in our cohort.

In terms of limitations of this study, we point the small sample, in part caused by the costly access to this therapy. Only a few studies have been published describing SLIT to Pru p 3 and randomized double-blind controlled trials with larger samples are required to assess efficacy.

One patient from the standard protocol group that was on the twelfth month of treatment, decided herself to interrupt the treatment when she found out that she was pregnant. This was in contradiction to medical recommendations, since she was on maintenance doses which is not a contraindication to the continuation of the immunotherapy in pregnancy.

Conclusions

SLIT with Pru p 3 is a safe treatment for patients with LTP syndrome, including for those with severe manifestations, such as anaphylaxis. The ultra-rush build-up protocol here presented requires two days of medical supervision, which is more convenient than the four days expected with the standard build-up schedule. Our study demonstrates that this novel protocol is safe and well tolerated.

Conflicts of interest

The authors declare that they have no conflict of interests

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Favorable clinical efficacy of mepolizumab on the upper and lower airways in severe eosinophilic asthma: a 48-week pilot study

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Key words

eosinophilic asthma; forced expiratory volume in one second; Lund-MacKay CT scoring; mepolizumab; SNOT-22 score

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Summary

Objective. *Assessing efficacy of mepolizumab on the upper and lower airways in severe eosinophilic asthma patients.* **Patients and methods.** *This study was a 48-week prospective open-label analysis of mepolizumab in 11 asthmatics with chronic rhinosinusitis (CRS). It was administered every 4 weeks. Six patients were aspirin-exacerbated respiratory disease (AERD).* **Results.** *Blood eosinophil count was reduced after the first administration, and was continued until 48 weeks. The Sino-Nasal Outcome Test scores, the Lund-MacKay CT scoring, and forced expiratory volume in 1 second were improved. Symptom scores of anosmia and nasal congestion were not improved in the patients with AERD. All oral corticosteroid-dependent patients successfully withdrew from corticosteroids.* **Conclusions.** *This pilot study showed mepolizumab improved nasal symptoms and lung function in severe eosinophilic asthma patients with CRS, suggesting efficacy of mepolizumab on the upper and lower airway symptoms in eosinophilic asthma.*

Introduction

It has been clearly demonstrated that the upper and lower airway diseases share common immunopathological mechanisms (1,2). The term "one airway disease" has been established between not only allergic rhinitis, but also chronic rhinosinusitis (CRS) and asthma (3,4). Clinical relationship between CRS and asthma has been a growing health concern, and reported incidence of asthma is at least 50% in patients with CRS (5).

Current consensus in Europe and the United States discerns 2 major phenotypes defined as subgroups of patients homogeneous clinically observations: CRS with nasal polyps and CRS without nasal polyps (6,7). The former in white patients is characterized by eosinophilic inflammation with high interleukin 5 (IL-5) level in the tissue (8). Also, investigations of cytokine profiles in Japanese patients with CRS demonstrated that eosinophilic infiltration was a common histological feature, and cytokine profiles in the Japanese resembled those in Europe and the United States (9-11).

Because IL-5 plays a key role on chemotaxis, differentiation, activation, and survival of eosinophils (12), and because those cells represent such prominent characteristics in the polyps, antagonism of IL-5 has been considered a therapeutic target. The first pilot study with reslizumab, a humanized IL-5 antibody, showed a significant reduction of the size of nasal polyps after a single intravenous injection (13). Hence, the principle of IL-5 antagonism was established in eosinophilic nasal polyps.

Mepolizumab, a humanized IgG_1 monoclonal antibody that blocks human IL-5 from binding to the IL-5 receptor, has been shown to be a potential novel therapeutic approach in patients with severe eosinophilic nasal polyposis (14). An international randomized, double-blinded, placebo-controlled, multicenter study including 105 patients treated with mepolizumab or placebo showed that in patients with recurrent nasal polyposis re-

ceiving topical corticosteroids, mepolizumab treatment led to a greater reduction in the need of surgery and a greater improvement on symptoms than placebo (15).

Aspirin-exacerbated respiratory disease (AERD) is the triad of CRS with nasal polyposis, adverse reaction to aspirin, and asthma (16). A recent retrospective analysis of mepolizumab in 22 patients with AERD provided clinical evidences that IL-5 inhibition improved subject-reported upper and lower airway symptoms, but not improved forced expiratory volume in 1 second (FEV_1) (17). To our knowledge, no data has been reported on the benefit of mepolizumab in severe eosinophilic asthma patients with CRS. This is the first prospective open-label pilot study of 48-week subcutaneous administration of mepolizumab in Japanese patients of severe eosinophilic asthma with CRS. The primary objective of this study was to investigate whether mepolizumab treatment may improve the symptoms of CRS and the findings of computed tomography (CT) scan opacification of paranasal sinuses. Second, we compared the response to mepolizumab on nasal symptoms and the findings of CT scan opacification of paranasal sinuses between patients with AERD and those without AERD. Next, we assessed the changes of FEV_1 with mepolizumab treatment. Finally, we investigated whether mepolizumab possess oral corticosteroid-sparing effect in the patients.

Patients and methods

Patients

The study population comprised 11 subjects (6 males and 5 females), median age 55.0 years in the age range 29 - 69 years in males, and median age 50.4 years in the age range 44 - 56 years in females, respectively. The diagnosis of bronchial asthma was confirmed based on the Global Initiative for Asthma (GINA) guidelines (18). Enrolled patients in this study were required to have received a clinical diagnosis of bronchial asthma by experienced pulmonologists. All patients showed that FEV_1 measured with a spirometer was less than 80% of the predicted value for age, sex, and height, with documented short-acting $ß_2$ agonist reversibility of more than 12% after administration of 180 µg of salbutamol. Six patients (3 each in males and females) were AERD, who were diagnosed as reported (19). Four patients (3 males and 1 female, and 1 AERD male) had been receiving maintenance treatment with oral corticosteroids (5 to 10 mg per day of prednisone or its equivalent) for at least 6 months before entering the study.

The diagnostic guidelines established by the American Academy of Otorhinolaryngology - Head and Neck Surgery were met in each patient for the diagnosis of CRS (20). All patients had been diagnosed with the presence of nasal polyps using a nasal endoscope by experienced otolaryngologists at other hospitals before the treatment. Then, they had undergone a pretreatment CT scan of paranasal sinuses, and diagnostic evidence of CRS was defined by experienced radiologist of our hospital using the Lund-Mackay (LM) scores (21). The Japanese Epidemiological Survey of Refractory Eosinophilic Chronic Rhinosinusitis (JESREC), which was a retrospective study conducted by 15 institutions in Japan, has subdivided CRS into non-eosinophilic and eosinophilic CRS using the JESREC score criteria (22), and the score was assessed from each patient in this study. Clinical characteristics of the study patients are shown in **table I**.

All patients had to have experienced at least 2 asthma exacerbations in the previous year that were treated with systemic corticosteroids administered intravenously or orally for more than 3 days, or that required a visit to the emergency department and/or hospitalization. They were receiving treatment with an inhaled corticosteroid at high-dosage of more than 500 µg fluticasone dry powder or equivalent daily dosage / long-acting $ß_2$ agonists inhalers with an additional controller, for 12 months before enrollment. In addition, all patients had to have an eosinophil count at least 150 cells/µl in blood at screening or at least 300 cells/µl at some time during the previous year. Patients were allowed to continue their current therapy throughout the study. The exclusion criteria included present smoking, a past history of smoking greater than 10 pack-years, parasitic infection in the 6 months before study entry, substantial uncontrolled co-morbidity, possibility of pregnancy, and history of poor treatment adherence.

Mepolizumab 100 mg was administered subcutaneously at baseline (visit 1; week 0), and then every 4 weeks for a total 48 weeks as an add-on to appropriate standard care that could be adjusted at the physician's discretion. Thirteen visits were completed to 48 weeks. Patients were asked about exacerbations at every 4-week clinic visit from baseline to week 48 (exit visit). Safety was evaluated at each visit by assessment of adverse events, vital signs and electrocardiographic findings along with clinical labo-

Table I *- Clinical characteristics of the study patients.*

gender	**male**	**female**
number of patients	6	5
age (years) (median)	55.0	50.4
allergic	2	0
non-allergic	4	5
duration of asthma (years) (mean)	16.7	18.6
aspirin hypersensitivity (AERD)	3	3
oral corticosteroid-dependent	3	1

AERD, aspirin-exacerbated respiratory disease.

ratory testing variables at baseline (week 0) and at weeks 24 and 48. Blood eosinophil count was assessed from baseline and every 4 weeks until week 48. FEV_1 was measured at baseline and at weeks 24 and 48 (exit visit).

This study was performed in accordance with Good Clinical Practice guidelines, and the ethics principles outlined in the Declaration of Helsinki 2008, and approved by the Institutional Ethics Committee of Sutoh Hospital (IRB#20160051). Written informed consent was obtained from each individual before the study commenced. This study was conducted between June 2016 and December 2018.

Clinical measurements

Eosinophils in peripheral blood were counted automatically using a counter (Beckman Coulter, Fullerton, CA, USA) and MAXM A/L system (Beckman Coulter). Serum levels of total immunoglobulin E (IgE) were measured using the CAP system (Phadia, Uppsala, Sweden). The percentages of predicted FEV_1 were measured using a spirometer (FUKUDA-77, Fukuda Denshi, Tokyo, Japan), and the best of 3 expirations was recorded.

The Sino-Nasal Outcome Test (SNOT-22) questionnaire (23) is a modification of a pre-existing instrument, the SNOT-20 (24) with 2 additional questions about anosmia and nasal congestion. The SNOT-22 is a validated questionnaire quantifying upper respiratory tract symptoms. Each subject completed the SNOT-22 by answering all questions based on a 0-5 scale, where 0 defines no problems with the given symptom and 5 defines maximal problems. The scores range from 0 to 110, with high scores indicating greater symptoms, and a change of 8.9 or more points represents a minimally important difference (23). In this study, each subject completed the SNOT-22 at baseline and at week 48.

