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ACCELERATED BUILD-UP SCHEDULES OF SPECIFIC IMMUNOTHERAPY FOR THE TREATMENT OF RESPIRATORY ALLERGY IN PEDIATRIC PATIENTS - SYSTEMATIC REVIEW
ARTIGO DE REVISÃO

ÁREA CIENTÍFICA DE PEDIATRIA

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Accelerated build-up schedules of specific immunotherapy for the treatment of respiratory allergy in pediatric patients – systematic review

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Table of contents

Abstract

Background: Allergen-specific subcutaneous immunotherapy (SCIT) is an important therapeutic approach for children and adolescents with respiratory allergy (rhinitis, rhinoconjunctivitis and asthma). However, only few allergic patients accept this therapy mainly because of the inconvenience of the conventional time-consuming schedules. Accelerated (rush and cluster) immunotherapy schedules represent an alternative to conventional SCIT, getting immunotherapy benefits in a shorter period. The objectives of this systematic review were to assess clinical and immunological efficacy as well as safety of accelerated build-up schedules of SCIT for the treatment of respiratory allergy in pediatric patients.

Methods: Studies were located by searching PubMed database, using "immunotherapy" and "desensitization" as keywords. The selection of studies, published from 2005 to July 24th, 2015, was performed in two stages: screening of titles and abstracts, and subsequent assessment of the full papers identified as relevant, considering the inclusion criteria. Data from the included randomized controlled trials (RCTs) were extracted in a standardized way by one reviewer and synthesized qualitatively to assess efficacy and safety of accelerated schedules in respiratory allergy.

Results: Eleven RCTs, including four in pediatric population, three in adult population and four in mixed population, were included. Two evaluated rush SCIT and the remaining assessed cluster SCIT. Regarding clinical efficacy, this descriptive analysis demonstrated that rush and cluster schedules are clinically efficacious, with more rapidly effects when compared with conventional regimens. In general, no relevant difference with respect to clinical outcomes was noticed between subgroups (pediatric, adult and mixed populations). This systematic review also determined important immunological effects of accelerated SCIT: increased allergen-specific IgG4 antibodies levels and blocking activity, and increased allergen-specific Treg cells frequency and IL-10 production. Accelerated regimens induced

immunogenicity more rapidly than conventional schedules. Concerning safety, most local adverse reactions were mild and there were neither life-threatening systemic reactions nor fatal events. No relevant differences in the incidence and severity of either local or systemic reactions between the accelerated schedule group and control group were registered, demonstrating in general a good safety profile for these accelerated regimens in both pediatric and adult populations.

Conclusion: The current evidence provides some support for the efficacy and safety of accelerated subcutaneous immunotherapy schedules for respiratory allergy treatment. However, additional RCTs of accelerated immunotherapy schedules enrolling patients younger than 18 years are still needed to conclude affirmatively that these schedules, besides less time-consuming, are effective and safe for respiratory allergy treatment in pediatric patients.

Keywords: Immunotherapy; Accelerated subcutaneous immunotherapy schedules; Pediatrics.

Resumo

Introdução: A imunoterapia subcutânea alérgeno-específica constitui uma importante abordagem terapêutica para crianças e adolescentes com alergia respiratória (rinite, rinoconjuntivite e asma). Contudo, apenas uma parte dos doentes alérgicos aceita esta terapêutica, principalmente devido à inconveniência dos esquemas convencionais de longa duração. Os esquemas de imunoterapia acelerados (*rush* e *cluster*) representam uma alternativa aos esquemas convencionais, atingindo os benefícios da imunoterapia num menor período de tempo. Os objetivos desta revisão sistemática foram avaliar a eficácia clínica e imunológica assim como a segurança dos esquemas de indução acelerados da imunoterapia subcutânea para o tratamento das doenças respiratórias alérgicas em doentes pediátricos.

Métodos: Os estudos foram identificados através da pesquisa na base de dados PubMed, usando as palavras-chave ''immunotherapy'' e ''desensitization''. A seleção dos estudos, publicados desde 2005 até 24 de Julho de 2015, foi realizada em duas etapas: triagem dos títulos e resumos, e subsequente avaliação integral dos artigos identificados como relevantes, levando em consideração os critérios de inclusão. Os dados dos estudos randomizados e controlados incluídos na revisão foram extraídos, de um modo padronizado, por um revisor e sintetizados qualitativamente para avaliar a eficácia e a segurança dos esquemas acelerados na alergia respiratória.

Resultados: Foram incluídos onze estudos randomizados e controlados, incluindo quatro em população pediátrica, três em população adulta e quatro em população mista. Dois estudos avaliaram esquemas *rush* e os restantes estudaram esquemas *cluster*. Relativamente à eficácia clínica, esta análise descritiva demonstrou que os esquemas de imunoterapia *rush* e *cluster* são clinicamente eficazes, alcançando efeitos mais precocemente em comparação com os esquemas convencionais. No geral, não foi notada diferença relevante no que respeita aos resultados clínicos entre os subgrupos (populações pediátrica, adulta e mista). Esta revisão sistemática também determinou importantes efeitos imunológicos dos esquemas SCIT

acelerados: aumento dos níveis e da atividade de bloqueio dos anticorpos IgG4 alérgeno-específicos, e aumento da frequência de células Treg e da produção de IL-10. Os regimes acelerados induziram imunogenicidade mais precocemente que os esquemas convencionais. No que concerne à segurança, a maioria das reações adversas locais foram ligeiras e não ocorreram reações sistémicas ameaçadoras da vida nem eventos fatais. Não foram registadas diferenças relevantes na incidência e na severidade das reações locais e sistémicas entre o grupo com esquema acelerado e o grupo controlo, demonstrando-se no geral um bom perfil de segurança para estes regimes acelerados, quer na população pediátrica quer na população adulta.

Conclusão: A atual evidência suporta, em certa medida, a eficácia e a segurança dos esquemas de imunoterapia acelerados para o tratamento da alergia respiratória. Contudo, são necessários mais ensaios clínicos randomizados e controlados com esquemas acelerados de imunoterapia específica envolvendo doentes com menos de 18 anos, para poder concluir afirmativamente que estes esquemas, além de consumirem menos tempo, são seguros e eficazes para o tratamento da alergia respiratória em doentes pediátricos.

Palavras-chave: Imunoterapia; Esquemas acelerados de imunoterapia subcutânea; Pediatria.

Introduction

Respiratory allergic diseases, including rhinitis, rhinoconjunctivitis and asthma, have shown an increasing prevalence over the past decades, especially in Western countries, where up to 20% of the population is affected.[1,2] This condition generally develops in childhood and may persist, impairing quality of life and performance at school and/or work.

Currently, three therapeutic approaches are employed for IgE-mediated respiratory allergies treatment. Specific allergen avoidance is decisive, however it may be difficult (or even impossible) to achieve. Symptomatic drugs such as antihistamines, corticosteroids, mast cell stabilizers, antileukotrienes, β_2-agonists and anti-IgE monoclonal antibodies are the most used therapeutic approaches in respiratory allergy. Although effective and safe these anti-allergic drugs don't modify the natural course of allergy. In contrast, allergen-specific immunotherapy (SIT), also known as immunologic desensitization, is an immune-modifying therapeutic since it restores mechanisms of immune tolerance to allergens, resulting in a significant reduction of symptoms and symptomatic medication usage, as well as in an improvement of quality of life and productivity at school and/or work.[1-4] It is therefore a critical component in respiratory allergy treatment, both in adults and children, being of particular interest in pediatric population because of its capacity to change the response to allergens at an early phase and, thus, to prevent disease progression.[5]

In general, SIT involves the gradual administration of increasing concentrations of standardized allergen extracts, with the aim of inducing clinical and immunological tolerance to the disease-causing allergen. Subcutaneous immunotherapy (SCIT) is the most common mode of administering SIT, even though other administration routes may be used. SCIT protocols are performed in two stages: build-up (up-dosing) phase which involves the administration of increasing doses of allergen extracts until the effective (or maintenance) dose is reached, and maintenance phase. Conventional immunotherapy schedules generally involve one or two weekly injections during up-dosing phase, over a 16-week period,

followed by monthly maintenance injections for a period of three to five years. Rush and cluster immunotherapy schedules are accelerated build-up schedules which allow the patient to reach the maintenance dose and, thus, the benefits of immunotherapy, more rapidly. In a cluster up-dosing regimen, two to four repeated injections are given to the patient in a single day of treatment on nonconsecutive days, in most cases reaching the maintenance dose in four to eight weeks. A rush up-dosing schedule involves the subcutaneous administration of increasing amounts of allergen extracts at intervals of 15 to 60 minutes over a period ranging from one to three days. Independently of the performed schedule, the patient must remain under medical attendance for 30 minutes following each dose, due to the risk of allergic reactions and, for that reason, the clinical center should be adequately equipped to support emergency treatment.[4,6]

Even though SCIT has been considered an important treatment modality for respiratory allergy with clear benefits, it is estimated that only few allergic patients accept this therapeutic option. One of the main reasons for this is the treatment's inconvenience, mainly related to its duration. Thus, accelerated schedules represent an alternative to conventional time-consuming schedules, allowing a reduced number of office visits (and associated costs), while preserving clinical efficacy. Although their advantages, these schedules haven't been widely used, mainly regarding safety issues.[6]

The main objectives of this systematic review were to evaluate clinical and immunological efficacy as well as safety of accelerated SCIT build-up schedules for the treatment of respiratory allergy in pediatric patients.

Methods

The protocol was developed following international guidelines for systematic reviews [7] regarding three objectives: to assess clinical and immunological efficacy, and safety of accelerated SCIT schedules for the treatment of respiratory allergy in pediatric patients.

Search strategy and study selection

Studies were located by searching an electronic database, PubMed, from 2005 to July 24th, 2015. The search strategy used two keywords: "immunotherapy" and "desensitization". In addition to searching electronic databases, bibliographies of all potentially relevant studies and international guidelines were searched by hand. The search was conducted at *Serviço de Documentação do Centro Hospitalar e Universitário de Coimbra,* Portugal.

Inclusion criteria used to select studies for this systematic review were: (i) population: studies of participants diagnosed with IgE-mediated allergic respiratory disease, confirmed by objective measures (positive skin prick test and/or serum-specific IgE to sensitizing allergens); (ii) intervention: accelerated subcutaneous immunotherapy schedules (rush or cluster SCIT); (iii) comparative intervention: placebo, conventional SCIT or pharmacotherapy; (iv) outcomes: symptoms and medication scores, quality of life, functional measures (lung function, rhinometry), allergen specific reactivity (cutaneous, nasal, conjunctival, and bronchial allergen reactivity), immunological and inflammatory parameters, safety and tolerability; and (v) study design: randomized controlled trial (RCT). Only studies written in English were included.

Studies selection was performed in two stages. The first stage was a screening of titles and abstracts against the inclusion criteria to identify potentially relevant articles. When a definite decision based on title or abstract wasn't possible, the full papers were assessed. Rejected studies were grouped in those that didn't meet the review objectives and those that addressed the topic of interest but failed on one or more criteria (population, intervention, comparative intervention, outcomes and/or study design). When the abstract wasn't available,

the studies were also excluded. The second stage was the assessment of the full papers identified as relevant at the initial screening. If full papers weren't accessible, the studies were excluded.

