

The effect of allergen immunotherapy in the onset of new sensitizations: a meta-analysis

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Background: Although the preventive efficacy of allergen immunotherapy (AIT) in the onset of new allergen sensitizations has been asserted by many reviews, position papers, and consensus conferences, the evidence available is from only 3 studies. The objective of this work was a systematic review to evaluate the preventive efficacy of AIT in the onset of new allergen sensitizations. The end-point was the risk difference (RD) in the onset of new allergen sensitizations between patients treated with AIT and pharmacotherapy.

Methods: Computerized bibliographic searches of MEDLINE, EMBASE, and the Cochrane Library (until November 30th, 2016) were done. Random-effects and fixed-effects model meta-analyses were performed. Randomized controlled trials or observational studies comparing children treated with AIT with house dust mite (HDM) to subjects who did not receive AIT, with a long-term observation period (at least 3 years including treatment and follow-up) have been included.

Results: Eight studies totaling 721 children (390 treated with AIT and 331 with pharmacotherapy) met the inclusion criteria. The risk of bias was high. Low evidence supports

the conclusion that AIT prevents the onset of new allergen sensitizations, with 3 of 8 studies reporting a reduction in the onset of new sensitizations in patients treated with AIT vs pharmacotherapy. Our meta-analysis found no difference between AIT and pharmacotherapy, with high heterogeneity (RD, -0.10 ; 95% confidence interval [CI], -0.31 to 0.11 ; $p = 0.32$; $I^2 = 91.4\%$).

Conclusion: The data of this systematic review do not support a preventive effect in the onset of new allergen sensitizations, in children treated with AIT in comparison with those treated with pharmacotherapy. © 2017 ARS-AAOA, LLC.

Key Words:

allergen immunotherapy; new allergen sensitizations; asthma; meta-analysis; allergic rhinitis

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Allergen immunotherapy (AIT) has been proven effective with variable clinical benefit, in reducing symptoms and the use of anti-symptomatic medications in

patients with allergic rhinoconjunctivitis.^{1,2} The Opinion Leaders (OL) of AIT, in contrast to anti-symptomatic medications, have reported that AIT administered both by the subcutaneous (SCIT) or sublingual (SLIT) route in clinical practice, acts through a modification of the immune system. Among these effects AIT is claimed to reduce the onset of new allergen sensitizations in monosensitized or polysensitized patients.^{3,4}

A preventive effect of AIT may be particularly important in children, since monosensitization is prevalent, and it could be easier to turn off the allergen specific immunoglobulin E (IgE) response. The transition from monosensitization to polysensitization has been observed in children with house dust mite (HDM) sensitization, and to a lesser degree in children with pollen sensitization. HDMs seem to play a “triggering” role in the development of polysensitization, as a high proportion of children originally monosensitized

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to HDM become polysensitized.⁵ Another study reported that polysensitized patients had more severe symptoms than monosensitized patients.⁶ Moreover, it has been observed that polysensitized patients with rhinitis more frequently present associated asthma than monosensitized patients.⁷ The preventive effects of AIT in the onset of new allergen sensitizations has been reported in many reviews, position papers, and consensus conferences.^{3,4,8–17}

However, the scarce data reported in these documents indicate that a preventive effect of AIT in the onset of new allergen sensitizations is based more on expert opinion rather than objective and extensive review of the literature.^{4,15,17,18} The primary objective of our study is to review the quality of the available studies on this issue using the Cochrane Risk of Bias Assessment Tool for Non-Randomized (ACROBAT-NRSI) and Randomized Studies. We examined the results of the studies on “HDM and immunotherapy and onset of new sensitization” using χ^2 test and a meta-analysis including only the studies with HDM in children that evaluated this outcome.

Methods

Data sources and searches

We carried out a systematic review and meta-analysis of observational studies following the recommendations of the Meta-analysis of Observational Studies in Epidemiology (MOOSE) group Checklist.¹⁹ The primary sources of the reviewed studies were Medline, EMBASE, and the Cochrane Library (from January 1965 until November 30, 2016) with a specific search strategy using the following medical subject headings: rhinitis OR hay fever OR rhinosinusitis OR rhinoconjunctivitis OR conjunctivitis OR asthma AND allergen-specific immunotherapy OR immunotherapy OR specific hyposensitizing therapy AND new sensitization AND long term OR follow-up AND house dust mite. The computer search was supplemented by manual searches of reference lists for all available review articles, primary studies, and abstracts from meetings. There was no language restriction.