The findings of CT scan opacification of paranasal sinuses in each patient was blindly staged by the same radiologist using the LM score system at baseline and at week 48. In the scoring system, each paranasal sinus (anterior ethmoid, posterior ethmoid, maxillary, frontal, and sphenoid sinus on the right and left sides) was assigned a score (0 for no opacification, 1 for partial opacification, and 2 for total opacification), and the ostiomeatal complex on each side was also assigned a score (0 for patent, 1 for partially obstructed, and 2 for completely obstructed). So, the total score ranges from 0 to 24. An LM score less than 4 was classified as no CT abnormality, and an LM score greater than or equal to 4 was classified as CT abnormality, suspecting the presence of CRS.

The JESREC scoring system (22) assessed either unilateral or bilateral, the presence of nasal polyps, number of peripheral blood eosinophils, and dominant shadow of ethmoid sinuses in CT scans of paranasal sinuses. A JESREC score higher than or equal to 11 was determined as eosinophilic CRS (22). We evaluated the JESREC score from each patient before starting mepolizumab treatment.

Statistical analyses

Data are presented as mean ± SD or numbers of observations, unless stated otherwise. Difference in study variables over time was analyzed using the Dunnett multiple comparison test. The Wilcoxon signed-rank test was used to compare paired data. All statistical analyses were performed using Microsoft Excel for Mac 2011. A p value < 0.05 was considered significant.

Results

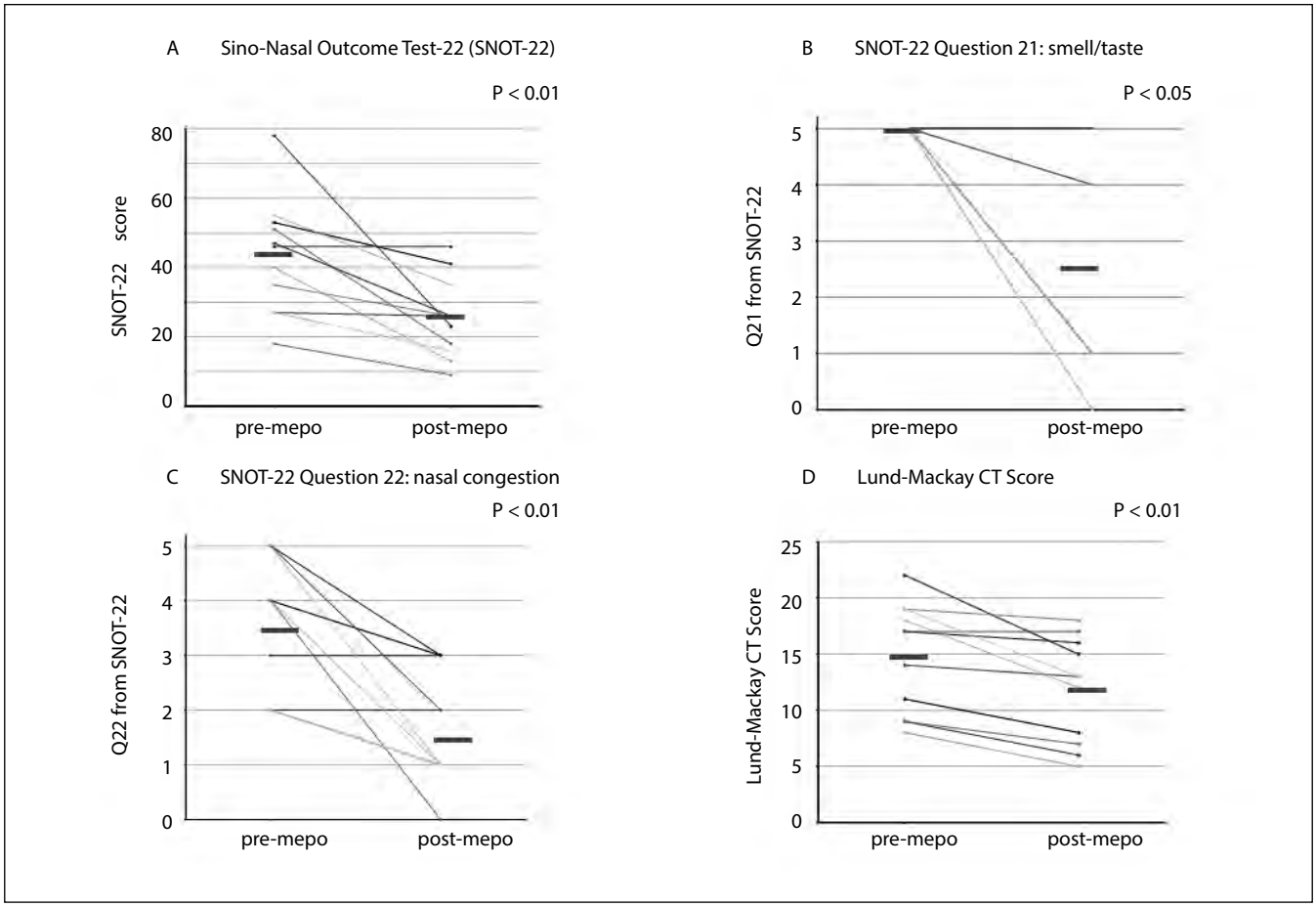
No patients failed to continue mepolizumab treatment because of adverse events, such as local injection site reactions and anaphylactic reactions, and none of them complained of headache or signs of nasopharyngitis. No clinically relevant trends were observed in vital signs, electrocardiographic findings, or clinical laboratory testing data. All patients continued to receive mepolizumab throughout the trial period without exacerbations. The mean of the JESRES score before starting mepolizumab treatment was 12.6 in the score range 11-17.

Blood eosinophil counts at baseline were 409.8 ± 259.1 (mean ± SD), and it showed a rapid and sustained reduction with mepolizumab (at weeks 4, 24 and 48, the counts were 82.9 ± 42.3, 55.7 ± 60.9 and 49.5 ± 35.5, respectively; each $p < 0.01$).

The total SNOT-22 scores decreased by 18.0 points ($p < 0.01$; **figure 1**, **A**). Symptom scores of anosmia (SNOT-22 question 21, **figure 1**, **B**) and nasal congestion (SNOT-22 question 22, **figure 1**, **C**) decreased by 2.5 points ($p < 0.05$), and decreased by 1.9 points ($p < 0.01$). The LM score decreased by 3.0 points ($p < 0.01$; **figure 1**, **D**). In aspirin-tolerant patients, the total SNOT-22 scores (**figure 2**, **A**), the SNOT-22 question 21 score (**figure 2**, **B**), the SNOT-22 question 22 score (**figure 2**, **C)**, and the LM score (**figure 2**, **D**) decreased by 15.2 points, 3.8 points, 2.0 points, and 3.6 points, respectively (each $p < 0.01$). In AERD patients, the total SNOT-22 scores (**figure 3**, **A**) and the LM score (**figure 3**, **D**) decreased by 20.3 points and 2.5 points (both $p < 0.05$), but not the SNOT-22 question 21 score (**figure 3**, **B**) and the SNOT-22 question 22 score (**figure 3**, **C**). Some patients did not provide a change of SNOT-22 and/or LM score after the period of observation (**table II**). Namely, among 6 AERD patients 2 patients did not provide a change of SNOT-22 score, and 3 patients did not provide a change of LM score change. On the other hand, a patient with allergic, oral corticosteroid-dependent asthma did not show a change of LM score.

FEV_1 at week 24 and at week 48 was 73.3 ± 8.4 % and 73.9 ± 8.8 % respectively, and increased compared with 69.0 ± 10.5 % at baseline (both $p < 0.05$) (**figure 4**).

Figure 1 *- Change in SNOT scores and the Lund-MacKay CT scorings before mepolizumab therapy (0 week) and 48 weeks after start of the therapy in 11 patients. A significant reduction of total SNOT-22 scores and the CT scorings was seen at week 48 after mepolizumab treatment. Also, SNOT-22 Question 21 and 22 were significantly reduced with the treatment, indicating an improvement in sinonasal symptoms of the patients with mepolizumab treatment.* **A**, *total SNOT-22 scores;* **B**, *SNOT-22 question 21 (smell / taste);* **C**, *SNOT-22 question 22 (nasal congestion);* **D**, *Lund-MacKay CT scorings. Gray horizontal lines represent group means. mepo: mepolizumab.*



In the present study, 4 patients required daily oral corticosteroid therapy before starting the trial, and all of the patients successfully withdrew from daily use of oral corticosteroids without exacerbations and in parallel with sustained reduction in peripheral eosinophil count after initiation of the therapy (**figure 5**).