Data extraction and quality assessment

Data extraction was performed by one reviewer. Data extraction forms were standardized and decision rules about coding data were used. Only essential information for descriptive purposes of the systematic review were included in data extraction forms, namely: first author; publication year; study design; subjects characteristics (age, disease and co-morbidities) and number of subjects allocated to intervention and control groups; intervention description (type of vaccine, build-up schedule, duration and number of injections per up-dosing visit, gap between increasing doses) and control group; co-interventions description; treatment duration; outcome measures; and key results of the study analysis.

The Cochrane Collaboration's recommended tool for assessing risk of bias was the quality assessment process used, in which critical judgment was made independently for the most important domains (random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment and completeness of outcome data).[7] Primarily, these outcomes were summarized in order to draw conclusions about the overall risk of bias. Risk of bias judgment ("Low risk", "High risk", or "Unclear risk" of bias) was followed by a description of the observations that supported it. The process was a blind assessment (to the names of the authors, institutions, journals and results of the study).

Data synthesis and analysis

Data were stratified according to subjects age (18 years or under - pediatric population; over 18 years – adult population; or mixed population – pediatric and adult populations), up-dosing schedule (rush or cluster) and outcomes (clinical assessment, quality of life, functional measures, surrogate markers, safety assessment), and were synthesized qualitatively.

Clinical efficacy was evaluated by means of the following outcomes: symptoms and medication scores, quality of life assessment, functional measures and allergen specific reactivity. Immunological efficacy was determined according to objective parameters: allergen-specific serum antibodies analysis, lymphocyte subsets and cytokines, and local and systemic inflammatory markers. Regarding safety, adverse reactions were analyzed according to location (local or systemic reactions), and compared between groups concerning severity, time of appearance (immediate or delayed reactions), requirement of symptomatic treatment, dose adjustments or withdrawals, and phase of SCIT protocol (induction or maintenance phase).

Treatment efficacy was established by statistical significance (p-value) knowing that values of $p < 0.05$ were considered statistically significant, and/or by 95% confidence intervals which point to a clinically relevant difference when the zero value isn't included in the 95% confidence limits.

Results

Study identification and selection

A preliminary database search identified a total of 337 potentially relevant articles. All of these articles were submitted to an initial screening of titles and abstracts against inclusion criteria, resulting in exclusion of 273 studies: 44 didn't meet the review objectives and 229 failed on one or more criteria (population, intervention, comparative intervention, outcomes and/or study design). Two studies were also excluded, because abstracts weren't available.

A definite decision based on title or abstract wasn't possible in 52 cases, hence the full papers were obtained for detailed assessment aiming to evaluate if the inclusion criteria were or not satisfied. In addition to these, more 10 potentially relevant articles (not excluded based on title and abstract) were assessed for eligibility. In total, 62 full-text articles were retrieved and assessed for eligibility. Of them, 45 were excluded: 42 failed on one or more criteria, and three weren't accessible.

The remaining 17 publications met all inclusion criteria. However, six of these studies were excluded: one didn't elucidate the study design, one was part of another study, three didn't perform a real accelerated build-up schedule (rush or cluster SCIT), and one wasn't accessible.

At the end, 11 studies were included in the systematic review. The flowchart of studies selection is shown in Fig. 1.
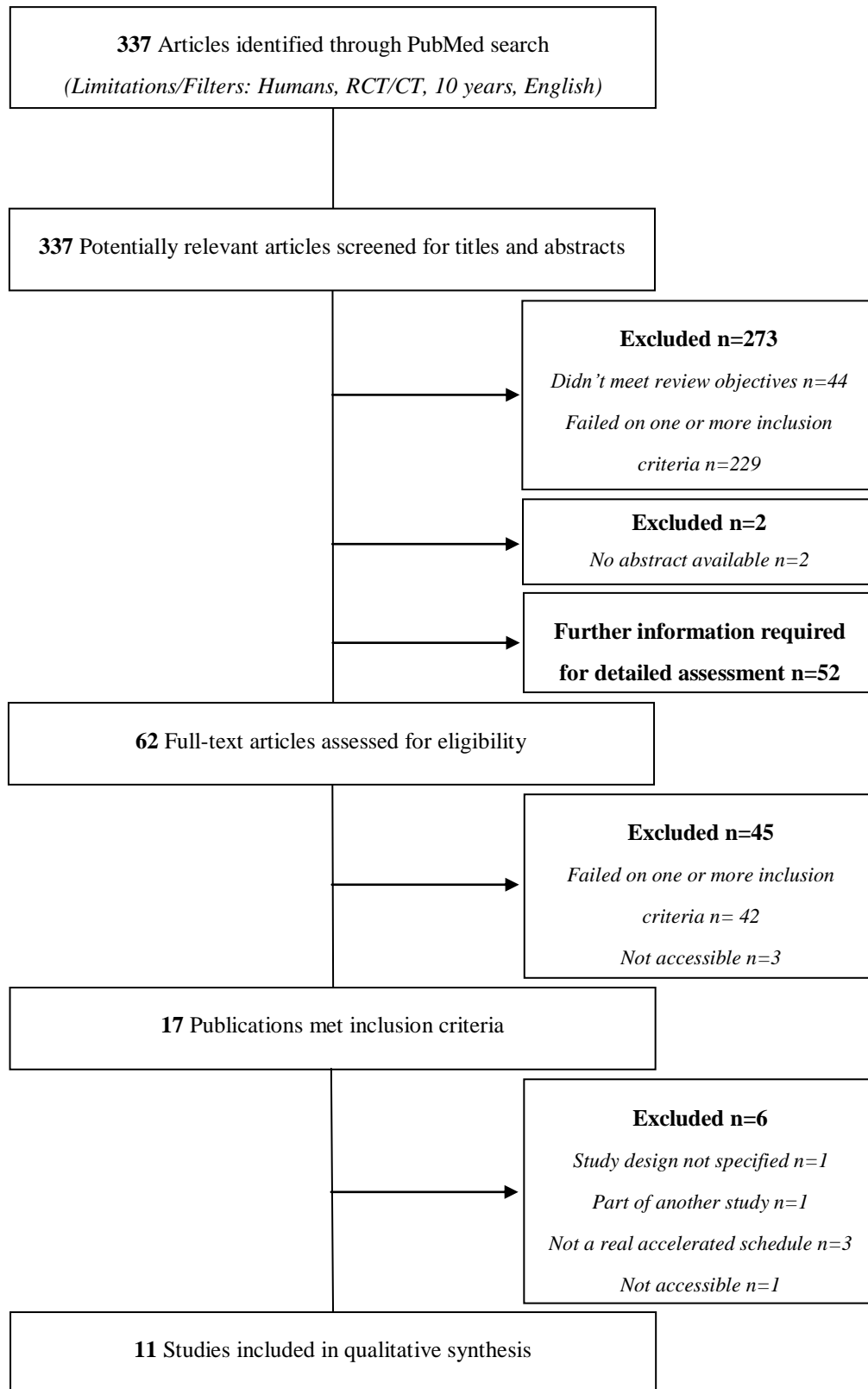
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│  337 Articles identified through PubMed search            │
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         │──────────▶│  Excluded n=273                   │
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         │──────────▶│  Excluded n=6                     │
         │          │  Study design not specified n=1    │
         │          │  Part of another study n=1         │
         │          │  Not a real accelerated schedule n=3│
         │          │  Not accessible n=1                │
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│  11 Studies included in qualitative synthesis             │
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Figure 1. Flowchart of studies identification and selection process. RCT/CT, randomized controlled trial/controlled trial.

Study and population characteristics

Overall characteristics of included studies and subjects are listed in Table 1.

In total there were seven randomized double-blind placebo-controlled trials ("gold standard" of study design to assess SIT efficacy) and four randomized controlled open-label parallel studies.

Four studies [8-11] integrated participants at age of 18 years or under, three [12-14] included adult participants, and the remaining four [15-18] were of mixed population. The primary diagnosis was allergic rhinitis/rhinoconjunctivitis in six studies [8,12,14-16,18] and asthma in the other five studies [9-11,13,17].

Two studies [15,16] described a rush induction schedule, and compared this accelerated regimen with placebo. The vaccine was based on a chemically modified allergen extract (allergoid) in both studies and each individual received two injections at up-dosing visit, with a 30 minutes gap between increasing doses. The build-up phase duration was one day and these studies were conducted over a period of two years.

A cluster administration schedule was described in the remaining nine studies [8-14,17,18]. This up-dosing schedule was compared with placebo in five studies [10,12-14,17], with conventional SCIT in two studies [9,18], and with pharmacotherapy in two studies [8,11]. In three studies [11,12,14] immunotherapy protocol was performed with a chemically modified allergen extract (allergoid), and with an unmodified allergen extract in six studies [8-10,13,17,18]. During the build-up phase, the number of injections received by each individual ranged from four to 15, with a 15 to 60 minutes gap between increasing doses. Time required for induction phase ranged from one to 11 weeks. These studies were carried out between four months and three years.

All included studies explained in detail the immunotherapy schedule, except in one case [13]. A great heterogeneity in the allergen dose delivered and the reported pharmacological units was observed. In general, treatment groups compared in each study

were balanced regarding demographic, physical, and anamnestic baseline characteristics. In six studies [10,12,14-16,18] it was specified that patients were observed for 30 to 60 minutes after each injection in the clinical center. Only two studies [10,18] used pretreatment before each immunotherapy injection in order to reduce adverse effects associated with immunotherapy. All studies allowed routine and/or rescue medications.

Risk of bias assessment of included studies

Table 2 provides a summary of methodological quality assessment of included studies, according to the Cochrane Collaboration's recommended tool for risk of bias assessment.[7]

Selection bias

The method used to generate random sequence and to conceal allocation sequence was adequately performed in four studies [10,12,15,18] ("low risk"). In the remaining seven studies [8,9,11,13,14,16,17] the methods used for random sequence generation and concealment of allocation were poorly reported making it difficult to judge whether the methods were or not susceptible to bias ("unclear risk").

Performance bias

Blinding of participants and investigators was clearly stated and not broken in seven studies [10,12-17] ("low risk"). Four [8,9,11,18] of the included studies weren't blinded ("high risk").

Detection bias

Blinding of outcome assessors is mostly important for subjective measures such as patient-reported outcomes (symptoms and medication scores, quality of life assessment, and safety evaluation). Three studies [9,11,18] didn't conduct a blinding assessment of patient-reported outcomes and had a "high risk "of detection bias. Eight studies [8,10,12-17] described all measures used to blind outcome assessors ("low risk").

The assessment of objective measures such as functional measures, allergen specific reactivity, immunological and inflammatory parameters aren't likely to be influenced by lack

of blinding. Thus, all included studies [8-18] were classified with "low risk" of detection bias regarding objective outcomes.

Attrition bias

In all included studies [8-18] missing data was imputed using appropriate methods such as intention-to-treat (ITT) analysis or, if an ITT analysis wasn't performed, the numbers and reasons of withdrawals or exclusions from the study were reported ("low risk").

Statistical analysis

Statistical techniques used to analyze outcome data were clearly described in all studies. Only six clinical trials [10-14,17] reported the sample size calculation process prior to the start of the study.

Table 1. Characteristics of studies included in the systematic review.