Study selection

We required that studies: (1) were prospective or retrospective observational studies with a long-term observation period (here defined as at least 3 years including treatment and follow-up) or long term follow-up of randomized control trials of SCIT or SLIT comparing subjects treated with AIT to subjects who did not receive AIT;^{3,4} (2) included monosensitized patients with and without asthma with a positive allergen-specific skin-prick test, and/or serum allergen-specific IgE to HDM; (3) reported new sensitization onset as outcome measure of the treatment effect, regardless of whether this was the primary endpoint. Studies were excluded if they did not report on our outcome of interest, or the follow-up was less than 3 years, or they did not include a control population.

Data extraction and quality assessment

Two separate reviewers (M.S.L.B., S.L.P.) independently extracted detailed information on the study characteristics, study participants, interventions, outcomes, and their methods of ascertainment. The accuracy of data extraction was confirmed by 2 other reviewers (D.D.B., G.D.L.). Disagreements were solved by consensus adjudication. We used the ACROBAT-NRSI to evaluate the risk of bias in observational nonrandomized studies,²⁰ and a modification of the Cochrane Collaboration Tool for Assessing Risk of Bias from the Cochrane Handbook for Systematic Reviews of Interventions to evaluate the risk of bias in the results of the randomized studies.

Data synthesis and analysis

The statistical analysis was performed by using the χ^2 test and meta-analysis of only child studies including subjects treated with SCIT or SLIT for HDM. With this choice we did not pool the results of adult or child studies performed with allergens other than HDM because of their extreme clinical and statistical heterogeneity, especially in the differences of allergic diseases between children and adults but also because of clinical differences between allergic disease to pollens and HDM allergens.

Using the χ^2 test we evaluated the odds ratio, using the Woolf [logit] between the patients treated with AIT and with pharmacotherapy, with regard to the onset of new sensitizations. Through meta-analysis we evaluated the overall risk difference (RD) with 95% confidence intervals (CIs) for the development of new sensitizations among patients treated with AIT and patients treated with pharmacotherapy, estimated using models based on fixed-effects and random-effects assumptions. Data were combined using the method by DerSimonian and Laird.²¹ The I^2 statistic, which describes the percentage of variability due to heterogeneity rather than sampling errors, was used to quantify heterogeneity in the meta-analysis. Moreover, we used the Abbe plot for investigating the potential sources of heterogeneity in the meta-analysis. Publication bias was assessed with Funnel plots, and Egger's test for asymmetry.

Statistical analysis

All of our analyses were computed using StatsDirect (version 2.8.0; StatsDirect Ltd., Cheshire, UK) statistical packages.

Results

Our search strategy identified 1378 unique publications, including over 338 peer-reviewed studies published from inception to November 30, 2016. The full text of 11 studies was finally retrieved.^{22–32} Of these, only 8 studies have the inclusion criteria.^{22–29} We excluded the studies of Acquistapace et al.,³⁰ Szépfalusi et al.,³¹ and Shao et al.³² because they were not long-term studies (<3 years treatment/follow-up). Table 1 shows descriptive data for

TABLE 1. Characteristics of studies assessing the effects of AIT on the development of new allergen sensitizations