Discussion

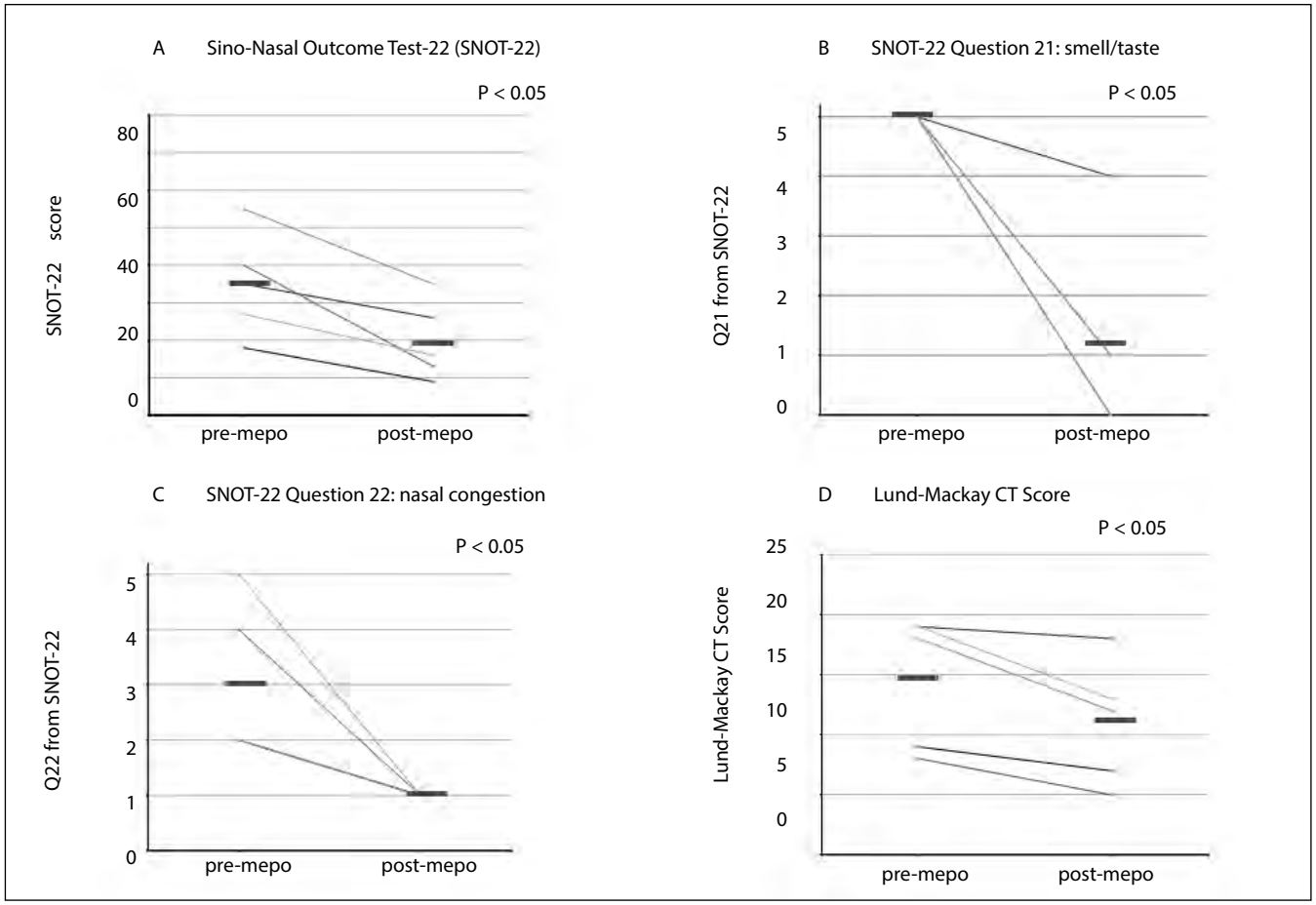
This was the first open-label pilot study of subcutaneous administration of mepolizumab that showed safely improvements on nasal symptoms, the CT findings of the paranasal sinuses and lung function in Japanese patients of severe eosinophilic asthma with CRS. None of the patients experienced exacerbations during 48-week administration.

Before starting mepolizumab treatment, all patients had been diagnosed with the presence of nasal polyps using a nasal endoscope by experienced otolaryngologists at other hospitals. In addition, a JESREC score was assessed from each patient, and the mean JESREC score of the patients was 12.6 in the score range 11-17, supporting that all patients were eosinophilic CRS.

A recent review described blood eosinophil counts as a predictive biomarker for the efficacy of treatment with mepolizumab in patients with severe eosinophilic asthma (25). Following mepolizumab administration, a rapid and pronounced reduction in peripheral blood eosinophil levels was observed in this study, which was consistent with previous studies (25-27).

Unfortunately, after mepolizumab treatment less than half of the patients agreed to an endoscopic evaluation performed by expe-

Figure 2 - *Change in SNOT scores and the Lund-MacKay CT scorings before mepolizumab therapy (0 week) and 48 weeks after start of the therapy in 5 patients without aspirin hypersensitivity. A significant reduction of total SNOT-22 scores and the CT scorings was seen at week 48 after mepolizumab treatment. Also, SNOT-22 Question 21 and 22 was significantly reduced with the treatment.* **A**, *total SNOT-22 scores;* **B**, *SNOT-22 question 21 (smell / taste);* **C**, *SNOT-22 question 22 (nasal congestion);* **D**, *Lund-MacKay CT scorings. Gray horizontal lines represent group means. mepo: mepolizumab.*



rienced otolaryngologists at other hospitals, and the rest of them refused to visit otolaryngologists. So, the primary objective of this study was to investigate whether mepolizumab may improve the symptoms of CRS and the findings of CT scan opacification of paranasal sinuses. Total SNOT-22 scores, symptom scores of anosmia (Question 21 from the SNOT-22) and nasal congestion (Question 22 from the SNOT-22) significantly decreased after the treatment. The next objective was the comparison of the response to mepolizumab on nasal symptoms and the CT findings between patients with AERD and aspirin-tolerant patients. In aspirin-tolerant patients, the total SNOT-22 scores, Question 21 score from the SNOT, Question 22 score from the SNOT-22, and the LM score significantly decreased with mepolizumab treatment. However, in AERD patients, the total SNOT-22 scores and the LM score significantly decreased, but not Question 21 and Question 22 scores from the SNOT-22. Some patients did not provide a change of SNOT-22 and/or LM score after the period of observation.

A recent investigation indicated that patients with AERD showed more olfactory loss, but no difference in the total SNOT-22, in comparison with patients without AERD (28). Peripheral blood eosinophil count has been shown to be higher in AERD patients than in aspirin-tolerant asthma (19,29,30). On the other hand, it has been reported that while nasal congestion is a common symptom in patients with eosinophilic CRS, reduction in or loss of the sense of smell precedes nasal congestion (31), and characteristic CT images of the sinuses are opacification of posterior ethomoid sinus and the olfactory cleft (22,32). Taking all into

Figure 3 *- Change in SNOT scores and the Lund-MacKay CT scorings before mepolizumab therapy (0 week) and at week 48 after start of the therapy in 6 patients with aspirin-exacerbated respiratory disease. A significant reduction of total SNOT-22 scores and the CT scorings was seen at week 48 after mepolizumab treatment. However, SNOT-22 Question 21 and 22 was not with the treatment.* **A**, *total SNOT-22 scores;* **B**, *SNOT-22 question 21 (smell / taste);* **C**, *SNOT-22 question 22 (nasal congestion);* **D**, *Lund-MacKay CT scorings. Gray horizontal lines represent group means. mepo: mepolizumab, NS: not significant.*

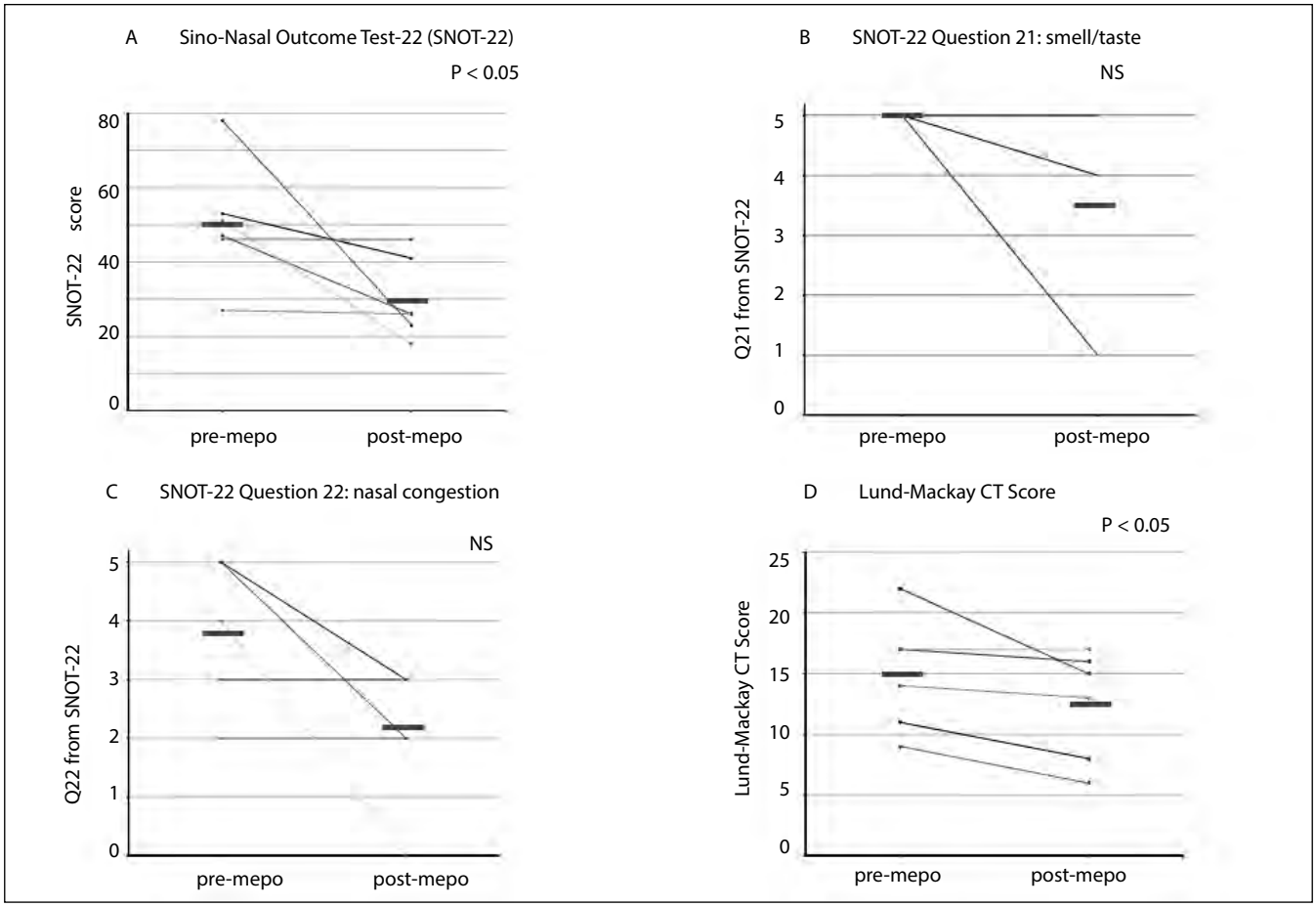


Table II *- Patients and their clinical characteristics that did not provide changes of SNOT-22 score and/or Lund-Mackay CT score after mepolizumab treatment.*

Patients (gender, age-years)	**SNOT-22 score**		**Lund-Mackay CT score**	
	pre-mepo	**post-mepo**	**pre-mepo**	**post-mepo**
patient 1 (female, 47, AERD)	27	26	14	13
patient 2 (female, 44, AERD)	46	46	9	6
patient 3 (female, 56, AERD)	51	18	17	17 (oral corticosteroid-dependent)
patient 4 (male, 46, AERD)	47	26	17	16
patient 5 (male, 56, allergic)	35	26	19	18 (oral corticosteroid-dependent)

AERD, aspirin-exacerbated respiratory disease; mepo, mepolizumab.