Study (Year)	Study design	Study's Group (N)	Age (mean ± SD)	Disease (co-morbidities)	Intervention/ Comparator	Vaccine type	Build-up schedule (duration)	No. injections/visit (gap between doses)	Total duration	Outcome measures
Klimek *et al.* (2014) [12]	RCT (DBPC)	I (N=61)	37.1 ± 10.4 yrs	Rhinitis/ Rhinoconjunctivitis (with or without asthma)	Grass Rye	Allergoid	Cluster (1 wk)	2/2 (30 min)	1 yr	SMS/SS/MS NCT Immune parameters Safety
		C (N=59)	36.2 ± 10.7 yrs		Placebo	-	Cluster (1 wk)	2/2 (30 min)		
Pfaar *et al.* (2013) [15]	RCT (DBPC)	I (N=186)	31.3 ± 12.4 yrs 15.3 ± 1.9 yrs[a]	Rhinitis/ Rhinoconjunctivitis (with or without asthma)	Birch Grass	Allergoid	Rush (1 day)	2 (30 min)	2 yrs	SMS/SS/MS QoL Immune parameters Safety
		C (N=99)	31.3 ± 11.8 yrs 15.3 ± 1.8 yrs[a]		Placebo	-	Rush (1 day)	2 (30 min)		
Lou *et al.* (2012) [8]	RCT (OPS)	I (N=25)	12 yrs	Rhinitis/ Rhinoconjunctivitis (without asthma)	Der p	Depot	Cluster (6 wk)	3/2/2/2/2/1 (1h)	1 yr	SS/MS Immune parameters
		C (N=25)	11 yrs		Pharmacotherapy	-	-	-		
Pfaar *et al.* (2012) [16]	RCT (DBPC)	I (N=135)	32.9 ± 13.8 yrs	Rhinitis/ Rhinoconjunctivitis (with or without asthma)	Grass	Allergoid	Rush (1 day)	2 (30 min)	2 yrs	SMS/SS/MS QoL Immune parameters Safety
		C (N=60)	33.8 ± 13.3 yrs		Placebo	-	Rush (1 day)	2 (30 min)		
Vidal *et al.* (2011) [17]	RCT (DBPC)	I (N=21)	25.9 yrs	Asthma (with or without rhinoconjunctivitis)	Der p	Depot	Cluster (3 wks)	2/2/2/2 (30 min)	1 yr 5 mon	ST Immune parameters Safety
		C (N=24)	28.3 yrs		Placebo	-	Cluster (3 wks)	2/2/2/2 (30 min)		
Schubert *et al.* (2008) [9]	RCT (OPS)	I (N=22)	10 yrs	Asthma	Der p	Depot	Cluster (5 wks)	3/3/3/2/1/1 (NAD)	16 wks	Immune parameters Safety
		C (N=12)	8.5 yrs		Der p	Depot	Conventional (13 wks)	1/1/1/1/1/1/1/1/1/1/1/1/1 (1 wk)		
Zhang *et al.* (2008) [18]	RCT (OPS)	I (N=48)	25 yrs	Rhinitis/ Rhinoconjunctivitis (with or without asthma)	Der p	Depot	Cluster (6 wks)	3/2/2/2/2/2/1 (30 min)	1 yr	SS/MS QoL ST Immune parameters Safety
		C (N=48)	25 yrs		Der p	Depot	Conventional (14 wks)	1/1/1/1/1/1/1/1/1/1/1/1/1/1 (1 wk)		
Blumberga *et al.* (2006) [13]	RCT (DBPC)	I (N=26)	29.8 ± 10.7 yrs	Asthma	Der p	Depot	Cluster (8 wks)	2-3/ visit (NAD)	3 yrs	SS/MS Lung function Safety
		C (N=28)	28.5 ± 7.1 yrs		Placebo	-	Cluster (8 wks)	2-3/ visit (NAD)		
Colás *et al.* (2006) [14]	RCT (DBPC)	I (N=43)	34 yrs	Rhinitis/ Rhinoconjunctivitis (with or without asthma)	Sal k	Allergoid	Cluster (1 wk)	3/3 (15-20 min)	1 yr 1 wk	SS/MS QoL ST Safety
		C (N=20)	33 yrs		Placebo	-	Cluster (1 wk)	3/3 (15-20 min)		
Roberts *et al.* (2006) [10]	RCT (DBPC)	I (N=19)	9.2 ± 4.4 yrs	Asthma (with rhinoconjunctivitis)	Grass	Depot	Cluster (11 wks)	3/2/2/2/2/2/1/1 (30-60 min)	1 yr 2 mon	SMS/SS/MS Lung function ST/CCT/BCT Immune parameters Safety
		C (N=18)	10.6 ± 2.9 yrs		Placebo	-	Cluster (11 wks)	3/2/2/2/2/2/1/1 (30-60 min)		

Ibero *et al.* (2006) [11]	RCT (OPS)	I (N=15)	10 yrs	Asthma (with rhinoconjunctivitis)	Der p	Allergoid	Cluster (1 wk)	2/2 (30 min)	4 mon 1 wk	SS/MS ST/BCT
		C (N=15)	12 yrs		Pharmacotherapy	-	-	-		Safety

N, Number of participants; SD, Standard deviation; RCT, Randomized controlled trial; DBPC, Double-blind placebo-controlled trial; OPS, Open-label and parallel study; I, Intervention group; C, Control group; wk, week; yr, year; mon, month; SMS, Combined symptoms-medication score; SS, Symptoms score; MS, Medication score; QoL, Quality of life; ST, Skin test; NCT, Nasal challenge test; CCT, Conjunctival challenge test; BCT, Bronchial challenge test; Der p, *Dermatophagoides pteronyssinus;* Sal k, *Salsola kali;* NAD, No available data.
[a] Data relative to adolescent subjects.

Table 2. Assessment of risk of bias summary.

Type of bias	Judgment	Support for judgment	Study (reference)
Random sequence generation Allocation concealment **(Selection bias)**	"Low risk" of selection bias	The investigators described the method used to obtain random sequence and to conceal the sequence of treatment allocation.	(10,12,15,18)
	"High risk" of selection bias	The investigators described a non-random approach and the method for allocation concealment wasn't robust.	-
	"Unclear risk" of selection bias	Insufficient information to permit judgment of "Low risk" or "High risk" of bias.	(8,9,11,13,14,16,17)
Blinding of participants and personnel **(Performance bias)**	"Low risk" of performance bias	The investigators described the methods used to blind study participants and personnel during the study.	(10,12-17)
	"High risk" of performance bias	The participants and personnel weren't blinded during the study.	(8,9,11,18)
	"Unclear risk" of performance bias	Insufficient information to permit judgment of "Low risk" or "High risk" of bias. This outcome wasn't reported in the study.	-
Blinding of outcome assessment **(Detection bias)** (Patient-reported outcomes)	"Low risk" of detection bias	The investigators described the methods used to blind outcome assessors during the study and the blinding wasn't broken.	(8,10,12-17)
	"High risk" of detection bias	The outcome assessors weren't blinded during the study and the outcome measure can be influenced by lack of blinding.	(9,11,18)
	"Unclear risk" of detection bias	Insufficient information to permit judgment of "Low risk" or "High risk" of bias. This outcome wasn't reported in the study.	-
Blinding of outcome assessment **(Detection bias)** (Objective outcomes)	"Low risk" of detection bias	The assessment of objective measures isn't likely to be influenced by lack of blinding.	(8-18)
Incomplete outcome data addressed **(Attrition bias)**	"Low risk" of attrition bias	Adequately methods such as ITT analysis were used to impute missing data. Numbers and reasons of withdrawals or exclusions from the study were reported.	(8-18)
	"High risk" of attrition bias	Inadequate methods were used for imputation of missing data.	-
	"Unclear risk" of attrition bias	Insufficient information to permit judgment of "Low risk" or "High risk" of bias. This outcome wasn't reported in the study.	-

ITT, Intention-to-treat.

Outcomes summary of included studies

Clinical assessment

Ten studies [8,10-18] assessed clinical efficacy of SCIT. Data on individual symptoms (SS) and medication (MS) scores were quantified in nine trials [8,10-16,18]. Only four studies [10,12,15,16] used the recommended combined symptoms-medication score (SMS) as the primary efficacy parameter. The method performed to measure the individual symptoms severity was different among studies. Symptoms were graded using a 4-point rating scale (0= no symptoms, 1= mild symptoms, 2= moderate symptoms and 3= severe symptoms) in seven studies [8,10-12,14,16,18], and a 6-point and a 5-point rating scales in one study [13]. Four studies [13,14,15,18] used a validated 10-point visual analogue scale - continuous scale from zero (no symptoms) to 10 (very severe symptoms) centimeters (cm) - to evaluate disease severity and global treatment efficacy. With respect to medication score assessment, arbitrary scores for rescue medications were applied among clinical trials (different scores were attributed to each dose of symptomatic drugs according to degree and duration of their clinical effects). The SMS was calculated as the time-weighted area under the curve (AUC) of the sum of daily symptoms and daily rescue medication scores in three studies [10,15,16]. Klimek *et al.* [12] calculated SMS by means of the sum of total symptoms score plus total medication score in the observational period.

Quality of life

Among all included studies, four [14-16,18] calculated quality-of-life scores using the validated Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ), by which the overall quality of life and specific domains were evaluated.

Functional measures

Lung function is a functional measure evaluated in two clinical trials [13,14].

Surrogate markers

Five studies [10,11,14,17,18] reported results about cutaneous allergen reactivity. Nasal

challenge tests were performed in one clinical trial [12], conjunctival challenge tests in one study [10], and bronchial challenge tests in two studies [10,11].

Immunological parameters were assessed in eight studies [8–10,12,15–18]. Allergen-specific serum antibodies analyses were performed in seven studies [8,9,12,15–18]. The effect of allergen-specific non-IgE antibodies (blocking antibodies) on mechanisms induced by IgE antibodies was evaluated in two studies [9,17]. The frequency of T-cell subsets in peripheral blood and their functions as well as production of cytokines from peripheral blood mononuclear cells were expressed in two clinical trials [8,9]. SCIT effects on airway inflammation were appraised in two studies [9,10].

Safety assessment

Adverse events were reported in 10 studies [9-18]. The method used to evaluate local reactions was specified in six of these studies [11-15,18]. Klimek *et al.* [12] classified local adverse events according to European Academy of Allergy and Clinical Immunology (EAACI) guidelines, and the remaining five studies quantified local reactions by measuring the longest diameter of the local reaction. In eight clinical trials [9,11-16,18] systemic side effects were graded according to EAACI guidelines. The remaining studies didn't report the measurement tools used to assess SCIT safety.

Accelerated SCIT build-up schedules in pediatric population

The search generated four studies [8-11] of accelerated subcutaneous immunotherapy schedules for the treatment of respiratory allergy in pediatric population, all of them evaluating cluster SCIT. Data on clinical efficacy, immunological efficacy and safety of accelerated SCIT schedules in pediatric patients were summarized in Table 3, 4 and 5, respectively.

Cluster SCIT in pediatric patients

Clinical assessment

Three studies [8,10,11] evaluated clinical efficacy of cluster SCIT in pediatric patients using symptoms and/or medication scores.

Cluster SCIT vs. placebo. Roberts *et al.* [10] evaluated cluster SCIT for control of asthma symptoms during two consecutive pollen seasons using as primary outcome parameter the asthma SMS which showed a significant reduction in the active group compared to placebo in the second pollen season (mainly during the peak pollen season). Although this study demonstrated an improvement in individual SS and MS in the cluster SCIT group, the differences between study groups weren't statistically significant.