Study (year)	Country	Study type	Groups	Participants ^a	Male (%)	Age (years)	Sensitization allergen(s)	Asthma (%)	Rhinitis (%)	Type of AIT	Allergen, maintenance dose, and manufacturer	Treatment duration (years) ^b	Evaluation period
Des Roches et al. ²² (1997)	France	Open, prospective	AIT C	22 → 22 22 → 22	68 64	5 ^c (4–6) 4 ^c (3–5)	HDM Mono-S	100 100	73 77	SCIT	Der p1, 1500 BU (2 µg) every week for 6 weeks, then every 2 weeks (1st year); then every month	3	EOT
Pajno et al. ²³ (2001)	Italy	Open, prospective	AIT C	75 → 69 63 → 54	56 ^d 51 ^d	7.1 (6–8) 6.4 (5–7)	HDM Mono-S	100 100	37.7	SCIT	D.pt/D.f.a, 50/50%; 50,000; once/month; Alutard SQ, Alk	3	3 years after EOT
Piffert et al. ²⁴ (2002)	Italy	Open, prospective, randomized	AIT C	15 → 15 14 → 10	– 55 ^e	10.7 (6–14) 10.3 (6–14)	HDM Mono-S	100 100	47 43	SCIT	D.pt, 800 U; every 4–6 weeks; Conjuvac, Bayer	3	EOT
Di Rienzo et al. ²⁵ (2003)	Italy	Open, prospective	AIT C	35 → 35 25 → 25	51 52	8 (3–17) 9 (4–17)	HDM Mono/Poly-S	88.6 92	100 100	SLIT	D.pt/D.f.a, 50/50%; 1.12 µg group 1 and 0.56 µg group 2, twice weekly; ALK-Abellö	4–5	4–5 years after EOT
Gulen et al. ²⁶ (2007)	Turkey	Open, prospective	AIT C	70 → 68 59 → 55	58 ^d 54 ^d	8.4 (6–10) 8.7 (6–10)	HDM Mono-S	100 100	n.r.	SCIT	D.pt/D.f.a, 50/50%; 50,000 SQU once a month; Stallergenes	4	2 years after EOT
Inal et al. ²⁷ (2007)	Turkey	Open, prospective	AIT C	85 → 85 62 → 62	41 55	14.4 (6–16) 13.5 (6–16)	HDM Mono-S	88.9 90	45 55	SCIT	AIT-ad: ALK; Stallergenes, Allergopharma; AIT-aq: Greer Lab.; Dose n.r.	5	EOT
Reha and Ebru ²⁸ (2007)	Turkey	Open, prospective	AIT C	43 → 43 51 → 51	71 65	10.3 (4–15) 10.9 (4–11)	HDM Mono-S	95 100	5 12	SCIT	D.pt/D.f.a: 800 U/mL every 4–6 weeks, Stallergenes	4	EOT
Harmanci et al. ²⁹ (2010)	Turkey	Open, prospective	AIT C	62 → 53 60 → 52	65 ^d 46 ^d	12.7 (8–18) 12 (8–18)	HDM Mono-S	46 42	100 100	SCIT	D.pt/D.f.a: 50/50% (IR 10); 0.8 mL every 4–8 weeks; ASPI retard, Stallergenes	4	EOT

AIT = allergen immunotherapy; AIT-ad = aluminum hydroxide or calcium phosphate adsorbed extracts; AIT-aq = aqueous extracts; ALK in ALK-Abellö; ASPI in CPAE (calcium phosphate adsorbed extracts); BU = biological units; C = controls, subjects not treated with AIT; D.f.a = *Dermatophagoides farinae*; D.pt = *Dermatophagoides pteronyssinus*; DB = double blind; EOT = end of treatment; HDM = house dust mites; IR = index of reactivity; Mono-S = mono-sensitized; n.r. = not reported; PC = placebo-controlled; PNU = protein nitrogen units; Poly-S = polysensitized; R = randomized, RCT = randomized controlled trial; SCIT = subcutaneous immunotherapy; SLIT = sublingual immunotherapy; SQU = standardized quality units; TU = therapeutic units.

^aPatients analyzed at the first of the study → patients analyzed at the last of the study.

^bData calculated on the subjects at the end of follow-up.

^cMedian.

^dDiscrepancy between data in the text and in the tables in the original studies; data reported in the text are shown here.

^ePercentage of males for both groups.

the 8 systematically reviewed studies.^{22–29} The studies included a total of 721 patients (390 treated with AIT, and 331 treated with pharmacotherapy). Four of 8 studies were conducted in Turkey,^{26–29} three in Italy,^{23–25} and the remaining one in France.²² All the studies are observational studies with a case-control/cohort design. In the Pifferi et al.²⁴ study, the participants were allocated to the AIT or pharmacotherapy groups after randomization. In the remaining observational studies patient preference or parent preference was taken into account for inclusion of participants in each group.

The sample size of the studies varied greatly, from 25 patients²⁴ to 147 patients²⁷ (median, 99.5 patients).