Figure 4 *- Change in forced expiratory volume in one second (FEV_1) before mepolizumab therapy (0 week), and 24 weeks, 48 weeks after start of the therapy. Significant improvements in FEV_1 were seen at weeks 24 and at 48. $^*p < 0.05$.*

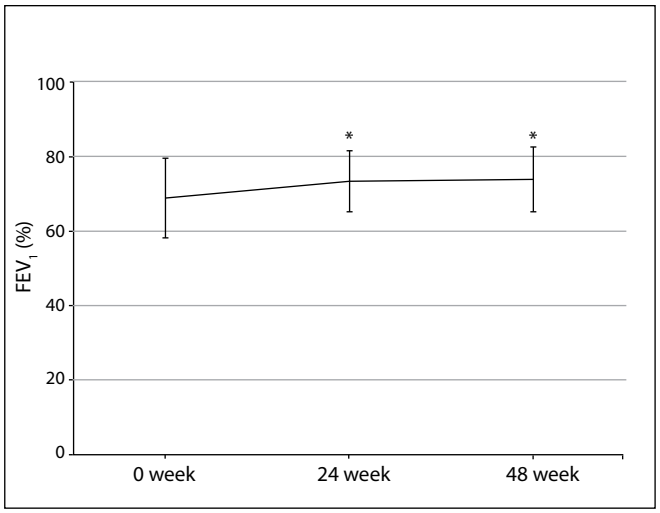
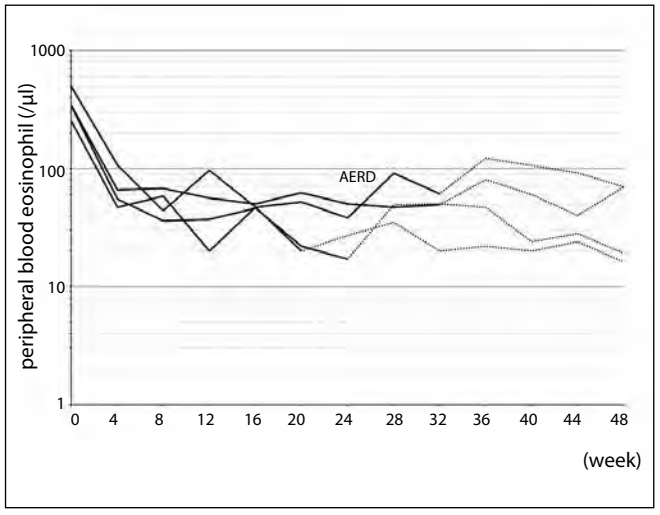


Figure 5 *- Change in peripheral blood eosinophil count in oral corticosteroid-dependent asthma patients before mepolizumab treatment (week 0) and every 4 weeks thereafter. Solid lines show the eosinophil count under corticosteroid administration, and dotted lines show the eosinophil count without corticosteroids. All 4 corticosteroid-dependent asthma patients (3 aspirin-tolerant patients and 1 AERD patient) successfully withdrew from daily use of oral corticosteroids without exacerbations and in parallel with sustained reduction in peripheral blood eosinophil count after initiation of mepolizumab treatment. AERD: aspirin-exacerbated respiratory disease.*



account, the findings in this study may suggest a possibility that local biologic activity of eosinophils, which induces symptoms of anosmia and nasal congestion, might be more severe in eosinophilic CRS with AERD, and longer duration of the treatment may be needed. Further studies are required.

In this study, some patients did not provide a change of SNOT-22 and/or LM score after the period of observation. Because this is a pilot study of 11 patients, we could not investigate their precise characteristic differences into responders and non-responders.

The Asthma Control Test (ACT) scores (33) is often used for assessment of asthma control. However, the ACT mainly depends on patient's reported outcome, and furthermore the presence of rhinitis has been shown to heavily affect the patient's perception of asthma control (34,35). Some studies showed an evidence that rhinitis was associated with an incremental adverse impact on the disease-specific quality of life in asthmatic patients (34). Because the presence of rhinitis may affect the patient's perception of asthma, it was suggested that the accuracy of the ACT has not been systematically evaluated (36). Therefore, we assessed FEV_1 in this study. The results showed FEV_1 at week 24 and at week 48 were increased significantly compared with that at baseline.

Finally, we evaluated the corticosteroid-sparing effect of meplozimab, because 4 patients required daily use of oral corticosteroids before initiating mepolizumab. All patients successfully withdrew from daily use of oral corticosteroids without exacerbation and in parallel with a sustained reduction in peripheral blood eosinophil count, which was consistent with the results of a previous report (37).

Needless to say, this pilot study has limitations. First, a modified LM CT system, which uses a 3-dimensional, computerized method to qualify the volume of mucosal inflammation in the sinuses, has been reported to better correlate with symptoms and disease-specific quality of life of the patients with CRS (38). However, the decrease of SNOT-22 scores was associated with that of LM scorings in the present study. Second, nasal IL-5 levels have been shown to determine the response to anti-IL-5 treatment in patients with nasal polyps (11). In this study, local biologic activity assessment and endoscopic evaluation could not be performed, because no experienced otolaryngologists work at our hospital. As concerns to nasal polyps, investigations about changes of polyp sizes before and after mepolizumab treatment will be required. In addition, number of subjects in the study was 11, and this trial was not randomized, blinded, nor placebo-controlled. So, multicenter, double-blinded, controlled studies are necessary to confirm our data.

Conclusions

Our results showed a favorable long-term safety and clinical efficacy of mepolizumab on the upper airway symptoms and lung function in severe eosinophilic asthma patients with CRS.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical disclosures

Institutional ethics committee approved this study and written informed consent from each individual was obtained before the study.

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Quality of life improvement with allergen immunotherapy treatment in patients with rhinoconjunctivitis in real life conditions. Results of an observational prospective study (ÍCARA)

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Key words
allergic rhinoconjunctivitis; allergen immunotherapy; AIT; quality of life; HRQoL; RQLQ

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Summary
Objectives. *Evaluate the changes in quality of life of patients with allergic rhinoconjunctivitis (AR), with or without asthma, after one-year treatment with allergen immunotherapy.* **Methods.** *This was an observational prospective multicenter study. RQLQ questionnaire and VAS scale to assess treatment satisfaction were used. Impact on AR and asthma was also analyzed. Any adverse reaction was recorded.* **Results.** *127 patients were recruited. Mean values in RQLQ decreased from 2.61 to 1.34 points, reflecting a statistically and clinically significant improvement ($p < 0.01$). The percentage of asthmatic patients decreased significantly ($p < 0.01$). Mean value of patients' satisfaction was 7.24 (SD = 1.90). Only 11 patients presented systemic reactions (9.17%), none of them serious.* **Conclusions.** *One-year AIT treatment significantly increases QoL in patients with AR. Moreover, high patients' satisfaction values were reported, together with an adequate safety profile.*

List of abbreviations

AEMPS, Agencia Española de medicamentos y productos sanitarios; AIT, allergen immunotherapy; AR, allergic rhinoconjunctivitis; ARIA, allergic rhinitis and its impact on asthma; CEIm, Comité de ética de la investigación con medicamentos; GINA, Global initiative for asthma; HRQoL, health-related quality of life; IRB, institutional review board; MID, meaningful importance difference; QoL, quality of life; RQLQ, Rhinoconjunctivitis quality of life questionnaire; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; STROBE, Strengthening the reporting of observational studies in epidemiology; VAS, Visual analogue scale.

Introduction

Allergic rhinoconjunctivitis (AR) is an allergic disorder of the nose and eyes, resulting in a chronic, mostly eosinophilic, in-

flammation of the nasal mucosa and conjunctiva (1). It is characterized by symptoms of nasal obstruction, watery nasal discharge, sneezing and itching and, when it affects conjunctiva, ocular itching, injection and tearing (2). It is mediated by IgE antibodies and it is secondary to exposure to offending allergens in previously sensitized patients. Depending on exposure patterns and the nature of the allergen triggers, the symptoms may be intermittent, persistent or persistent with intermittent exacerbations (3). It is considered the most prevalent allergic disease, affecting around 25% of population in Western Europe (4), and it is frequently associated with other allergic manifestations, both respiratory and otherwise (5). Although AR does not endanger patients' lives, it can result in considerable morbidity (6,7), and can cause a significant deterioration in patients' quality of life (QoL) (8,9). AR is also a risk factor for the development of asthma (10).