Cluster SCIT vs. pharmacotherapy. Lou *et al.* [8] reported that the rhinoconjunctivitis total SS was significantly decreased from baseline for both SCIT-treated and drug-treated patients, however without significant differences between groups. In contrast, the MS was significantly reduced from baseline and the differences between groups were statistically significant. Ibero *et al.* [11] conducted a study involving asthmatic children and showed a significant reduction in the total SS of the active group after four months of treatment. In the control group there was a reduction in SS but this wasn't statistically significant. The MS was reduced from baseline for both groups, but the values didn't achieve statistical significance.

Functional measures

Data on functional measures to assess clinical effects of cluster SCIT in pediatric patients were available in one trial [10].

Cluster SCIT vs. placebo. Roberts *et al.* [10] evaluated lung function of asthmatic children using the parameter Forced Expiratory Volume at one second (FEV_1) obtained by spirometry. This study didn't demonstrate a significant effect of immunotherapy on lung function of asthmatic subjects.

Allergen specific reactivity

Two studies [10,11] assessed clinical efficacy of cluster SCIT in pediatric patients by means of skin prick tests, and conjunctival and bronchial challenge tests.

Cluster SCIT vs. placebo. Roberts *et al.* [10] showed a statistically significant increase in the concentration of allergen extract needed to produce positive cutaneous, conjunctival and bronchial reactions in the active group compared to placebo, with significant differences between groups at the end of the pollen seasons.

Cluster SCIT vs. pharmacotherapy. Ibero *et al.* [11] reported that cutaneous and bronchial allergen reactivity was similar in the two study groups at baseline, reaching statistically significant differences after four months of treatment due to a significant increase in concentration of allergen extract needed to induce positive reactions in the active group.

Allergen-specific serum antibodies analysis

Two studies [8,9] assessed immunogenicity of cluster SCIT in pediatric patients through allergen-specific serum antibodies analysis.

Cluster SCIT vs. conventional SCIT. Schubert *et al.* [9] quantified Der p-specific IgE, IgG and IgG4 levels at week one, eight and 16 of treatment. Specific IgE concentrations increased significantly at week eight of treatment (end of the rapid build-up phase) in the cluster group. The conventional SCIT group didn't show a significant increase in specific IgE levels. On the other hand, concentrations of specific IgG and IgG4 showed a significant increase at week eight in the cluster group, and at week 16 (end of the classic

build-up phase) in the control group.

Cluster SCIT vs. pharmacotherapy. Lou *et al.* [8] measured Der p-specific serum IgE and IgG4 levels, prior to and at the end of the study. The results demonstrated that the changes in allergen-specific IgE levels from baseline weren't statistically significant in both groups, after SCIT for one year. In contrast, the concentration of allergen-specific IgG4 showed a significant increase from baseline in actively-treated patients compared with those in the control treatment group, after one year of immunotherapy.

Effects of allergen-specific non-IgE antibodies (blocking antibodies) on immunological phenomena

One clinical trial [9] evaluated immunological effects of non-IgE antibodies induced by specific immunotherapy (blocking antibodies).

Cluster SCIT vs. conventional SCIT. Schubert *et al.* [9] evaluated inhibitory capacities of serum IgG antibodies induced by specific immunotherapy on the allergen-induced cysteinyl leukotrienes (cysLT) release and CD63 expression on basophils at week one, eight and 16 of treatment. Prior to treatment, both groups showed an important cysLT secretion after allergen stimulation and it significantly decreased after eight weeks in the cluster group, and after 16 weeks in the conventional SCIT group, registering a significantly lower cysLT release in the cluster patients after eight weeks. Allergen-induced CD63 expression showed a significant reduction in both treatment groups after eight weeks, compared to baseline, but in the conventional SCIT group it continued to decrease at week 16 and in the cluster group it reached a plateau level at week eight.

T-cell subsets and cytokines assessment

Two clinical trials [8,9] analyzed T-cell subsets and cytokines secretion to determine immunological efficacy of cluster SCIT in pediatric population.

Cluster SCIT vs. conventional SCIT. Schubert *et al.* [9] evaluated the effect of cluster and conventional SCIT schedules on the balance of Treg, Th1 and Th2 cells

transcription factors (Foxp3, T-bet and GATA-3, respectively). The investigators didn't find significant differences within and between treatment groups.

Cluster SCIT vs. pharmacotherapy. Lou *et al.* [8] assessed the frequency of allergen-specific IFN-y^+IL-4$^-$CD4$^+$, IL-4$^+$IFN-y^-CD4$^+$, and IL-10$^+$IL-4$^-$CD4$^+$ T cells (Th1, Th2 and Tr1 cells, respectively) in peripheral blood, and found that the frequencies of Th1 and Th2 cells weren't significantly changed from baseline in active and control groups, after one year of SCIT. By contrast, this study demonstrated a significant increase in Tr1 cells in SCIT-treated patients, but not in the drug-treated patients, after one year of treatment compared with baseline levels. In the cluster SCIT group, compared with control group, the ratio of Tr1/Th2 cells was significantly increased at the end of the treatment. Significant correlations were found between increased numbers of Tr1 cells and improvements in clinical severity, particularly in nasal symptoms, in SCIT group after one year of treatment. The same investigators measured the frequency of CD4$^+$CD25$^+$FOXP3$^+$ Treg cells in CD4$^+$ T cells and evaluated the suppressive capacity of isolated CD4$^+$CD25^{high} T cells after allergen stimulation on T cell proliferation and cytokines (IFN-y, IL-4 and IL-10) synthesis, at baseline and after SCIT for one year. The results demonstrated that levels of CD4$^+$CD25$^+$FOXP3$^+$ Treg cells in peripheral blood weren't significantly altered from baseline in the two treatment groups at the end of the treatment. The suppressive capacity of CD4$^+$CD25^{high} T cells was observed for T cell proliferation and IFN-y production at baseline, without significant changes after one year of immunotherapy. In relation to IL-4 synthesis, it wasn't significantly suppressed by CD4+CD25^{high} T cells at baseline and at the end of the treatment, and IL-10 production wasn't altered at baseline in both groups however it was significantly increased after one year in SCIT group. Finally, the allergen-induced production of IFN-y, IL-4 and IL-10 from peripheral blood mononuclear cells (PBMCs) cultures was measured, and the levels of IL-10 were significantly increased in the active treatment group, but not in the control group, after one year, as opposed to the levels of IFN-y and IL-4 which didn't change in both groups.

Airway inflammatory markers

Two clinical trials [9,10] used airway inflammatory markers to evaluate efficacy of cluster SCIT in pediatric patients.

Cluster SCIT vs. placebo. Roberts *et al.* [10] assessed levels of exhaled nitric oxide (eNO) and number of eosinophils per gram of sputum as secondary outcomes to investigate cluster SCIT efficacy in pediatric patients with allergic asthma, and found that there were no significant differences in the levels of airway inflammation between the actively-treated and placebo subjects.

Cluster SCIT vs. conventional SCIT. Schubert *et al.* [9] evaluated eNO and eosinophilic cationic protein (ECP) levels after one, eight and 16 weeks of treatment. The authors reported that there was a decrease of eNO levels in the cluster group over the treatment period, without significant differences when compared with that seen in the conventional SCIT group. Both groups showed a reduction of ECP levels compared with baseline values, but the cluster group had a more rapid decline of ECP and the conventional SCIT group reached a significant decline only after 16 weeks. When comparing the two treatment groups after eight weeks of immunotherapy, a significant difference was found.

Safety assessment

Three studies [9–11] evaluated safety of cluster SCIT in pediatric population.

Cluster SCIT vs. placebo. Roberts *et al.* [10] compared cluster SCIT with placebo and reported a total of 54 treatment-related adverse reactions. Thirteen of 18 patients (72% of subjects) in the intervention group experienced 34 reactions, of which 13 were local and 21 were systemic. The remaining 20 reactions occurred in the placebo group, reported by seven of the 17 subjects (41% of subjects): 11 local reactions and nine systemic reactions. All local reactions (pruritus, pain and swelling) were mild and well tolerated with specific treatment (antihistamines and ice). Most systemic reactions consisted in episodes of eczema, urticaria and rhinoconjunctivitis, for both groups. There was one child in the active group who

experienced cough and another one in the placebo group who had chest tightness, both two hours after respective injection. Finally, the investigators reported seven mild pulmonary adverse reactions, four in the active group and three in the placebo group, all of them adequately controlled with inhaled bronchodilator. Four pulmonary events occurred during up-dosing phase, and the remaining three were reported during maintenance phase. None of the adverse reactions led to withdrawals from the study. Of note, in this trial pretreatment with topical anesthetic cream and an antihistamine was administered before immunotherapy injections.

Cluster SCIT vs. conventional SCIT. Schubert *et al.* [9] evaluated the safety of treatment for 30 children, 20 included in the cluster SCIT group and 10 in the conventional SCIT group. The authors reported 185 local adverse effects in the cluster group which corresponds to 54.2% of a total of 341 cluster injections, and these reactions were: 97 (28.4%) erythema/redness, 57 (16.7%) swelling with less than five cm, 22 (6.5%) swelling with more than five cm, and eight (2.3%) painful swelling with more than three hours. On the other hand, 80 local adverse reactions occurred among patients in the conventional SCIT group, this is, 53% of a total of 151 classic injections. Of them, 40 (26.5%) reactions were erythema/redness, 20 (13.2%) swelling with less than five cm, 17 (11.3%) swelling with more than five cm, and three (2%) painful swelling with more than three hours. In all cases, local side effects were classified as mild and didn't require specific drugs or dose adjustments. Regarding systemic side effects, subjects of the cluster group experienced 12 (3.5% of cluster injections) systemic adverse reactions: 10 cases of cough (2.9%) and two cases of dyspnea (0.6%). Overall systemic side effects observed in the intervention group, 2.9% of cluster injections were classified as nonspecific or mild (grade I-II), 0.6% were grade III (bronchial asthma), and none was grade IV. In the conventional SCIT group, the investigators reported seven (4.6% of classic injections) systemic adverse events: six cases of cough (4%) and one case of dyspnea (0.7%). Of them, 3.9% were nonspecific or mild (grade I-II), 0.7% were

grade III (bronchial asthma), and none of classic injections was grade IV. There were no significant differences between the two treatment groups regarding local and systemic adverse reactions. None subject dropped out because of local or systemic adverse events. However, due to an increase of systemic side effects, mainly cough, five patients in the cluster group received a lower dose of vaccine at week four of treatment.

Cluster SCIT vs. pharmacotherapy. Ibero *et al.* [11] described three local reactions in three patients and two systemic reactions in two patients, among 15 patients included in the active group. One child experienced a local reaction characterized by pain and heat over a period of 24 hours after the first two injections (up-dosing phase). The other two local reactions occurred during maintenance phase: one was an episode of pain at the injection site, and the other one was induration (one cm in diameter) and pruritus. All local reactions were evaluated as mild. Regarding systemic reactions, one patient had dyspnea and another one had an exacerbation of asthma and rhinitis. Both systemic reactions were classified as grade II on the EAACI scale and occurred during maintenance phase. Symptomatic treatment or changes in dosing schedule weren't necessary. There were no withdrawals from the study because of adverse events. There was no available data for the control group.

Table 3. Clinical efficacy evaluation of accelerated SCIT build-up schedules in pediatric population.