A high rate of new sensitizations at follow-up (77%) was reported in the Des Roches et al.²² study, and a low rate of new sensitizations at follow-up (8%) in the Di Rienzo et al.²⁵ study. The median of the mean age of patients at inclusion was 9.3 years (range, 4 [in Des Roches et al.²²] to 14.4 [Inal et al.²⁷]). All the studies enrolled children with bronchial asthma and all patients were monosensitized to HDM. The severity of the main disease at baseline or comorbidities was not clearly reported in the majority of the studies. Seven of 8 studies,^{22–24,26–29} used SCIT, while only the Di Rienzo et al.²⁵ study used SLIT. The duration of treatment ranged from 3 to 5 years. Five studies evaluated patients for the onset of new sensitizations at the end of treatment,^{22–24,27–29} whereas the remaining 2 studies evaluated patients from 2 (Gulen et al.²⁶) to 10 years (Di Rienzo et al.²⁵) after the end of treatment. There was great heterogeneity in the reporting of the maintenance or cumulative dose delivered, and a variety of units were used to report dosing (Table 1).

The risk of bias was estimated as serious in the 7 observational studies with a nonrandomized design,^{22,23,25–29} mainly because important confounding domains were not appropriately assessed and adjusted (Table 2), and in the only randomized study, owing to serious bias such as the limitation of the study design.²⁴ Two studies included employees of the sponsor of the study^{23,25} among the authors (Table 2).

We found low evidence to support the use of AIT to reduce the likelihood of the onset of new allergen sensitizations (Table 3). The magnitude of association was strong ($RR < 0.5$) in 2 out of 8 studies (25%).^{23,24} The RR was between 0.5 and 1.0 in 2 studies.^{22,28} Finally, in the remaining 4 studies RR was > 1.0 .^{25–27,29} In the studies with $RR < 0.5$ the direction of the association was in favor of AIT^{23,24}; in those with RR between 0.5 and 1.0, only in 1 study was the direction of the association in favor of AIT,²² while in the remaining 2 studies the direction of the outcome was in favor of pharmacotherapy.^{26,27} Finally, in 3 studies we did not find any difference between AIT and pharmacotherapy.^{24,28,29}

The comparisons of groups of studies sharing similar characteristics evidenced that small studies and studies with a shorter follow-up showed the highest benefit of AIT^{22,24} (Table 4). No difference was found with respect to allergen

used, route of administration (SCIT vs SLIT), and age of participants (Table 4). Table 5 shows the χ^2 test through which we evaluated the odds ratio, using the Woolf (logit), between the patients treated with AIT and with pharmacotherapy, regarding the onset of new sensitizations. The incidence of new sensitizations is not statistically significant between the patients treated with AIT and the patients treated only with pharmacotherapy (odds ratio = 0.90; 95% CI, 0.66 to 1.21; $p = 0.55$).

Figure 1A-D shows the relative risk meta-analysis plot (fixed effects), relative risk meta-analysis plot (random effects), bias assessment plot (funnel plot), and Abbe plot (symbol size represents sample size), respectively. The relative risk meta-analysis plot (fixed effects) was as pooled risk difference = -0.03 (95% CI, -0.09 to 0.04), $p = 0.39$, while relative risk meta-analysis plot (random effects) was as pooled risk difference = -0.10 (95% CI, -0.31 to 0.11), $p = 0.32$.

Discussion

We found that the available evidence for the position that AIT is effective in reducing the likelihood of developing new allergen sensitizations in allergic monosensitized children is low in strength. It is opinion based rather on belief than on evidence-based medicine (EBM). Nevertheless, the preventive effects of AIT on the onset of new allergen sensitizations has been claimed in many reviews, position papers and consensus conferences (Table 6).

To our knowledge, this work is the first to systematically report on this aspect of the preventive AIT effect, through a comprehensive review of the literature and meta-analysis of evidence for this outcome. This issue of AIT in children with skin prick test and/or serum specific IgE to HDM (*Dermatophagoides pteronyssinus* and/or *Dermatophagoides farinae*) was the subject of only 8 studies and the preventive effect of AIT in new sensitizations was demonstrated in only 3 of 8 studies.^{22–24}

The low study quality is likely responsible for the high level of inconsistency between the results of individual studies. Comparisons of groups of studies sharing similar characteristics to explain the heterogeneity between the results of individual studies did not show any difference with respect to age of participants and allergen used (Table 4). In contrast, the comparisons of these subgroups highlighted that studies with the most serious limitations, such as small sample size at baseline, report the highest AIT effect. Furthermore, the benefit of AIT was mainly reported in studies with shorter follow-up, while studies with longer follow-up, which is critical for this specific outcome, did not show any difference. No study reported on the possible correlation between the onset of new sensitizations and the primary clinical benefit of AIT,^{33–35} which is the reduction of symptom or medication scores, rendering any inference impossible as to the primary efficacy and long-term benefit of AIT.