The effects of AR on Health-related Quality of Life (HRQoL) extend to learning, sleep, vitality / alertness, perception of general health, cognitive and emotional functioning, and psychomotor performance (11,12). All these possible limitations in patients' day to day can have considerable negative effects on the person's performance both at work or school, and at home, having a direct and indirect economic impact on society (11,13). Symptoms can, in many cases, be controlled with avoidance measures and pharmacological therapies such as oral, intranasal and topical H1 antihistamines, intranasal corticosteroids and antileukotrienes, as monotherapy, or in combination (14,9). Allergen immunotherapy (AIT) with the subcutaneous or sublingual administration of the causative allergen(s), is an additional potential treatment option, particularly for those patients with more troublesome diseases which remain inadequately controlled despite avoidance measures and regular pharmacotherapy (15,9). The problem of inadequately controlled AR, despite optimal medical treatment, continues to represent a therapeutic challenge in the majority of patients, since consequently a significant number of patients continue to experience symptoms that affect their HRQoL. AIT has also been shown to have a disease-modifying effect (16), since it can not only desensitize a patient, thereby ameliorating symptoms, but also deliver long-term clinical benefits that may persist for years after discontinuation of treatment (15,17). For the above mentioned, nowadays AIT is considered the only etiological treatment of allergic diseases caused by inhalant allergens and *Hymenoptera* venom (15,18).

Improvements of the disease-specific HRQoL are especially important for long-term treatments like AIT, and assessment of treatment effectiveness in real life is essential. Currently, a number of studies with AIT have been reported, where the improvement in HRQoL is evaluated mostly as a secondary efficacy variable (19,20,21,22). These studies had shown positive results in HRQoL, but most are clinical trials with sublingual immunotherapy (SLIT), and data from real life studies are still scarce (23,24). Therefore, there is still a need for more clinical evidence, specially with non-interventional studies, where a higher representation of patient's population can be included, as children, patients with comorbidities, etc. are not usually included in clinical trials. Moreover, it is also very important to identify possible factors that may be associated with HRQoL improvement, that could help in the decisions of physicians' day-to-day clinical practice.

The main objective of this study was to evaluate the changes in HRQoL in patients with AR with or without asthma, after one-year treatment with ROXALL subcutaneous immunotherapy (SCIT). As secondary objectives, the impact on AR and asthma symptoms, satisfaction reported either by patients and physicians, patients' adherence to treatment and treatment safety were evaluated. Moreover, the identification of possible patient and treatment factors associated with the AIT efficacy were also analyzed.

Material and methods

This article was written following the Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines (25). The study was a non-interventional prospective multicenter clinical study performed in 13 allergy departments in Spain between June 2015 and May 2017. Patients were included in the study in a baseline visit in which patients' eligibility was checked and the informed consent was signed. Then, the patient started the treatment with the administration of the first dose of SCIT. All patients started the treatments between September 2015 and March 2016, and finalized the study follow-up 1-year after. The baseline RQLQ questionnaire was completed by the patients just before starting treatment, and the final RQLQ questionnaire was carried out after 1 year, corresponding therefore to the same moment during the year as the baseline RQLQ questionnaire was performed. Included patients were those diagnosed of allergic rhinoconjunctivitis, with or without asthma, in which subcutaneous AIT (ROXALL Medicina España S.A.) was prescribed in a routine clinical practice basis, in either formulation, composition or administration schedule. The study was approved by AEMPS (Agencia Española de medicamentos y productos sanitarios) and all the involved regional competent authorities, and by an Institutional review board (IRB) (CEIM hospitales Torrevieja, Elche-Vinalopó) according to Spanish regulation, and other local IRBs. Before participation, all patients gave their signed informed consent.

Patients selection criteria

The assignment of a patient to a specific AIT treatment was not decided in advance by the study protocol, whereas was decided

by the physician according to their usual clinical practice, and following EAACI recommendations for the use of AIT with aeroallergens. No intervention either diagnostic or of follow-up was applied to patients, other than the usual clinical practice.
Eligible patients were those over 12 years of age suffering from AR with or without asthma, with type I hypersensitivity to one or more aeroallergens, responsible for their clinical manifestations, according to:

- positive result Prick test, defined as: a positive result of at least 3 mm in diameter for one or more aeroallergens;
- specific IgE value ≥ class 2 (≥ 0.70 kU/L) (CAP/PHADIA) for one or more aeroallergens.

Patients were subsidiary to receive subcutaneous AIT (in any composition, formulation, or administration schedule) according to clinical recommendations, and thus including both monosensitized and polysensitized patients.
Pregnant or lactating women were not eligible, as they were not susceptible to receive treatment with immunotherapy, according to the usual clinical practice following EAACI recommendations (15). All patients were evaluated at baseline, and were recalled after 6 months of treatment for a follow-up visit, and after 1 year of initiating AIT treatment for the final visit evaluation performance.

Outcomes measures

Quality of Life. To assess the changes in patients' QoL after treatment, Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) developed and validated by Juniper (26,27) was used. The questionnaire consists on a self-administered version validated in Spanish for patients over 12 years. RQLQ involves 28 items-questions distributed in 7 domains (activities 3 items, sleep disturbances 3 items, general problems 7 items, nose symptoms 4 items, eye symptoms 4 items, and emotional function 4 items). Responses are scored on a 7-point Likert scale, while domains and overall score are scored on a 0-to-6 scale (0 = not troubled; 6 = extremely troubled), with lower scores indicating better QoL. Comparisons were made between the mean scores of the RQLQ obtained at baseline visit, and at the one-year post-treatment visit. As previously reported, a change greater than 0.5 on the RQLQ domain and overall scores is the critically meaningful "minimal important difference" (MID) or clinically significant difference (22,28).
Impact on AR and Asthma. The mean number of AR episodes suffered by patients within the last year before AIT treatment initiation, and after 1-year receiving treatment was described and compared between. On the other hand, the classification of AR, ARIA (3), was used to assess the clinical status of patients, before and after treatment (type and intensity). The classification of allergic asthma (GINA) (29) was also used to assess the presence or absence of asthmatic symptoms in patients, before and after AIT treatment. The intake of symptomatic medication before the start of treatment, as well as after 1-year SCIT treatment was also evaluated.
Adherence to AIT treatment. The percentage of patient's therapeutic compliance with treatment was evaluated. Patients who had completed at least 80% of established doses were considered to be good compliant.
Satisfaction with AIT treatment. After treatment, patients' and physician's satisfaction with received AIT was assessed using a visual analogue scale (VAS) from each point of view. This scale ranges from 0 to 10, being 10 the highest degree of imaginable satisfaction, and 0 the lowest degree of satisfaction that may exist.
Factors associated with AIT efficacy. Through a multivariate analysis, the identification of the possible patient and treatment associated factors that may had influenced in the patients' QoL changes after treatment, was analyzed through the assessment of the following variables:

- patient's age (children / adults);
- patient's sex (male / female);
- therapeutic compliance (good compliant / non-compliant);
- level of studies (without studies / primary studies / professional training / high school / higher technical degree / higher degree);
- socioeconomic level (very low / low / medium- low / medium / medium-high / high);
- type of center (public / private);
- degree of physician satisfaction with AIT (< or ≥ of the median);
- degree of patient satisfaction with AIT (< or ≥ of the median);
- prescribed AIT treatment: source (pollens / mites / others); composition (single source / mixture of extracts); schedule (cluster / fast / conventional / other); formulation (depot / polymerized);

Safety. For safety assessment, any adverse reaction occurred during treatment and detected either by patient or by physician was recorded. A patient's diary was used for these purposes.

Statistical analysis

Safety and descriptive analyses were performed using the safety population (receiving at least one dose). Efficacy statistical analyses were performed using the intention-to-treat, (ITT) population. The categorical variables were described by absolute and relative frequencies. For the description of the continuous variables, mean and standard deviation were used. For the comparison of the quantitative variables of two or more independent groups, parametric tests (Student's t test or ANOVA) or non-parametric tests (U of Mann-Whitney or Kruskal-Wallis)

were used. For the comparison of two or more paired groups parametric tests (Student's t-test for paired data or analysis of the variance of repeated measures) or non-parametric tests (Wilcoxon or Friedman), were used, according to the characteristics of the variables under study (normality) and the number of groups to compare. For the qualitative variables, the Chi-square test or Fisher's exact test was used to compare patients' subgroups, or either McNemar test or Bhapkar test (table KxK, k > 2) for comparisons between visits. Correlations (Pearson or Spearman's rho) were used to study the relationship between 2 quantitative variables. In all statistical tests, a bilateral statistical significance level of 0.05 was applied.

To study the possible associated variables of influence on patients' QoL through a multivariate analysis, a multiple linear regression was performed using the "backward" procedure with an exit probability of 0.10. All the statistical analysis of the data was carried out with the support of the statistical package SAS version 9.4.