Subgroup	Intervention/ Comparator	Study	Outcome	Summary of findings
Cluster SCIT	Cluster vs. placebo	Roberts *et al.* (2006) [10]	Asthma SMS	Improvement in SMS in the active group; significant differences between groups in favor to the active group (second season).
			Asthma SS	Improvements in SS in the active group; **no** significant differences between groups.
			Asthma MS	Improvement in MS in the active group; **no** significant differences between groups.
			Lung function	**No** significant effects of immunotherapy on lung function of asthmatic patients.
			Allergen specific reactivity	Improvement in ST, CCT and BCT in the active group; significant differences between groups in favor to the active group.
	Cluster vs. pharmacotherapy	Lou *et al.* (2012) [8]	Rhinoconjunctivitis SS	Improvement in SS in the active group; **no** significant differences between groups.
			Rhinoconjunctivitis MS	Improvement in MS in the active group; significant differences between groups in favor to the active group.
		Ibero *et al.* (2006) [11]	Total SS	Improvement in SS in the active group; significant differences between groups in favor to the active group.
			Total MS	Improvement in MS in the active group; **no** significant differences between groups.
			Allergen specific reactivity	Improvement in ST and BCT in the active group; significant differences between groups in favor to the active group.

SMS, Combined symptoms-medication score; SS, Symptoms score; MS, Medication score; ST, Skin test; CCT, Conjunctival challenge test; BCT, Bronchial challenge test.

Table 4. Immunological efficacy evaluation of accelerated SCIT build-up schedules in pediatric population.

Subgroup	Intervention/ Comparator	Study	Outcome	Summary of findings
Cluster SCIT	Cluster vs. placebo	Roberts *et al.* (2006) [10]	Airway inflammatory markers	**No** significant differences in eNO levels between groups. **No** significant differences in sputum eosinophilia levels between groups.
	Cluster vs. conventional	Schubert *et al.* (2008) [9]	Serum antibodies	Significant increase in IgE levels in the active group (wk 8); significant differences between groups in favor to the active group (wk 8). Significant increase in IgG and IgG4 levels in the active group (wk 8) and in the control group (wk 16); significant differences between groups in favor to the active group (wk 8).
			Non-IgE antibodies (blocking antibodies) effects	Significant reduction in cysLT release in the active group (wk 8) and in the control group (wk 16); significant differences between groups in favor to the active group (wk 8). Significant reduction in CD63 expression in the active group (wk 8) and in the control group (wks 8, 16).
			T-cell subsets and cytokines	**No** significant changes in the expression of T cell subset transcription factors (Foxp-3, T-bet and GATA-3) in both groups.
			Airway inflammatory markers	**S**ignificant reduction in eNO levels in the active group; **no** significant differences between groups. **S**ignificant reduction in ECP levels in the active group; significant differences between groups in favor to the active group (week 8).
	Cluster vs. pharmacotherapy	Lou *et al.* (2012) [8]	Serum antibodies	**No** significant changes in IgE levels in both groups. Significant increase in IgG4 levels in the active group; significant differences between groups in favor to the active group.
			T-cell subsets and cytokines	**No** significant changes in Th1 and Th2 cells frequencies in both groups. Significant increase in Tr1 cells frequency in the active group; significant differences between groups in favor to the active group. Significant increase in Tr1/Th2 cells ratio in the active group; significant differences between groups in favor to the active group. **No** significant changes in $CD4^+CD25^+Foxp3^+$ Treg cells frequency in both groups. **No** significant changes in $CD4^+CD25^{high.}$T cells function in both groups. **No** significant changes in IL-4 and IFN-γ production in both groups. Significant increase in IL-10 production in the active group; significant differences between groups in favor to the active group.

eNO, Exhaled nitric oxide; cysLT, Cysteinyl leukotrienes; ECP, Eosinophilic cationic protein; wk, Week.

Table 5. Safety evaluation of accelerated SCIT build-up schedules in pediatric population.

Subgroup	Study	Intervention/ Comparator	Adverse reactions														
Cluster SCIT			Type of reactions	Frequency of reactions (r/%r)		Description of reactions (r/%r)		Severity of reactions (r/%r)		Specific treatment/Dose adjustment (yes/no)		Study withdrawals (n)		Protocol phase (r)	
	Roberts *et al.* (2006) [10]	**Cluster vs. placebo**		I	C	I	C	I	C	I	C	I	C	I	C
			Local	13	11	Pruritus, Pain, Swelling (13)	Pruritus, Pain, Swelling (11)	Mild (13)	Mild (11)	Yes	Yes	0	0	NAD	NAD
			Systemic	21	9	Eczema, Urticaria, RCA (most) Cough (1) Pulmonary (4)	Eczema, Urticaria, RCA (most) Chest tightness (1) Pulmonary (3)	Mild (21)	Mild (9)	Yes	Yes	0	0	Up-dosing (4)	Maintenance (3)
	Schubert *et al.* (2008) [9]	**Cluster vs. conventional**		I	C	I	C	I	C	I	C	I	C	I	C
			Local	185 54.2 %	80 53%	Erythema/Redness (97/28.4%) Swelling <5 cm (57/16.7%) Swelling >5 cm (22/6.5%) Painful swelling >3h (8/2.3%)	Erythema/Redness (40/26.5%) Swelling <5 cm (20/13.2%) Swelling >5 cm (17/11.3%) Painful swelling >3h (3/2%)	Mild (185)	Mild (80)	No	No	0	0	NAD	NAD
			Systemic	12 3.5%	7 4.6%	Cough (10/2.9%) Dyspnea (2/0.6%)	Cough (6/4%) Dyspnea (1/0.7%)	Gr I-II (2.9%) Gr III (0.6%)	Gr I-II (3.9%) Gr III (0.7%)	Yes	No	0	0	NAD	NAD
	Ibero *et al.* (2006) [11]	**Cluster vs. pharmacotherapy**		I	C	I	C	I	C	I	C	I	C	I	C
			Local	3	NAD	Pain (1) Pain and heat (1) Induration and pruritus (1)	NAD	Mild (3)	NAD	No	NAD	0	0	Up-dosing (1) Maintenance (2)	NAD
			Systemic	2	NAD	Dyspnea (1) Asthma and rhinitis (1)	NAD	Gr II (2)	NAD	No	NAD	0	0	Maintenance (2)	NAD

I, Intervention group; C, Control group; r, Number of adverse reactions; %r, Percentage of injections with adverse reactions; n, Number of subjects; RCA, Rhinoconjunctivitis; Gr, Grade; NAD, No available data.

Accelerated SCIT build-up schedules in adult population

The search resulted in three studies [12-14] of accelerated subcutaneous immunotherapy schedules for the treatment of respiratory allergy in adult patients, all of them evaluating cluster SCIT. Data on clinical efficacy, immunological efficacy and safety of accelerated SCIT schedules in adult patients were summarized in Table 6, 7 and 8, respectively.

Cluster SCIT in adult patients

Clinical assessment

Three studies [12-14] assessed clinical efficacy of cluster SCIT in adults by measuring changes in symptoms severity and medication usage, and evaluating global treatment effectiveness.

Cluster SCIT vs. placebo. Klimek *et al.* [12] assessed clinical efficacy of cluster SCIT in adults with allergic rhinoconjunctivitis and found a significant reduction in SMS in the actively-treated group compared to placebo for the whole pollen season and peak pollen season. Relating to rhinoconjunctivitis total SS, there was a significant reduction in the active group compared to placebo during the whole pollen season (34%) and peak pollen season (36%). The MS was significantly reduced in the active group compared to placebo, registering a reduction of 40% during the whole pollen season and of 43% during peak pollen season. Blumberga *et al.* [13] evaluated cluster SCIT for control of asthma, and demonstrated that there were no largely changes at the annual re-assessments in SS and MS. Visual scale analysis showed similar scores for the two study groups. Colás *et al.* [14] evaluated clinical effects of cluster SCIT in adults with allergic rhinoconjunctivitis and reported significant reduction in total SS (sum of nasal, ocular and bronchial symptoms scores) in the active group compared to placebo over the treatment period. This study also demonstrated a significant difference in MS between groups, with the active group registering lower intake of medication. Finally, VAS scores were higher in the active group compared to placebo, with statistically significant differences between groups.

Quality of life

One clinical trial [14] reported the Quality of Life Questionnaire as a secondary outcome for cluster SCIT clinical efficacy in adult patients.

Cluster SCIT vs. placebo. Colás *et al.* [14] demonstrated that there was a greater improvement in overall quality of life of patients in the active group, but not in the placebo group, with significant differences between groups. Activities, nasal symptoms and emotions domains showed significant differences between active and placebo groups, as opposed to other domains such as sleep, non-hay fever symptoms, practical problems, and eye symptoms**.**

Functional measures

One clinical trial [13] used lung function to assess clinical efficacy of cluster SCIT in adults.

Cluster SCIT vs. placebo. Blumberga *et al.* [13] measured peak expiratory flow (PEF) before starting treatment and after one, two and three years of SCIT. The authors reported that PEF didn't show significant changes overall the treatment period.

Allergen specific reactivity

Two studies [12,14] quantified allergen specific reactivity through skin tests and nasal challenge test to assess clinical efficacy of cluster SCIT in adults.

Cluster SCIT vs. placebo. Klimek *et al.* [12] performed a nasal challenge test in allergic adult patients, and both active and placebo groups showed an increase in allergen concentration required to induce a positive nasal reaction at the end of the treatment, compared with baseline values. However, the differences between groups weren't statistically significant. Colás *et al.* [14] assessed immediate cutaneous responses to allergen, at initial visit and at the end of the treatment. There were no significant differences between the two groups at baseline, but at the end of the study the allergen concentration needed to produce a positive cutaneous reaction was significantly higher in the active group compared to placebo.

Allergen-specific serum antibodies analysis

One clinical trial [12] analyzed allergen-specific IgE and IgG4 levels as immunological efficacy markers of cluster SCIT in adults.

Cluster SCIT vs. placebo. Klimek *et al.* [12] described a significantly higher increase of IgG4 levels in the active group than in the control group, when comparing pre- and post-SCIT values of this immunoglobulin, with significant differences between groups at the end of the treatment. The investigators reported that the concentration of specific IgE was decreased in both groups at the end of the treatment compared to baseline values, but the differences between groups weren't statistically significant.

Safety assessment

All adult studies [12-14] evaluated the safety of cluster SCIT.