TABLE 2. Potential bias

References	Confounders (1)	Selection (2)	Measurement of intervention (3)	Missing data (4)	Overall	Comments
Des Roches et al. ²² (1997)	Serious	Moderate	Serious	Low	Serious	No assessment/adjustment of confounders (1): –severity of disease at baseline; –severity/presence of comorbidities; –socioeconomic status/education. No assessment of disease duration: inception bias (2). Information bias (3): –house status (old/new)/location (city/country); –presence of animals. No missing data (4)
Pajno et al. ²³ (2001)	Moderate	Low	Serious	Low	Serious	No assessment/adjustment of confounders (1): –severity of disease at baseline not precisely reported; –severity of comorbidities (rhinitis) at baseline not precisely measured; –socioeconomic status/education. Disease duration assessed (2). Information bias (3): –house status (old/new); –presence of animals. Low rate of loss to follow-up (4).
Pifferi et al. ²⁴ (2002) ^a	Moderate	Low	Serious	Low	Serious	No assessment/adjustment of confounders (1): –severity of disease at baseline not precisely reported; –severity of comorbidities (rhinitis) at baseline not precisely measured; –socioeconomic status/education. Disease duration assessed (2). Information bias (3): –house status (old/new); –presence of animals. Low rate of loss to follow-up (4).
Di Rienzo et al. ²⁵ (2003)	Serious	Moderate	Serious	Low	Serious	No assessment/adjustment of confounders (1): –socioeconomic status/education. No assessment of disease duration (2). Information bias (3): –house status (old/new)/location (city/country); –presence of animals. No missing data (4).
Gulen et al. ²⁶ (2007)	Serious	Moderate	Serious	Low	Serious	No assessment/adjustment of confounders (1): –severity of disease at baseline; –severity of comorbidities (rhinitis) at baseline not precisely measured. Information bias (3): –house status (old/new)/location (city/country); –presence of animals. No assessment of disease duration (2). Low rate of loss to follow-up (4).
Inal et al. ²⁷ (2007)	Serious	Moderate	Serious	Low	Serious	No assessment/adjustment of confounders (1): –severity of disease at baseline; –comorbidities; –socioeconomic status/education. No assessment of disease duration (2). Information bias (3): –house status (old/new)/location (city/country); –presence of animals. No missing data (4).

(Continued)

TABLE 2. Continued

References	Confounders (1)	Selection (2)	Measurement of intervention (3)	Missing data (4)	Overall	Comments
Reha and Ebru ²⁸ (2007)	Serious	Moderate	Serious	Low	Serious	No assessment/adjustment of confounders (1): –severity of disease at baseline not precisely measured, but patients matched for severity of the disease; –severity of comorbidities (rhinitis) at baseline not precisely measured, but patients matched for severity of the disease; –socioeconomic status/education. No assessment of disease duration (2). Information bias (3): –house status (old/new)/location (city/country); –presence of animals. No missing data (4).
Harmanci et al. ²⁹ (2010)	Serious	Moderate	Serious	Low	Serious	No assessment/adjustment of confounders (1): –socioeconomic reasons or convenience were the main reasons for refusal of AIT. No assessment of disease duration (2). Information bias (3): –house status (old/new)/location (city/country); –presence of animals. Low rate of loss to follow-up (4).