Results

Descriptive data

A total of 127 patients from 13 Allergy Departments were recruited, and 120 of them could be included and analyzed (7 patients did not start AIT treatment). All study sites were distributed in different regions of the inland area of Spain, except one site that was in the coastal area. Patients' mean age was 32.93 years (SD = 13.2), 22 of them were under 18 years old (18.3%). 45.8% of patients were men. 77.5% of patients expressed a socioeconomic level classified as medium and high, and a 21.7% as low or medium-low. Regarding levels of education-formation, 35.7% of patients (or tutors) had a bachelor's degree, as the most frequent one (**table I**). Forty out of the 120 patients (33.3%) had allergy family background. According to ARIA classification (3), most patients were classified as persistent AR (82.5%) and moderate / severe intensity (80.0%), being concomitantly persistent and moderate / severe in the 67.5% of all cases (**table II**). Following the criteria of GINA guidelines (29), 66.7% of patients presented associated asthma at baseline, being 95.5% of them mild asthma cases. The mean number of episodes of AR/year suffered by patients at baseline was 18.59 (SD = 28.4) episodes. At baseline, almost half of the patients (48.1%) were previously on symptomatic treatment, 82.76% of them with antihistaminic drugs, and 55.17% with nasal corticosteroids. Regarding subcutaneous AIT composition, 60.0% of treatments contained a single allergenic source, while the remaining 40.0% contained some extracts mixture. 52.9% of cases were in native depot, and 47.1% in polymerized formulation (**table I**). Abbreviated conventional administration schedule was the most prescribed (70.0%), followed by clustered one (25.0%). AIT treatments contained some type of pollen in its composition in 92.5% of cases, either as unique source (56.8%) or as any

Table I *- Patients' demographic and baseline characteristics.*

Baseline characteristics	**n = 120**
Age (years), mean (SD)	32.93 (13.21)
Age categories, n (%)	
12-17 years	22 (18.3)
≥18 years	98 (81.7)
Gender, n (%)	
men	55 (45.8)
women	65 (54.2)
Race, n (%)	
Caucasian	102 (85.0)
Sub-saharan	1 (0.8)
Iberoamerican	16 (13.3)
Asiatic	1 (0.8)
Concomitant asthma, n (%)	80 (66.7)
Extract type, n (%)	
pollen	111 (92.5)
mites	6 (5.0)
others	3 (2.5)
Extracts source type, n (%)	
unique	72 (60.0)
mixtures	48 (40.0)
SCIT formulation n (%)	
polimerized	56 (47.1)
native depot	63 (52.9)

Table II *- Patients' AR classification in frequencies and percentages (ARIA)*

		Intensity		**Total**
Type		mild	moderate / severe	
	intermittent	6 (5.0%)	15 (12.5%)	21 (17.5%)
	persistent	18 (15.0%)	81 (67.5%)	99 (82.5%)
	Total	24 (20.0%)	96 (80.0)	120 (100%)

pollen's mixture (43.2%). An additional 5% contained mites (single source or mixtures), and 2.5% other extracts, reflecting that almost all patients belonged to inland areas of Spain. Grass pollen as unique source (68.1% of the total number of unique treatments) and the combination of grass pollen with *Olea europaea* pollen (43.8% of the combined mixtures treatments) were the most frequently prescribed compositions.

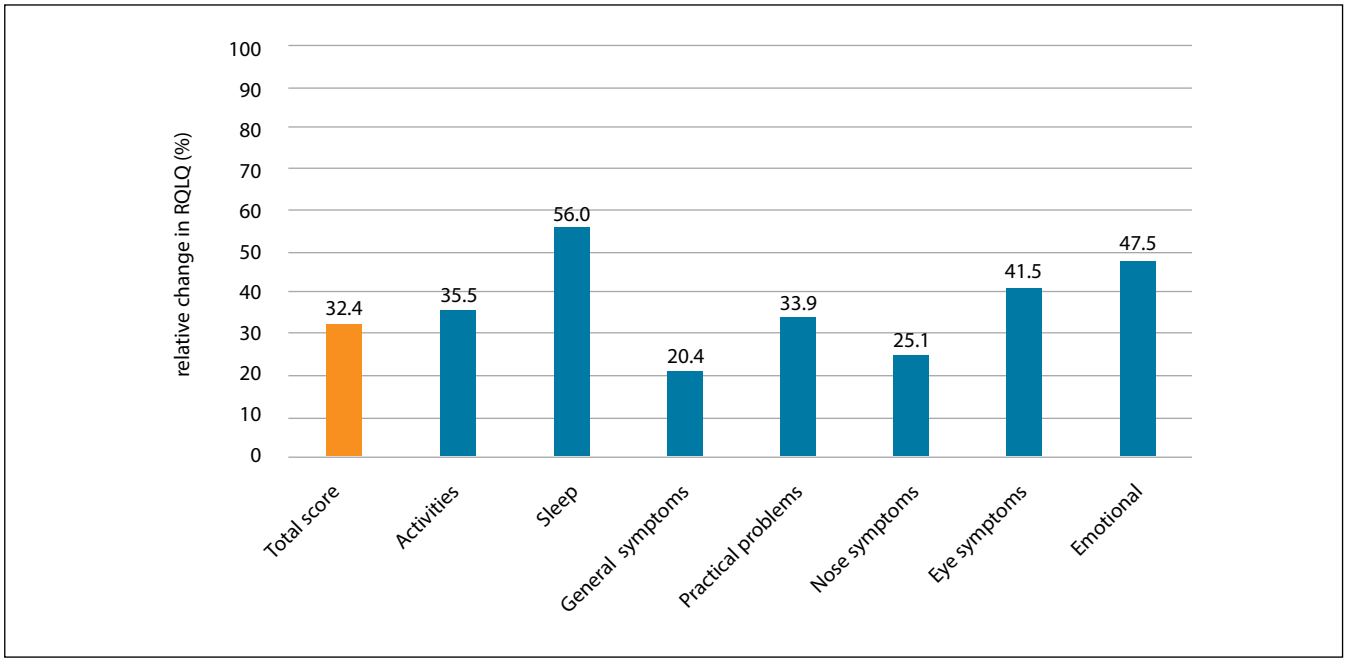
Quality of Life (RQLQ)

One hundred and three patients completed the study providing primary outcome data. Total score means values in RQLQ questionnaire decreased from 2.61 to 1.34 points in final visit (one-year treatment), reflecting a clinically and statistically significant improvement ($p < 0.01$, Wilcoxon test). The absolute change in score is clearly above the established MID (0.5 points) in RQLQ values and, therefore, represents a clinically significant difference for patients. These values of 1.27 points in absolute change, constitute a mean value of 32.4% (SD = 80.59) in relative change between basal-final study visits (**figure 1**). Complete data are described in **table III**. There were clinically and statistically significant reductions in all of the 7 different domains that constitute the RQLQ questionnaire. The domain with the greatest improvement in absolute change was the eye symptoms, with 1.50 points, 41.5% of relative change from baseline ($p < 0.01$), followed by sleeping (1.44 points, 56.0% relative change) and practical problems (1.41 points, 33.9% relative change). Every remaining domain improved in more than 1.0 point in absolute change and at least in 20% in relative change.

Impact on AR and asthma

In addition, the average number of AR annual episodes decreased from 18.77 (SD = 29.31) to 8.75 (SD = 30.39) after one-year AIT treatment, being a statistically significant difference ($p < 0.01$, Wilcoxon test) and representing a 46.6% of reduction. The 43.7% of patients improved after 1-year post-treatment from persistent to intermittent AR ($p < 0.01$), and 40.8% from moderate/severe to mild intensity (ARIA) ($p < 0.01$) (**table IV**). Significantly, a 29.1% of patients improved from persistent and moderate-severe AR to an intermittent and mild AR. An additional 15.5% of the former patients improved at the end of the follow up, being categorized in lower grades according to the basal gradation. Regarding the classification of allergic asthma (GINA), 17.5% of asthmatic patients at baseline, did not have any bronchial symptoms after 1-year AIT treatment (**table V**) (p

Figure 1 *- Mean relative changes (%) in RQLQ global score and different RQLQ items between baseline and final visits.*



Relative change between visits: ([value of baseline visit - value one-year visit] / value of baseline visit) x 100; percentages higher than 0 indicate improvement in the QoL.
Note. Calculated only in patients with baseline values > 0.

Table III - *Evolution of the RQLQ scores after one-year AIT treatment.*

	Baseline visit	One-year visit	Absolute change[1]	p[2]
RQLQ total score				
mean (SD)	2.61 (1.58)	1.34 (1.21)	1.27 (1.64)	< 0.0001
95% CI	(2.30 - 2.92)	(1.11 - 1.58)	(0.95 - 1.59)	
median	2.96	0.89	1.43	
Activities				
mean (SD)	2.81 (1.76)	1.51 (1.54)	1.29 (2.07)	< 0.0001
95% CI	(2.46 - 3.15)	(1.21 - 1.81)	(0.89 - 1.70)	
median	3.00	1.00	1.67	
Sleep				
mean (SD)	2.37 (1.98)	0.93 (1.38)	1.44 (2.05)	< 0.0001
95% CI	(1.98 - 2.76)	(0.66 - 1.20)	(1.04 - 1.84)	
median	2.33	0.33	1.00	
General symptoms				
mean (SD)	2.41 (1.62)	1.35 (1.23)	1.06 (1.57)	< 0.0001
95% CI	(2.10 - 2.73)	(1.11 - 1.59)	(0.76 - 1.37)	
median	2.57	1.00	0.86	
Practical problems				
mean (SD)	3.24 (1.96)	1.83 (1.68)	1.41 (2.11)	< 0.0001
95% CI	(2.86 - 3.62)	(1.50 - 2.16)	(1.00 - 1.82)	
median	3.67	1.33	1.00	
Nose symptoms				
mean (SD)	3.19 (1.90)	1.81 (1.61)	1.38 (2.20)	< 0.0001
95% CI	(2.82 - 3.56)	(1.49 - 2.12)	(0.95 - 1.81)	
median	3.75	1.25	1.25	
Eye symptoms				
mean (SD)	2.72 (1.90)	1.22 (1.46)	1.50 (2.04)	< 0.0001
95% CI	(2.35 - 3.09)	(0.93 - 1.50)	(1.10 - 1.90)	
median	2.75	0.75	1 - 25	
Emotional				
mean (SD)	1.82 (1.57)	0.80 (1.07)	1.02 (1.52)	< 0.0001
95% CI	(1.51 - 2.12)	(0.59 - 1.01)	(0.72 - 1.31)	
median	1.75	0.25	0.75	

Note. Low scores in the RQLQ questionnaire indicate a better QoL (scale 0-6). [1]Absolute change between visits: (value of baseline visit - value of one-year visit). [2]Wilcoxon test

< 0.01, McNemar test). Moreover, none of the patients without asthma symptoms at basal visit developed bronchial symptoms at the end of one-year treatment.

In the case of patients who had previously taken antihistamine medications, 68.5% of them (26 of 38 patients) decreased or stopped their intake after 1-year treatment with SCIT. Only 1 patient increased its use. In the case of patients taking nasal corticosteroids, 67.8% of them (26 of 28 patients) decreased or stopped their use after 1-year treatment. Only 2 patients increased their use.