Cluster SCIT vs. placebo. Klimek *et al.* [12] assessed the safety of treatment for 120 patients, 61 in the active group and 59 in the placebo group, receiving 1778 injections, of which 928 were allergoid vaccines and 850 were placebo. Local reactions occurred after 0.7% of the total of 928 injections given to the subjects in the active group. All of them were classified as immediate (onset within the first 30 minutes after injection) and occurred during up-dosing phase. These local side effects were grade ≥I on the EAACI scale. Two mild systemic events (rhinitis and nasal obstruction) also occurred during up-dosing phase, after 0.2% of the total of 928 injections, and were classified as immediate reactions. Delayed grade I systemic reactions were reported (fatigue, nasal obstruction, skin reaction) after 0.6% of allergoid injections. No grade II, III or IV systemic reactions were observed. There were no severe adverse events related to treatment that justified withdrawals from the study. The tolerability of the treatment was classified by the investigators as good or very good for 95% of actively-treated patients and for 100% of placebo subjects. Blumberga *et al.* [13] reported that subcutaneous nodules were more often observed in patients under immunotherapy, with a difference of 26% in favor of the active treatment group. In the intervention group, 10 of the

26 patients (38%) experienced at least one systemic adverse reaction, totalizing 43 systemic reactions, and in that way a rate of 4.7% systemic reactions per cluster injection was observed. Of them, 41 were grade II systemic reactions and two were grade III. On the other hand, there were a total of 21 systemic adverse events in the placebo group experienced by eight of the 28 patients in this group (29%), resulting in a rate of 2.1% of systemic reactions per placebo injection. Nine systemic reactions classified as grade I were observed in five patients, and 12 grade II systemic reactions were detected in six patients. There were no grade III or IV adverse reactions. Most systemic adverse events occurred during up-dosing phase in both groups. There were no withdrawals from the study due to adverse reactions, but one patient in the intervention group experienced a severe bronchospasm which required specific treatment (inhaled β_2-agonist and oral corticosteroids). Colás *et al.* [14] reported 16 local adverse reactions in eight SCIT-treated patients and 10 local side effects in four placebo subjects. All local reactions were delayed (later than 30 minutes after injection), with nine and eight subcutaneous nodules caused by aluminum hydroxide in the intervention and placebo groups, respectively. The remaining local events were clinically irrelevant (diameter of less than 10 cm). Regarding systemic adverse reactions, there were 16 (four immediate and 12 delayed systemic reactions) in the active group, against four (all delayed) in the placebo group (experienced by three patients). All reactions were mild, classified as grade II according to EAACI guidelines (rhinoconjunctivitis or otic pruritus). None of these adverse events needed dose adjustment or specific treatment, and didn't lead to withdrawals from the study.

Table 6. Clinical efficacy evaluation of accelerated SCIT build-up schedules in adult population.

Subgroup	Intervention/ Comparator	Study	Outcome	Summary of findings
Cluster SCIT	**Cluster vs. placebo**	Klimek *et al.* (2014) [12]	Rhinoconjunctivitis SMS	Improvement in SMS in the active group; significant differences between groups in favor to the active group.
			Rhinoconjunctivitis SS	Improvement in SS in the active group; significant differences between groups in favor to the active group.
			Rhinoconjunctivitis MS	Improvement in MS in the active group; significant differences between groups in favor to the active group.
			Allergen specific reactivity	Improvement in NCT in the active group; **no** significant differences between groups.
		Blumberga *et al.* (2006) [13]	Asthma SS	**No** significant improvement in SS in both groups.
			Asthma MS	**No** significant improvement in MS in both groups.
			VAS (10 cm)	VAS scores were similar for the two groups.
			Lung function	**No** significant improvements in lung function of asthmatic patients in both groups.
		Colás *et al.* (2006) [14]	Total SS	Improvement in SS in the active group; significant differences between groups in favor to the active group.
			Total MS	Improvement in MS in the active group; significant differences between groups in favor to the active group.
			VAS (10 cm)	VAS scores were higher in the active group; significant differences between groups in favor to the active group.
			QoL score	Improvement in QoL score in the active group; significant differences between groups in favor to the active group.
			Allergen specific reactivity	Improvement in ST in the active group; significant differences between groups in favor to the active group.

SMS, Combined symptoms-medication score; SS, Symptoms score; MS, Medication score; VAS, Visual analogue scale; QoL, Quality of life; ST, Skin test; NCT, Nasal challenge test.

Table 7. Immunological efficacy evaluation of accelerated SCIT build-up schedules in adult population.

Subgroup	Intervention/ Comparator	Study	Outcome	Summary of findings
Cluster SCIT	**Cluster vs. placebo**	Klimek *et al.* (2014) [12]	Serum antibodies	Reduction in IgE levels in the active group; **no** significant differences between groups.
				Significant increase in IgG4 levels in the active group; significant differences between groups in favor to the active group.

Table 8. Safety evaluation of accelerated SCIT build-up schedules in adult population.

Subgroup	Study	Intervention/ Comparator	Adverse reactions														
Cluster SCIT			Type of reactions	Frequency of reactions (r/%r)		Description of reactions (r/%r)		Severity of reactions (r/%r)		Specific treatment/Dose adjustment (yes/no)		Study withdrawals (n)		Protocol phase (r/%r)	
	Klimek *et al.* (2014) [12]	**Cluster vs. placebo**		I	C	I	C	I	C	I	C	I	C	I	C
			Local	0.7%	NAD	Immediate[a] (0.7%)	NAD	Gr ≥I (0.7%)	NAD	NAD	NAD	0	0	Up-dosing (0.7%)	NAD
			Systemic	0.8%	NAD	Immediate[a] (0.2%): rhinitis, nasal obstruction Delayed[b] (0.6%): fatigue, nasal obstruction, skin reaction	NAD	Gr I (0.8%)	NAD	NAD	NAD	0	0	Up-dosing (0.2%)	NAD
	Blumberga *et al.* (2006) [13]	**Cluster vs. placebo**		I	C	I	C	I	C	I	C	I	C	I	C
			Local	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD	NAD
			Systemic	43	21	NAD	NAD	Gr II (41) Gr III (2)	Gr I (9) Gr II (12)	Yes	NAD	0	0	Up-dosing (most)	Up-dosing (most)
	Colás *et al.* (2006) [14]	**Cluster vs. placebo**		I	C	I	C	I	C	I	C	I	C	I	C
			Local	16	10	Delayed[b] (16): subcutaneous nodules (9)	Delayed[b] (10): subcutaneous nodules (8)	Irrelevant (d<10cm)	Irrelevant (d<10cm)	No	No	0	0	NAD	NAD
			Systemic	16	4	Immediate[a] (4) Delayed[b] (12): RCA, otic pruritus	Delayed[b] (4): RCA, otic pruritus	Gr II (16)	Gr II (4)	No	No	0	0	NAD	NAD

I, Intervention group; C, Control group; r, Number of adverse reactions; %r, Percentage of injections with adverse reactions; n, Number of subjects; RCA, Rhinoconjunctivitis; Gr, Grade; d, Diameter of local reaction; NAD, No available data.

a. Immediate reaction = onset within the first 30 minutes after injection.
b. Delayed reaction = onset after 30 minutes post-vaccine.

Accelerated SCIT build-up schedules in mixed population

The search generated four studies of accelerated subcutaneous immunotherapy schedules for the treatment of respiratory allergy in mixed population. Two [15,16] of them evaluated rush SCIT and another two [17,18] evaluated cluster SCIT. Data on clinical efficacy, immunological efficacy and safety of accelerated SCIT schedules in mixed population were summarized in Table 9, 10 and 11, respectively.

Rush SCIT in mixed population

Clinical assessment

Two clinical trials [15,16] evaluated clinical efficacy of rush SCIT in mixed population using symptoms and/or medication scores.

Rush SCIT vs. placebo. Two placebo-controlled trials [15,16] evaluated the clinical efficacy of rush SCIT in mixed population over two consecutive pollen seasons, using as primary efficacy endpoint the combined SMS. One study [15] enrolled patients with allergic rhinoconjunctivitis and found a significant reduction (19.4%) in SMS, comparing patients treated with birch and grass pollen extract and placebo, over the second birch and grass pollen season. For the first season, differences between active and placebo groups weren't statistically significant. The individual SS and MS weren't significantly different between groups for both seasons. Other study [16] included grass pollen allergic patients and reported a significant reduction in SMS for the active group during the peak of the first grass pollen season, as well as during the peak of the second grass pollen season (33% less for active treatment group) and for the whole second pollen season (39.5% less for active treatment group). This study demonstrated an improvement in rhinoconjunctivitis SS for both seasons, but the differences between intervention and control groups were statistically significant only for the second season. The MS was lower for both groups, mainly in the active one, with significant differences between them. Both studies noted that adolescent patients showed similar results to those of adults, but numbers were too small to allow subgroup analyses [15]

or to lead to statistically significant differences [16].

Quality of life

Two clinical trials [15,16] reported the Quality of Life Questionnaire as a secondary outcome for rush SCIT clinical efficacy in mixed population.

Rush SCIT vs. placebo. One study [15] demonstrated a significant reduction in total quality-of-life score for the active group compared to placebo in the second year of treatment, as opposed to the results obtained in the first year. Sleep and nasal symptoms were the domains that showed significant differences between groups. It has to be noted that the investigators made an adaptation of questionnaire for adolescent patients. Other study [16] revealed an improvement in overall quality of life and specific domains such as activities and non-nasal symptoms for the first treatment year, in addition to emotion domain for the second year. The reported domains showed significant differences or a trend for difference between active and placebo groups. Available data on quality of life was only related to adults.

Allergen-specific serum antibodies analysis

Two clinical trials [15,16] determined allergen-specific IgE and IgG4 levels to evaluate immunological efficacy of rush SCIT.

Rush SCIT vs. placebo. Two clinical trials [15,16] determined allergen-specific IgE and IgG4 levels before and after two years of treatment, and reported similar findings. Over the treatment period both trials reported a significantly higher increase of allergen-specific IgG4 concentration in the active group than in the placebo group. Noteworthy, one of these studies [15] assessed the correlation between either levels of specific IgG4 at the end of the treatment or increase in specific IgG4 from baseline and the combined SMS evaluated in the same study, and didn't find a significant correlation. Regarding allergen-specific IgE, different results were reported, but in general no significant changes in its levels were observed throughout the treatment.

Safety assessment

Two studies [15,16] assessed safety of rush SCIT in mixed population.

Rush SCIT vs. placebo. Two placebo-controlled trials [15,16] assessed safety of rush SCIT in mixed population. One study [15] observed local side effects in 66 of 186 (35.5%) actively-treated patients and in 29 of 99 (29.3%) placebo subjects. None of the local adverse reactions were serious. In addition, 18 systemic reactions were observed. Of them, 13 reactions appeared in 10 patients treated with rush SCIT: two episodes of vertigo/anxiety, two of asthma, two of conjunctivitis, one of rhinitis, one of throat irritation, one of headache, two of chills, one of pruritus and one case of feeling hot. The two asthmatic reactions were grade II, and the remaining reactions were grade I on the EAACI scale. The remaining five systemic adverse events occurred in four placebo patients, and all were grade I. All adverse reactions didn't require specific treatment, and all patients completed rapid up-dosing phase without dose adjustment. There were no withdrawals from the study because of adverse events. In global, treatment was well tolerated. The other study [16] reported local reactions in 95 of the 135 (70.4%) subjects from the active group and in 24 of the 60 (40%) placebo subjects, none requiring specific treatment or dose adjustment. Twenty seven systemic reactions occurred in 16 patients treated with rush SCIT and seven appeared in three placebo subjects. All of them were classified as grade I and II, according to EAACI guidelines. In total, four patients from the intervention group and two patients from the control group withdrew from the study because of adverse reactions.

Cluster SCIT in mixed population

Clinical assessment

One study [18] evaluated clinical efficacy of cluster SCIT in mixed population through symptoms and medication scores, and visual scale analysis.

Cluster SCIT vs. conventional SCIT. Zhang *et al.* [18] determined clinical efficacy of cluster SCIT for patients with allergic rhinoconjunctivitis at week zero (baseline),

six (end of the cluster up-dosing phase), 14 (end of the conventional up-dosing phase) and 52 (end of the treatment). The changes in SS, MS and VAS score were similar. At week six, the cluster group showed a significant decrease in all scores compared with baseline values, as opposed to that seen in the conventional group. At week 14, both groups showed a significant decrease in all scores compared with baseline, but while the reduction in SS was significantly greater in the cluster group, the differences between groups had disappeared in respect to MS and VAS scores. At the end of the study, the differences between groups were minimal in all scores.