The table does not report three domains of The Cochrane Risk of Bias Assessment Tool for Non-Randomized-Studies of Interventions, used for the risk of bias analysis: (a) departure from intended intervention; (b) measurement of outcome; and (c) selection of outcome, because all the studies were at low risk of bias for these domains. Declaring a study to be at a particular level of risk of bias for an individual domain will mean that the study as a whole has a risk of bias at least this severe (for the outcome being assessed). Regarding the inception bias (included in the selection bias domain), we assume that it may arise if the cohorts are not followed from the inception of the disease. The presence of potential inception bias (disease duration not assessed in the individual studies) was considered as a moderate risk of bias in child studies and severe in adult studies. It was assumed that this bias had a lower impact in studies on children due to their narrower age range preventing important differences in the estimate of the disease duration, compared to the adults. Regarding the information bias (included in the measurement of intervention domain), in this context we considered that it may arise if one group is more likely than the other to be exposed to a definite risk factor that may influence the risk of the outcome. For this review we selected the house status (old/new)/location (city/country) or the presence of animals (pets), since many new sensitizations were due to animal dander and molds.

^aThe Pifferi et al.²⁴ study was analyzed with a modification of the Cochrane Collaboration Tool for Assessing Risk of Bias from the Cochrane Handbook for Systematic Reviews of Interventions (Canonica et al.¹⁵) since the subjects included in these studies were randomized. The randomized study by Pifferi et al.,²⁴ potentially providing high-quality evidence, was downgraded to a moderate risk of bias for limitation in study design (risk of bias) such as lack of concealment of allocation and tie with a provider (Cox et al.¹³). The randomized Pifferi et al.²⁴ study was considered at serious risk of bias owing to its very small sample size and loss to follow-up.

AIT = allergen specific immunotherapy.

TABLE 3. AIT for the prevention of new allergen sensitization: evidence summary

Outcome	Participants (n = 721)	Studies	HDM	Summary of grading data	Findings	Strength of the evidence	
New allergen sensitizations:							
children	AIT = 390; Pharmacotherapy = 331	n = 8	22–29	All the studies have high risk of bias (bias due to confounders, inconsistency, imprecision, high rate of loss to follow-up).	Three studies were in favor of AIT ^{22–24} ; 3 reported no statistically significant difference ^{25, 28, 29} ; and 2 were in favor of pharmacotherapy. ^{26, 27}	Low	
				Two ^{23, 24} out of 3 statistically significant studies have strong magnitude of effect, in 2 studies the RR was between 0.5 and 1.0 (but with opposite directions). ^{22, 28} In the remaining 4 studies RR was > 1. ^{25–28}			

AIT = allergen-specific immunotherapy; HDM = house dust mites; RR = risk ratio.

The use of randomization and a double-blind placebo-controlled design, as well as a relatively large sample size, and a high-quality effect for AIT would make this evidence stronger. Although many RCTs on AIT have been published to date, there is no study of SLIT with HDM

reporting data on the onset of new sensitizations. Until some time ago, according to EBM criteria, conclusions from meta-analyses represented the most robust proof of the efficacy of an intervention. Meta-analyses are used where individual studies disagree, or where individual

TABLE 4. AIT for the prevention of new allergen sensitizations: comparisons of subgroups

	Participants (n = 721)	Studies (n = 8)	Favors AIT (number of studies) 3 ²²⁻²⁴	Favors pharma- cotherapy or null (number of studies) 5 ²⁵⁻²⁹	Comment
Sample size					
>100	498	4	1 ²³	3 ^{26, 27, 29}	The effect of AIT on the outcome is evident in the small studies, not in the big ones.
≤100	223	4	2 ^{22, 24}	2 ^{25, 28}	
Type of treatment					
SCIT	661	7	3 ²²⁻²⁴	4 ²⁶⁻²⁹	
SLIT	60	1	0	0 ²⁵	
Length of follow-up					
3 years	69	2	2 ^{22, 24}	0	The benefit of AIT on the outcome is more evident in studies with a shorter follow-up.
>3 years	642	6	1 ²³	5 ²⁵⁻²⁹	

AIT = allergen immunotherapy; SCIT = subcutaneous immunotherapy; SLIT = sublingual immunotherapy.

TABLE 5. Incidence of new sensitization sensitizations in patients treated with AIT and with pharmacotherapy*

	New sensitizations	No new sensitizations	Total
Patients treated with AIT	153	237	390
Patients treated with pharmacotherapy	138	193	331
Total	291	430	721

*Yates-corrected $\chi^2 = 0.35$ ($p = 0.55$). Odds ratio = 0.90 (95% CI, 0.66 to 1.21).
AIT = allergen immunotherapy; CI = confidence interval.