Table IV *- AR classification (ARIA) evolution after one-year AIT treatment.*

Description				
		one-year visit		p[1]
		intermittent	persistent	
baseline visit	intermittent	16 (15.5%)	2 (1.9%)	< 0.0001
	persistent	**45 (43.7%)**	40 (38.8%)	
Intensity				
		one-year visit		p[1]
		mild	moderate / severe	
baseline visit	mild	18 (17.5%)	4 (3.9%)	< 0.0001
	moderate / severe	**42 (40.8%)**	39 (37.9%)	

Classification					
		one-year visit			p[1]
	intermittent and mild	intermittent and moderate / severe		persistent and mild	persistent and moderate / severe	
baseline visit	intermittent and mild	4 (3.9%)	0 (0.0%)	0 (0.0%)	1 (1.0%)	< 0.0001
	intermittent and moderate / severe	**6 (5.8%)**	6 (5.8%)	0 (0.0%)	1 (1.0%)	
	persistent and mild	**5 (4.9%)**	**0 (0.0%)**	9 (8.7%)	3 (2.9%)	
	persistent and moderate / severe	**30 (29.1%)**	**10 (9.7%)**	**6 (5.8%)**	22 (21.4%)	

n (%): (% calculated with n = 103). [1]Bhapkar test (table KxK, k > 2).

Table V *- Evolution of the of allergic asthma classification (GINA) after one-year AIT treatment.*

Asthma presence				
		one-year visit		p[1]
		yes	no	
baseline visit	yes	51 (49.5%)	**18 (17.5%)**	< 0.0001
	no	0 (0%)	34 (33%)	

[1]McNemar Test.

Adherence

Regarding patients' adherence with AIT treatment, mean percentage of therapeutic compliance in SCIT was 92.0% of patients (patient who completed at least 80% of established doses). When comparing compliance between different treatment types, a minor compliance was seen in patients treated with allergen mixtures (85.0% for mixtures vs 95.1% for unique source treatments), whereas no statistically significant difference was detected. No differences were also detected between pollen vs mites AIT treated patients (p > 0.05 Fisher test).

Satisfaction

After one-year AIT treatment, patients' and physician's satisfaction was assessed using a Visual Analogue Scale (VAS) from both perspectives. Mean values of treatment satisfaction were 7.24 (SD = 1.90) and 7.05 (SD = 1.83) for patients and physicians respectively. A clear correlation was observed between both values obtained by patients and physicians (p < 0.1, Rho Spearman).

Factors associated with AIT efficacy

The pre-requisite for analyzed variables to enter in the multiple linear regression model, was to obtain a p-value lower to 0.10 in the univariate analysis. The variables that complied and were included in the multivariate analysis were the following:

- degree of physician satisfaction with AIT;
- degree of patient satisfaction with AIT;
- composition (single source / mixture of extracts);
- formulation (depot / polymerized).

The introduced variables were eliminated one by one by the "backward" method, with an exit probability of 0.10. Only the variable "patient satisfaction" remained in the multivariate re-

gression model ($p < 0.01$), meaning that after treatment, patients who reported greater satisfaction values with the treatment, also improved significantly more in their QoL, than those with lower satisfaction values.

Safety assessment

The safety analysis was performed considering the total number of patients recruited in the study, with data from a follow-up visit (safety population, n = 120 patients who had at least some information during the 6-month visit). Of the 238 adverse events recorded, 233 were definitely, probably or possibly related to the study medication, therefore were considered adverse reactions. Thus, 54 patients (45.0%) had a total of 233 adverse reactions. None of the adverse reactions was serious, being the majority of them (90.8%) classified as mild, thus only 9.2% were of moderate intensity. 69.1% of the adverse reactions were registered within 0-6 months period, while the rest (30.9%) within the 6-12 months treatment period. 54.3% of the adverse reactions occurred in the treatment initiation phase, and the rest (45.7%) in the maintenance phase.

Local reactions in the injection area accounted for 89.7% of local reactions (209 reactions) being 62.2% of them delayed. Only 6.2% of these local reactions were clinically relevant (classified as moderate or severe intensity).

Only 24 systemic reactions were reported in 11 patients (9.17% of patients). Of them, 18 were of grade I (in 9 patients), and 6 of grade II (in 2 patients). One patient suffered a total of 5 grade II reactions, due to a dosage error in primary care, consisting in generalized itching, muscle pain, rhinitis and shortness of breath during 24-48 hours. The other patient with a grade II reaction, presented general discomfort, respiratory distress and ocular itching 48 hours after the AIT administration. Thus, only the 1.67% of patients suffered grade II reactions. There were no systemic reactions of grades higher than II (EAACI grading system) (**table VI**).

Taking into account the total number of doses administered (1712 doses), adverse reactions accounted for the 13.6% of them, being systemic reactions on the 1.4% of doses. Only the 1.2% of doses caused some moderate intensity adverse reactions (0.8% local and 0.4% systemic reactions).

Discussion

The efficacy and safety of immunotherapy has been very well documented in multiple well-designed and controlled clinical trials. Patients in these studies are usually very selected and rigorously controlled, which does not happen in daily clinical practice. This study evaluated the behaviour of immunotherapy in real life conditions, giving an idea of the profile of patients receiving immunotherapy and the type of treatment prescribed. The results of this study confirmed not only the improvement in the QoL, but also the impact on the symptoms of rhinitis and asthma, the decrease in medication, the good adherence to treatment, and the safety of AIT.

AIT is the only treatment option that can induce specific immune tolerance and has long-term disease-modifying effect, inducing desensitization (9,15,16,17,18). Several validated tools for assessing HRQoL in AR are currently available (30). The most frequently used specific and validated instrument, involved in AIT trials is the RQLQ questionnaire (23,26,27). Moreover, only the RQLQ allows calculating the MID, namely how much a score must change so that it is perceived as such by the patient, irrespective of its statistical significance (31).

***Table VI** - Adverse reactions classification and description.*

	N adverse reactions (%)		Description
local adverse reactions	209 (89.7%)	196 (84.1%)	mild local reaction in the injection area (inflammation, itching and/or pain)
		13 (5.6%)	moderate / severe local reaction in the injection area (inflammation, itching and/or pain)[1]
systemic adverse reactions	24 (10.3%)	9 (3.9%)	hypersensitivity with involvement of more than one organ
		1 (0.4%)	rhinitis
		12 (5.2%)	isolated symptoms of rhinoconjunctivitis
		1 (0.4%)	nonspecific symptoms
		1 (0.4%)	cough
Total adverse reactions	233 (100%)		

[1]Considered as clinically relevant.

In this study, a clear and important improvement in patients' HRQoL was observed compared to baseline, both globally in the total score, and in each of the different domains that form the validated and disease specific RQLQ questionnaire, after one-year AIT treatment. All observed improvements in RQLQ were clearly above the threshold of 0.5 points of change, for a clinically important improvement, previously defined by Juniper (31,32). Interestingly, the domain in which patients improved the most in absolute change, was the related to eye symptoms. On the other hand, there were also significant improvements in the number of AR episodes suffered by patients per year, as well as in the type and intensity of their pathology, according to the ARIA classification. In addition, focusing in patients with associated asthma at baseline, a significant percentage of them, did not present any bronchial symptoms after AIT treatment. Although with minor differences depending on the composition (mixtures vs single source), the patients' compliance with the treatment was very high, probably because subcutaneous AIT requires to be administered by a healthcare professional. This is important, since adherence to AIT in real-life, especially to the recommended prolonged courses, could be an issue and compromise the efficacy demonstrated in clinical trials. At the end of the study, both patients and physicians reported high and correlated satisfaction values with the treatment in VAS score, in concordance with the positive results observed also in patient's QoL improvement, and AR and asthma positive impact.

Limitations of this study are those of a non-interventional prospective, uncontrolled study in the real-life setting, like unpredictable bias, confusion bias and selection bias. In order to minimize a potential investigator and selection bias of the study, sites distributed all over Spain were involved. Moreover, given that in the study the different demographic, clinical and treatment factors that may had an influence on efficacy were analyzed, we consider that the possibility of confusion bias is reduced when interpreting these results.

On the other hand, treatments that contained pollen from grasses were the most widely received by the patients (either alone or in combination with, *Olea* or other extracts), involving 85.4% of total patients' treatments. Regarding a possible influence on the positive observed results, of a lower pollen counts in the spring in which patients were in treatment (2016), in comparison with the pollen counts in the spring before treatments started (2015), it must be clarified that grasses pollen counts in Spain in 2016 were much higher than in the previous year 2015. Given these facts, it is not possible to assign the improvement in patients' QoL to a lower level of pollens during the year receiving the treatments.

Whereas in the past traditional clinical measures were supposed to provide a comprehensive description of the impact of the disease on patients, it is now proved that HRQoL is a necessary parameter for achieving a more complete assessment of allergic diseases. In long-term treatments like AIT, improvements of the disease-specific HRQoL are especially important.

Exploring the improvements in absolute change values in the different domains of the RQLQ, the eye symptoms had the greatest change observed (1.50 points), followed by sleep (1.44 points) and practical problems (1.41 points). Eye symptoms is the aspect that, together with nasal symptoms, has been found to strongly affect HRQoL (33,8). Eye symptoms have a significant impact on daily activities and work or school performance. At the same time, they are some of the most difficult to control (23). These results go in accordance with those reported by Novakova et al recently (23). By the other hand, AR is known to affect nocturnal sleep and daytime sleepiness which may be related to nasal congestion (34). Additionally, lack of sleep has consequences for both social functioning and school performance (35). Given all of the above, sleep disturbances related to AR have clear significant implications on HRQoL (35).

By the other hand, as a result of their symptoms patients with AR run into daily practical problems such as the discomfort to carry tissues, the need to rub their nose / eyes, and have to blow their nose many times. These problems could potentially interfere with their social interaction, limiting their activities. The improvement of these "practical problems" such as that observed in this study, undoubtedly contributed to the overall HRQoL improvement as a result of AIT treatment.