Quality of life

One clinical trial [18] reported the Quality of Life Questionnaire as a secondary outcome for cluster SCIT clinical efficacy in mixed population.

Cluster SCIT vs. conventional SCIT. Zhang *et al.* [18] showed a significant improvement in overall quality of life and all other domains in both groups, except for non-hay fever symptoms domain in the conventional SCIT group, after one year of treatment, compared with baseline values. There were no significant differences between groups before and after the study.

Allergen specific reactivity

Two studies [17,18] quantified allergen specific reactivity through skin tests to assess clinical efficacy of cluster SCIT in mixed population.

Cluster SCIT vs. placebo. Vidal *et al.* [17] evaluated efficacy of cluster SCIT using skin tests which were performed prior to and at the end of the treatment. The investigators determined the immediate cutaneous response to allergen extract and observed that the Cutaneous Tolerance Index (factor by which it is necessary to multiply the allergen extract concentration after SCIT to obtain the same response as before the treatment) was significantly reduced in the active group as opposed to the placebo group, so there were statistically significant differences between groups.

Cluster SCIT vs. conventional SCIT. Zhang *et al.* [18] showed that both cluster and conventional SCIT decreased the Cutaneous Tolerance Index, but there were no significant differences between groups.

Allergen-specific serum antibodies analysis

Two clinical trials [17,18] determined allergen-specific IgE and IgG4 levels to evaluate immunological efficacy of cluster SCIT in mixed population.

Cluster SCIT vs. placebo. Vidal *et al.* [17] measured allergen-specific serum antibodies at the beginning and at the end of the treatment. A significant increase in specific IgG4 levels occurred in the active group, but not in the placebo. Therefore, the differences between groups became significant at the end of the study. The levels of specific IgE didn't show relevant differences between groups at the end of the treatment.

Cluster SCIT vs. conventional SCIT. Zhang *et al.* [18] reported that allergen-specific IgE levels didn't significantly change after one year of treatment, compared with baseline values, in both groups. There were no significant differences between cluster and conventional schedules.

Effects of allergen-specific non-IgE antibodies (blocking antibodies) on immunological phenomena

One clinical trial [17] evaluated immunological effects of non-IgE antibodies induced by specific immunotherapy (blocking antibodies).

Cluster SCIT vs. placebo. Vidal *et al.* [17] evaluated the inhibitory capacity of non-IgE antibodies on IgE binding to allergens (immunoglobulin E inhibition). This study demonstrated a significantly higher inhibitory effect in the active group, which wasn't found in the placebo group. Differences between groups were statistically significant.

Safety assessment

Two studies [17,18] evaluated safety of cluster SCIT in mixed population.

Cluster SCIT vs. placebo. Vidal *et al.* [17] found 14 local adverse events: 10 of

these events occurred in three of 21 (14.3%) patients from the intervention group and four adverse reactions were observed in three of 24 (12.5%) placebo subjects. The investigators observed 14 systemic reactions: eight of them occurred in six (28.6%) patients treated with SCIT, and the remaining six reactions occurred in the placebo group involving five (11.1%) subjects. Of them, eight systemic reactions were mild (five in the active group and three in the placebo group) and the remaining six were moderate (three in each group). Notably, among the total systemic reactions observed, only two were considered as treatment-related, one in each study group. It was reported one withdrawal in the intervention group due to adverse events.

Cluster SCIT vs. conventional SCIT. Zhang *et al.* [18] reported 11 local adverse reactions in six patients (13.3% of all patients) in the cluster group during up-dosing phase, in other words, local adverse reactions occurred in 1.7% of all up-dosing injections. On the other hand, nine local adverse events occurred in five patients (11.4% of all patients) in the conventional SCIT group, so local reactions were triggered by 1.4% of all classic injections. During maintenance phase, seven local adverse reactions were observed in four patients (8.9% of all patients), this is, 1.7% of all maintenance injections in the cluster group, and five reactions in four patients (9.1% of all patients), which corresponds to 1.6% of all maintenance injections in the conventional group. All local reactions were immediate (onset within 30 minutes after injection) and clinically irrelevant (diameter lesser than five centimeters), with no need of dose adjustment or specific treatment. There were no differences in frequency and severity of local reactions between groups. In relation to systemic side effects, six patients (13.4% of all patients) in the intervention group experienced 11 systemic reactions, five during up-dosing phase (1% of all up-dosing injections) and six during maintenance phase (1.5% of all maintenance injections). During up-dosing phase, there were three grade I and two grade II reactions, whereas during maintenance phase four grade I and two grade II reactions were observed. In the conventional SCIT group, three

patients (6.8% of all patients) experienced six systemic reactions during up-dosing phase (0.9% of all up-dosing injections): four classified as grade I and two as grade II. During maintenance phase, two patients (4.6% of all patients) experienced four systemic reactions (1.3% of all maintenance injections), with three grade I and one grade II reactions. No grade III or IV systemic reactions, according to EAACI guidelines, were reported. There were no differences in frequency and type of systemic reactions between groups. All systemic reactions were immediate, because they occurred within 20 min after the injection, and all of them were successfully treated. There were no study withdrawals because of adverse reactions. Of note, premedication (antihistamine) was used before each immunotherapy injection.

Table 9. Clinical efficacy evaluation of accelerated SCIT build-up schedules in mixed population.

Subgroup	Intervention/ Comparator	Study	Outcome	Summary of findings
Rush SCIT	**Rush vs. placebo**	Pfaar *et al.* (2013) [15]	Rhinoconjunctivitis SMS	Improvement in SMS in the active group; significant differences between groups in favor to the active group (second season).
			Rhinoconjunctivitis SS	Improvement in SS in the active group; **no** significant differences between groups.
			Rhinoconjunctivitis MS	Improvement in MS in the active group; **no** significant differences between groups.
			QoL score	Improvement in QoL score in the active group; significant differences between groups in favor to the active group (second season).
		Pfaar *et al.* (2012) [16]	Total SMS	Improvement in SMS in the active group; significant differences between groups in favor to the active group.
			Total SS	Improvement in SS in the active group; significant differences between groups in favor to the active group (second season).
			Total MS	Improvement in MS in the active group; significant differences between groups in favor to the active group.
			QoL score	Improvement in QoL score in the active group; significant differences between groups in favor to the active group.
Cluster SCIT	**Cluster vs. conventional**	Vidal *et al.* (2011) [17]	Allergen specific reactivity	Improvement in ST in the active group; significant differences between groups in favor to the active group.
		Zhang *et al.* (2008) [18]	Total SS	Improvement in SS in the active group; significant differences between groups in favor to the active group (wk 6, wk 14).
			Total MS	Improvement in MS in the active group; significant differences between groups in favor to the active group (wk 6).
			VAS (10 cm)	Improvement in VAS score in the active group; significant differences between groups in favor to the active group (wk 6).
			QoL score	Improvement in QoL score in the active group; **no** significant differences between groups.
			Allergen specific reactivity	Improvement in ST in the active group; **no** significant differences between groups.

SMS, Combined symptoms-medication score; SS, Symptoms score; MS, Medication score; VAS, Visual analogue scale; QoL, Quality of life; ST, Skin test; wk, Week;.

Table 10. Immunological efficacy evaluation of accelerated SCIT build-up schedules in mixed population.

Subgroup	Intervention/ Comparator	Study	Outcome	Summary of findings
Rush SCIT	**Rush vs. placebo**	Pfaar *et al.* (2013) [15]	Serum antibodies	**No** significant changes in IgE levels in both groups. Significant increase in IgG4 levels in the active group; significant differences between groups in favor to the active group.
		Pfaar *et al.* (2012) [16]	Serum antibodies	**No** significant changes in IgE levels in both groups. Significant increase in IgG4 levels in the active group; significant differences between groups in favor to the active group.
Cluster SCIT	**Cluster vs. placebo**	Vidal *et al.* (2011) [17]	Serum antibodies	**No** relevant changes in IgE levels in both groups. Significant increase in IgG4 levels in the active group; significant differences between groups in favor to the active group.
			Non-IgE antibodies (blocking antibodies) effects	Significant increase in inhibitory capacity of non-IgE antibodies on IgE binding to allergens in the active group; significant differences between groups in favor to the active group.
	Cluster vs. conventional	Zhang *et al.* (2008) [18]	Serum antibodies	**No** significant changes in IgE levels in both groups.

Table 11. Safety evaluation of accelerated SCIT build-up schedules in mixed population.

Subgroup	Study	Intervention/ Comparator	Adverse reactions														
			Type of reactions	Frequency of reactions (r/%r)		Description of reactions (r/%r)		Severity of reactions (r/%r)		Specific treatment/Dose adjustment (yes/no)		Study withdrawals (n)		Protocol phase (r)	
Rush SCIT	Pfaar *et al.* (2013) [15]	**Rush vs. placebo**		I	C	I	C	I	C	I	C	I	C	I	C
			Local	66/186 patients	29/99 patients	NAD	NAD	No serious	No serious	No	No	0	0	NAD	NAD
			Systemic	13	5	Vertigo/anxiety (2) Asthma (2) Conjunctivitis (2) Rhinitis (1) Throat irritation (1) Headache (1) Chills (2) Pruritus (1) Feeling hot (1)	NAD	Gr I (11) Gr II (2)	Gr I (5)	No	No	0	0	NAD	NAD
	Pfaar *et al.* (2012) [16]	**Rush vs. placebo**		I	C	I	C	I	C	I	C	I	C	I	C
			Local	95/135 patients	24/60 patients	NAD	NAD	NAD	NAD	No	No	4	2	NAD	NAD
			Systemic	27	7	NAD	NAD	Gr I-II (34)		NAD	NAD			NAD	NAD
Cluster SCIT	Vidal *et al.* (2011) [17]	**Cluster vs. placebo**		I	C	I	C	I	C	I	C	I	C	I	C
			Local	10	4	NAD	NAD	NAD	NAD	NAD	NAD	1	0	NAD	NAD
			Systemic	8	6	NAD	NAD	Mild (5) Moderate (3)	Mild (3) Moderate (3)	NAD	NAD		0	NAD	NAD
	Zhang *et al.* (2008) [18]	**Cluster vs. conventional**		I	C	I	C	I	C	I	C	I	C	I	C
			Local	18 3.4%	14 3%	Immediate[a]	Immediate[a]	Irrelevant (d<5cm)	Irrelevant (d<5cm)	No	No	0	0	Up-dosing (11) Maintenance (7)	Up-dosing (9) Maintenance (5)
			Systemic	11 2.5%	10 2.2%	Immediate[a]	Immediate[a]	Gr I (7) Gr II (4)	Gr I (7) Gr II (3)	Yes	Yes	0	0	Up-dosing (5) Maintenance (6)	Up-dosing (6) Maintenance (4)

I, Intervention group; C, Control group; r, Number of adverse reactions; %r, Percentage of injections with adverse reactions; n, Number of subjects; Gr, Grade; NAD, No available data.

a. Immediate reaction = onset within the first 30 minutes after injection.

Discussion

The present systematic review was performed to assess clinical and immunological efficacy and safety of accelerated SCIT schedules in respiratory allergy. Data from 11 RCTs, four in pediatric population, three in adult population, and four in mixed population, were summarized.