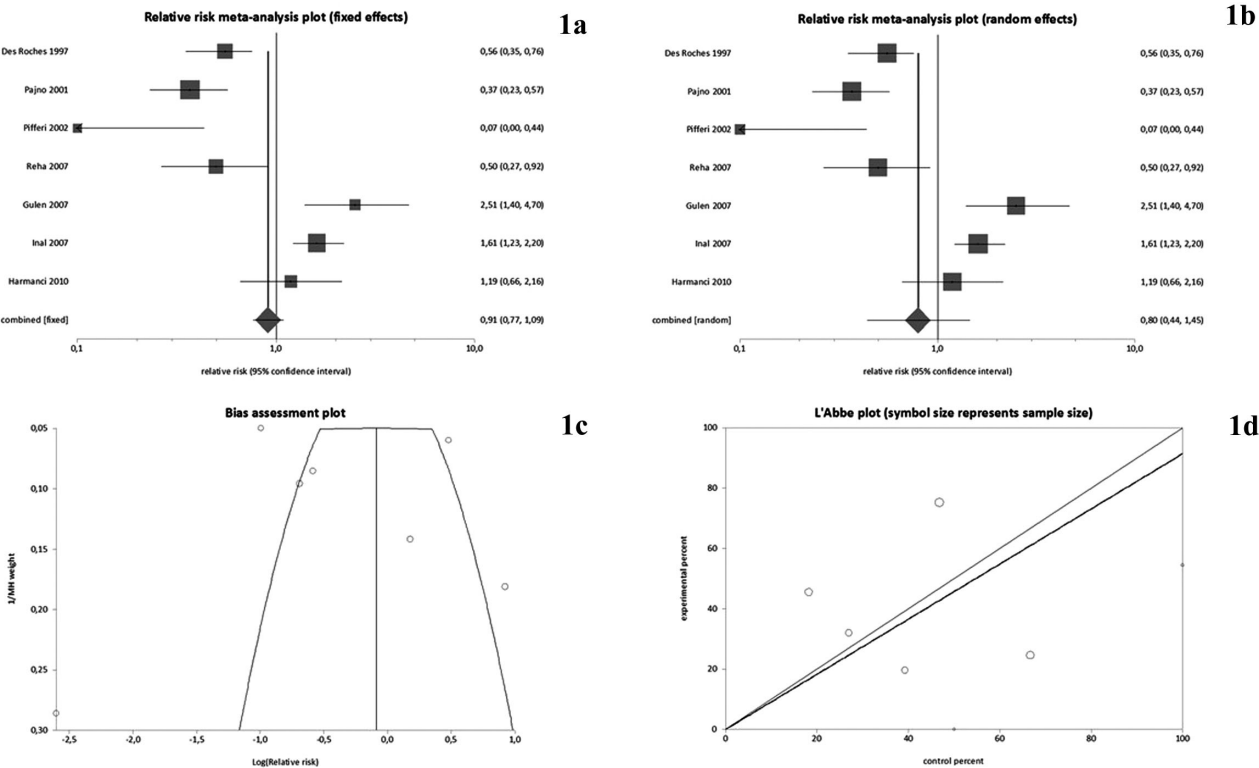


FIGURE 1. (A) Relative risk meta-analysis plot (fixed effects). (B) Relative risk meta-analysis plot (random effects). (C) Bias assessment funnel plot. (D) L'Abbe plot of the potential sources of heterogeneity in meta-analysis.

TABLE 6. List of the studies cited in some of the most important international guidelines or position papers reporting an assessment of the efficacy of AIT in the prevention of the onset of new allergen sensitizations