Also, significant improvements were observed in all the rest of the domains like "activities", general symptoms, nose symptoms and emotional aspects after one-year of treatment, contributing, to the global improvement shown of patients' HRQoL. Maybe the impact of AIT on all these aspects might explain at the same time the improvement of their emotional wellbeing.

The positive changes in RQLQ observed in this study, are in the same line that those previously reported in some randomized clinical trials where this effect has been evaluated for AIT (21,22,36,37). Nowadays, few observational studies, under clinical routine conditions, have shown the benefits of AIT treatment in improving the QoL of patients with AR (38,39). Interestingly, Schwanke et al. (40) in 2017 published a observational study with the objective to compare changes in QoL with sublingual immunotherapy (SLIT) and SCIT treatments in patients with AR in a real-world clinical setting. They concluded that although improvements in QoL were noted in both groups, changes in overall scores and the majority of domains only achieved statistical significance in the SCIT group. Some other non-interventional studies have also evaluated improvements in QoL in patients with AR, focusing only in SLIT treatments, and showing also positive results (23,24,41). Interestingly, in the study published by Horn (24), routine treatment with a grass SLIT-tablet resulted in clear improvements in disease-specific and general quality of life, while no

improvements were observed in patients treated only symptomatically.

Among the findings of this study, it is important to note the importance of seeing a significant reduction in the percentage of patients with associated asthma who showed bronchial symptoms after the year of treatment with AIT. As stated in the last GINA Report, in people with asthma and allergic sensitization, SCIT is associated with a reduction in symptom score and medication requirements, and improved allergen specific and non-specific airway hyper responsiveness (42). These findings go in accordance with the mentioned in last published GINA report, and other systematic reviews analyzing the benefits of AIT in asthma (43).

As part of the secondary objectives of this study, we explored the possible influence of some patient and treatment factors on the efficacy of treatment, regarding the improvement of HRQoL as the primary study endpoint. However, only "degree of patient satisfaction" appeared to be independent clinical predictor when multiple factors were accounted for, in the predictive model, as might be expected. On the other hand, it is worth noting that in this study, no significant differences were detected between the subgroups of analyzed patients, regarding demographic factors such as age (children vs. adults) or sex (male vs. female), with respect to the efficacy of treatment in patients' QoL improvement. Few studies evaluated the impact of the therapies on children and adolescent suffering from AR. Our findings in this regard are in accordance with those reported by Filanowicz et al. in 2016 (44), where no significant correlation between sex and age of examined people and the improvement of QoL was found, in patients with AR after AIT. No differences were neither found regarding the improvement in the QoL associated with the patients' socioeconomic characteristics.

Another aspect to be considered is the safety and tolerability of AIT treatment showed in this observational study. It can be affirmed that the safety profile is good, given that few systemic reactions associated with the treatment were reported, and at the same time few of them were of moderate intensity and in a small number of patients. The vast majority of adverse reactions reported were local, at the injection site, and consisted of erythema, inflammation, pain, and/or swelling, being only the 6.2% of them clinically relevant.

An important fact is that this study included different types of treatment composition (pollens and mites) sources of allergens (single and mixtures) as well as different formulations (polymerized / depot) not observing differences associated with the improvement of QoL, for the different subgroups analyzed.

The data observed in this study can be useful for physicians' decision-making when managing patients with AR, regarding whether a patient could be benefit from AIT treatment.

Conclusions

The results of this study provide evidence that a one-year treatment with subcutaneous AIT (ROXALL Medicina España S.A.), significantly increases QoL in patients with AR, together with a significant positive impact on AR type and intensity, and a reduction in the percentage of patients showing asthmatic symptoms after treatment.

Moreover, high patients' satisfaction values with treatment were reported, together with an adequate safety profile with a low number of systemic adverse reactions, none of them serious.

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Conflict of interest

LB, BM, MCG and AM belong to the R and D department of ROXALL Medicina España S.A, the company sponsor of this study.

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Availability of epinephrine auto-injectors and knowledge of community pharmacists about their use

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Key words
adrenaline; epinephrine; epinephrine auto-injectors; anaphylaxis; community pharmacists

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To the Editor

Epinephrine (adrenaline) is the medication of choice for anaphylaxis. Epinephrine auto-injectors (EAI) are prescribed to children and adults who have experienced an anaphylactic episode (1). Patients' training by an allergy specialist is extremely important in order to help them use EAI properly and with confidence, in a future episode of anaphylaxis. Structured educational programs improve the management of anaphylaxis by patients, parents, caregivers, and health-care professionals (2).
Filling an EAI prescription promptly after a medical visit is strongly advised. However, a study on filled prescriptions for EAI based on electronic medical records, showed unsatisfactory adherence (3). It seems that when a patient experiences anaphylaxis and has never acquired the prescribed EAI, or has neglected to carry it, or medication has expired, he/she could visit a community pharmacy, which is often easier and faster than driving to a Hospital Emergency Department.

Community pharmacists can play an important role by supplying EAI, offering instructions on its correct use and storage, and assisting patients who are experiencing anaphylaxis (4,5). However, it has been reported that there are gaps in pharmacists' knowledge on anaphylaxis and on EAI use (6-9). Therefore, it has been proposed that they should be appropriately trained and become able to intervene in an anaphylactic incident (10).
The aim of the present study was to assess the stock of epinephrine in community pharmacies of Cyprus and to evaluate community pharmacists' knowledge on epinephrine use.
Half of the community pharmacies registered in the "Nicosia-Kerynia district" of the Cyprus Pharmaceutical Association were randomly selected. The research team visited each pharmacy and invited the pharmacist on duty to participate in the study. Following a written informed consent, the research team proceeded with an interview. They were ensured about anonymity and researchers interviewed them in privacy, in order to avoid hesitation in front of pharmacies' clients. Due to our pro-

tocol and in order to preserve anonymity, no socio-demographic characteristics of the participants were kept.
The questionnaire was short in order to achieve a high response rate, and included questions that had been used in previous research in the Netherlands (6). Given that the only EAI device in Cyprus is Anapen, the brand name was used instead of EAI.

The following four questions were asked:
1. Do you have any of the following devices of epinephrine available now?
   a. Anapen 150 μg
   b. Anapen 300 μg
   c. Epinephrine ampoules for medical use
2. Which is the site of application for Anapen?
3. Which Anapen dose is recommended for a child of 27 kg?
4. Are there any contraindications for the use of epinephrine in the case of anaphylaxis, or is it indicated irrespective of patient's anamnesis?

The study was submitted for approval to Cyprus National Bioethics Committee and was exempted from full Board review (decision; EEBK 2017.01.47). All analyses were conducted in Stata 14.
The research team visited 57 pharmacies. Of these, 49 accepted to participate in the study (response rate 86%). Anapen (300 μg) was available in only one pharmacy while 3 pharmacies had epinephrine ampoules. The rest of the pharmacies had no stock either of EAI or of epinephrine ampoules.
Twelve pharmacists (24.4%) correctly indicated thigh as the application site for Anapen, while two (4%) replied both thigh and deltoid muscles. Twelve pharmacists (24.4%) answered that Anapen should be used intramuscularly without indicating the application site and 15 (30.6%) did not know the answer or refused to respond. Other answers included deltoid administration (n = 2, 4%) and other / non intramuscular application (n = 6, 12.2%).
Of 34 pharmacists who answered the question about the right device for a 27 kg child, 3 indicated correctly the 300 μg device (8.8%), while the rest (91.1%) said the 150 μg one. In terms of contraindications, 18 of 30 who replied to this question (60%) answered correctly that no contraindications apply.
Eleven community pharmacists, justifying themselves for their unwillingness to reply, mentioned that knowledge on anaphylaxis and education of patients on epinephrine's use is the responsibility of the prescribing physician.
It seems that there is significant lack of knowledge on the use of epinephrine among community pharmacists in the large district of Nicosia. In addition, there was unwillingness of some community pharmacists to be interviewed or to reply to certain questions, perhaps because they thought that they were tested for their knowledge, and less that they were participating in a scientific study with the aim of informing future policies that will benefit the population.
Deficiencies in the correct administration technique of EAI by patients, parents / caregivers, and health-care professionals (including physicians) have been recorded in many studies in the past (2,6-8,11,12). Factors that increase the correct use of EAI included patients' age over 18 years, training offered by an allergologist, prescription of an EAI for more than 30 months, anamnesis of severe anaphylaxis, and membership in a support group (11).
In an Australian study with mock patients, most of the pharmacists (who were unaware of the fact that they were assessed) demonstrated accurately the steps of safety cap's removal, the placement of the EpiPen and Anapen devices, and the injection. However, only 20% gave correct advices on what people should do after injection (7). In an online study in the Netherlands (6), the percentage of correct answers to three questions that were also used in our study (Q 2, 3 and 4) was 66.6%. The relative percentage in our study was 33.6%. A large questionnaire-based survey has also been contacted in Germany (8). A standardized written questionnaire containing items about anaphylaxis and its pharmacological treatment were handed out in person or sent by fax. The response rate was 28.5%, with pharmacists showing higher level of knowledge on anaphylaxis than on using and handling EAI (8). Pharmacists (n = 213) in that survey were also asked whether they were interested in receiving training, but only 35 replied positively (8).
In 2011, the Australasian Society of Clinical Immunology and Allergy launched the "ASCIA Anaphylaxis e-training for pharmacists". Anaphylaxis knowledge of the community pharmacists increased after the education program and remained high seven months later (4). However, teaching health-care professionals is feasible only if they are willing to be taught, which is not always true (8).
Concluding, besides patients, training on anaphylaxis and the use of EAI is very important for health-care professionals, including community pharmacists. Pharmacists should help in urgent situations like anaphylaxis and should be competent to do so. Educational programs and proper legislation adjustments that will remove barriers and encourage community pharmacists' help in certain emergencies are necessary.

Conflict of interest

Authors have no economic or other type of conflict of interest to declare, regarding the presented article.

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