Regarding clinical efficacy, and considering pediatric trials, one [10] pointed to the superior efficacy of cluster SCIT compared to placebo, through a significant reduction in the combined SMS in the active group during the second pollen season (differences between groups were small during the first season probably because many subjects reached the effective dose a few weeks before the season and there was a lower pollen exposure in this season). The same trial didn't show significant differences in individual SS and MS between groups. Indeed, a potential fault with SCIT trials is the assessment of symptoms and medication scores independently, because successful immunotherapy reduces both and symptoms severity and rescue medication usage are strictly interdependent.[19] Therefore, the primary efficacy measure is the combined symptoms-medication score.[19] Thence, this study demonstrated clinical efficacy of cluster SCIT which was confirmed by a significant improvement in allergen reactivity in the active group compared to placebo. This trial, however, didn't demonstrate a significant improvement on lung function of asthmatic children probably because not all subjects had available data. Other two pediatric studies showed superior efficacy of cluster SCIT in relation to pharmacotherapy: one trial [8] demonstrated significant differences in MS between study groups in favor to the active group, but not in SS; the other one [11] reported a significant improvement in SS, but not in MS, and an improvement in allergen reactivity in the active group compared with pharmacotherapy. In relation to adults, all studies pointed to the superior efficacy of cluster SCIT compared to placebo. One study [12] found a significant reduction in the combined SMS in the active group in the first pollen season (the use of an allergoid allowed the administration of larger allergen

concentration, which might explain the rapid onset of efficacy, as opposed to that observed in the previously reported pediatric trial [10]). Two studies [12,14] observed a significant improvement in SS and MS, and one [14] of them also showed a significant improvement in global efficacy of SCIT (VAS score). One study [14] demonstrated a significant improvement in total quality-of-life score in the active group compared to placebo. Two studies [12,14] described an improvement in allergen reactivity in the active group, but only one [14] reported significant differences between groups (in the other one [12] statistical significance wasn't reached probably due to a small number of evaluable data). Within adult studies, one [13] didn't find significant improvement in any of the evaluated outcomes (SS, MS, VAS and lung function), perhaps because this study included adults with long-term asthma which may be related with unsuccessful treatment because immunotherapy is more effective in earlier stages of respiratory disease. With respect to mixed population, two rush SCIT trials [15,16] found a significant reduction in the combined SMS in the active group compared to placebo, suggesting clinical efficacy of the investigational treatment. The same trials reported an improvement in SS and MS, with significant differences between groups in only one study [16] (the absence of significance in the other trial [15] probably reflects the use of a mixed extract with reduced concentration of each allergen, considering the dose-dependent effect of immunotherapy). These trials demonstrated a significant improvement in overall quality of life in the active group. On the other hand, one cluster SCIT trial [18] documented its efficacy and more rapid effect in relation to conventional regimen by reporting an improvement in SS, MS and VAS score in the active group at week six of treatment, this is, at the end of the rapid build-up phase, with significant differences between groups. The absence of efficacy of the conventional SCIT almost certainly reflects the low dose of allergen used at this point of classic schedule. The efficacy of conventional SCIT was only achieved at week 14, this is, at the end of the classic build-up phase. The clinical efficacy of cluster SCIT was confirmed in a DBPC study [17] through a significant improvement in allergen reactivity in the active group

compared to placebo.

This descriptive analysis demonstrated that rush and cluster SCIT were clinical efficacious and had more rapidly effects when compared with conventional schedule. No relevant differences between subgroups (pediatric, adult and mixed populations) were observed for cluster SCIT. Although rush SCIT was only evaluated in mixed population trials, the studies noted that pediatric patients showed similar results to those of adults. However, some concern is required in the interpretation of these data because there was a significant heterogeneity between studies, mainly related to the variety of methods and scoring systems used to determine clinical efficacy of immunotherapy. Moreover, it's difficult to determine what represents a clinically important difference on these different scales.

Regarding immunological efficacy, and concerning pediatric studies, one [9] found a rapid and significant increase in allergen-specific IgE and IgG4 levels, plus a significant reduction in cysLT release and CD63 expression on basophils, in the cluster group at week eight (end of the rapid build-up phase) compared with conventional SCIT group. The differences between groups disappeared at week 16 (end of the classic build-up phase). Other trial [8] showed a significant increase in IgG4 levels in the active group compared with pharmacotherapy, but not in IgE levels. In relation to T-cell subsets and cytokines, one study [9] didn't show significant changes in the expression of Treg, Th1 and Th2 cells transcription factors. In contrast, another study [8] demonstrated a significant increase in Tr1 cells frequency and allergen-induced IL-10 production in the active group compared with pharmacotherapy, but no significant changes in Th1 and Th2 cells frequency or IL-4 and IFN-ɣ production. These results pointed to the role of Treg cells in immunotherapy mechanisms, by suppressing allergic T cell responses. One study [9] didn't find significant differences in airway inflammatory markers (eNO and sputum eosinophilia) between active and placebo groups, suggesting a steroid-sparing anti-inflammatory effect of immunotherapy because airway inflammation was similar in both groups even though the immunotherapy group received only

half of the placebo group corticosteroid dose. Another study [9] found a more rapid reduction of eNO and ECP levels in the cluster group compared to the conventional SCIT group, with significant differences for ECP at week eight (end of the rapid up-dosing phase), but not for eNO (probably because eNO levels were already suppressed by inhaled corticosteroids in some subjects). Concerning adults, one study [12] reported a decrease in IgE levels in both groups at the end of the treatment, with no significant differences between groups. In contrast, a significant increase in IgG4 levels occurred in the cluster group compared to placebo. Relating to mixed population, two rush and one cluster SCIT trials [15–17] showed a significant increase in IgG4 levels in the active group compared to placebo. All mixed population studies didn't record significant changes in IgE levels. Finally, one study [17] showed a significant increase in blocking activity of IgG antibodies in the active group, but not in the placebo group.

This descriptive analysis showed that allergen-specific IgG4 significantly increased in the intervention group compared to the control group, reflecting treatment's immunogenicity, which was more rapidly achieved in the accelerated than in the classic schedules. Since changes in IgE levels weren't constant among studies, they aren't as consistent as IgG4 levels as indicators of successful immunotherapy. Additionally, blocking activity of IgG antibodies was an important finding that supports immunological efficacy of accelerated immunotherapy schedules. This review also showed allergen-specific Treg cells and IL-10 as important markers of effective desensitization. Nevertheless, clear correlations between immunological parameters and clinical outcomes are scarce, and in this review only one study [8] found significant correlations between increased numbers of Treg cells and improvement in clinical severity.

Looking at safety, and considering pediatric studies, one [10] demonstrated that the frequency of local and systemic adverse events were slightly more frequent in the cluster SCIT group compared to placebo, although similar in severity (mild). Noteworthy, in this

study topical anesthetic cream and antihistamines were given to the patients before each injection which might influence the risk of adverse reactions. Another study [9] didn't show significant differences in local and systemic reactions comparing cluster and conventional SCIT, with mild local reactions and no severe systemic reactions. Both studies didn't report withdrawals from the study due to adverse reactions. Regarding adults, an excellent safety profile for cluster SCIT was noted. Generally, local reactions were mild and well-tolerated, occurring within 30 min after injection (under medical observation) in one study [12], as opposed to another trial [14] in which all local reactions were delayed. No life-threatening systemic reactions (grade IV) occurred. There was no need to interrupt the treatment or adjust the dose, and only one patient with a severe bronchospasm required symptomatic treatment. Most adverse reactions occurred during up-dosing phase. Relating to mixed population, two rush SCIT trials [15,16] showed no serious side effects, allowing a rapid and safe treatment, without need of symptomatic treatment and/or dose adjustment. Only one [16] of these studies reported withdrawals from the study because of adverse reactions. Comparing cluster with conventional SCIT [18], the frequency and severity (mostly no serious) of local and systemic reactions were similar between groups, indicating that cluster schedule may be a safe alternative to the conventional one. Noteworthy, in this study premedication (antihistamine) was administered before each injection which might influence the occurrence of adverse reactions. A double-blind placebo-controlled trial [17] confirmed the excellent tolerability of cluster SCIT in mixed population.

The main obstacle to the widespread implementation of accelerated schedules is the potential risk of side effects, particularly in children. However, the present descriptive analysis didn't show relevant differences in the incidence of either local or systemic adverse reactions between the accelerated schedules and controls, demonstrating a good safety profile for these regimens in children and adults. Overall local adverse reactions were mild, only requiring symptomatic treatment in a few cases (with complete recovery). An important point

was the absence of life-threatening systemic reactions and fatal events. A carefully evaluation of patients health status before each injection and a correct medical observation of possible immediate reactions after each dose for a period of approximately 30 minutes, are important measures to improve immunotherapy safety. Additionally, patients should be alert to detect possible delayed reactions and report them to their doctor. Notably, some caution is required in the interpretation of safety data due to a significant heterogeneity between studies mainly related to differences in subjects disease and co-morbidities (it is know that side effects in allergic asthma are higher compared to allergic rhinitis, for example), and in measurement tools and units (mainly regarding local reactions). Moreover, premedication wasn't applied in all clinical trials which may impair the comparative analysis of adverse reactions between studies.

Conclusion

Several challenges and limitations were found in this review. Firstly, there was a great heterogeneity regarding sample size, subjects age, participants baseline characteristics, type and quality of allergen extracts, dosages and pharmacologic units, accelerated build-up protocols (mainly for cluster schedules), treatment duration, time-points to measure outcomes, methods and scoring systems. This heterogeneity didn't allow quantitative pooling of data and, accordingly, data was synthesized qualitatively. Additionally, RCTs incorporated in the review varied in their quality, i.e., in risk of bias, mainly because experimental designs and methods for allocation concealment and blinding of personnel and outcomes were quite heterogeneous among studies. It is important to note the introduction of language and publication bias in this review because only studies written in English and published in PubMed were included. Finally, a significant challenge in this review was the small number of studies exclusively enrolling children and adolescents (population of interest), adding the fact that most studies of mixed population didn't evaluate pediatric outcomes individually. The lack of pediatric studies preclude the collection of reliable data on efficacy and safety of accelerated immunotherapy schedules for the treatment of respiratory allergy, remaining the question to what extent collected data from adults could be applied to pediatric patients.

Considering the mentioned limitations, further studies should concentrate on the following points. It is necessary to standardize accelerated build-up protocols, mainly cluster schedules, regarding type of vaccines, dosage and pharmacologic units, duration, number of injections administered and gaps between increasing doses. It is also important to establish more appropriate time-points to measure and analyze the outcomes to assess the early effects of the accelerated schedules, including at least one measurement at the end of the build-up phase. Standardization of scoring systems is critical to evaluate the efficacy and safety of SCIT. Correct sample size estimation should be performed prior to the beginning of all clinical trials aiming to obtain sufficient patients to reach the study outcomes. Finally, further

RCTs exclusively enrolling pediatric patients are required to evaluate the real efficacy and safety of accelerated immunotherapy schedules in this population.

In conclusion, the current limited evidence provides support for the efficacy and safety of rush and cluster SCIT for the treatment of respiratory allergy. Concerning pediatric patients, additional large-scale and well-conducted randomized controlled trials of accelerated immunotherapy schedules (mainly rush SCIT) are still needed to conclude affirmatively that these schedules, besides less time-consuming, are safe and effective for the treatment of respiratory allergy in pediatric patients.

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