Study (year of publication)	Publication type	Cited studies	Findings
Bousquet et al. ³ (2001)	ARIA	Des Roches et al. ²²	Study in favor of AIT
Alvarez-Cuesta et al. ⁸ (2006)	EAACI SCIT	Des Roches et al. ²² ; Pajno et al. ²³	All studies in favor of AIT
Bousquet et al. ⁴ (2008)	ARIA	Des Roches et al. ²² ; Pajno et al. ²³	All studies in favor of AIT
Cox et al. ⁹ (2009)	AAAAI/ACAAI practice parameter	Des Roches et al. ²² ; Pajno et al. ²³	All studies in favor of AIT
Canonica et al. ¹⁰ (2009)	WAO SLIT Guidelines	Des Roches et al. ²² ; Pajno et al. ²³	All studies in favor of AIT
Saranz et al. ¹¹ (2010)	Argentina Guidelines	Des Roches et al. ²² ; Inal et al. ²⁷	One study in favor of AIT ²² ; one study in favor of pharmacotherapy ²⁷
Zuberbier et al. ¹² (2010)	GA ² LEN/EAACI; Pocket guide for AIT	Des Roches et al. ²²	One study in favor of AIT ²²
Cox et al. ¹³ (2011)	AAAAI/ACAAI practice parameter, 3rd update	Des Roches et al. ²² ; Pajno et al. ²³ ; Inal et al. ²⁷	Two studies in favor of AIT ^{22, 23} ; one study in favor of pharmacotherapy ²⁷
Walker et al. ¹⁴ (2011)	British Guidelines	Des Roches et al. ²² ; Pajno et al. ²³	All studies in favor of AIT
Canonica et al. ¹⁵ (2014)	WAO Position Paper	Des Roches et al. ²²	One study in favor of AIT
Pfaar et al. ¹⁶ (2014)	German, Austrian, and Swiss allergists' and specialists' consensus on AIT in patients with allergic airway diseases	Pajno et al. ²³ ; Di Rienzo et al. ²⁵	One study in favor of AIT ²³ ; one insignificant study ²⁵
Jutel et al. ¹⁷ (2015)	International Consensus on Allergy Immunotherapy	Des Roches et al. ²²	One study in favor of AIT ²²

AAAAI = American Academy of Allergy, Asthma and Immunology; ACAAI = American College of Allergy, Asthma and Immunology; AIT = allergen immunotherapy; ARIA = Allergic Rhinitis And Its Impact On Asthma; EAACI = European Academy of Allergy and Clinical Immunology; GA²LEN = Global Allergy and Asthma European Network; SCIT = subcutaneous immunotherapy; SLIT = sublingual immunotherapy; WAO = World Allergy Organization.

studies are thought to be of insufficient power to identify significant effects.³⁶ The importance of the evidence is greatest for systematic reviews with meta-analysis, and the risk of bias is minimal in systematic reviews with meta-analysis. Secondary sources are always the representation of primary studies but should be guaranteed by the critical analysis of the researchers. They utilize an explicit method and systematic examination of primary studies. Finally, the tertiary sources are represented by evidence-based clinical guidelines.³⁷

There are several sources of bias in the studies analyzed, including potentially unmeasured and unadjusted confounders, defined in this review as preintervention factors that predict whether an individual receives 1 or the other of 2 treatments. Socioeconomic status, education, severity of preexisting disease, and the presence of comorbidities are common confounding factors that have been shown to be particularly important when the choice (or refusal) of a treatment is left to the patients or children's parents, as in this case.³⁸ We also included as potential source of bias the lack of investigation of the presence of domestic animals or the age of the home (new/old) or location (city/country) that may influence the patient's exposure to different allergens, such as animal dander, mold, pollen, and other environmental conditions (pollution).³⁸

The presence of these biases may explain the considerable heterogeneity in the studies analyzed. This raises the possibility of the presence of inconsistency of the results owing to methodological weaknesses of the studies, including the small sample size of the majority of these studies affecting the precision of the estimation of the preventive effect of AIT. On the other hand, since meta-analysis confers a high grade of evidence to a certain phenomenon, these findings highlight the importance of studying a large population. This is desirable in order to focus the evidence mainly on children who should represent the best target population for immunotherapy for the prospect of modifying the natural history of allergic disease.

Hence, the new sensitization percentage of 100% reported in the control groups of the Des Roches et al.²² study in comparison to 8% reported by Di Rienzo et al.²⁵ after a follow-up of 10 years. The percentage of new sensitizations appears extremely high in 2 other studies: the Pajno et al.²³ and Pifferi et al.²⁴ studies in particular considering that 3 of 4 studies were performed on Italian children. The studies of Des Roches et al.²² and Pifferi et al.²⁴ are among the studies featuring the smallest sample size, and thus they are at the highest risk of imprecision. Note that the Des Roches et al.²² study is the most cited by the consensus on AIT (Table 6). Utilizing SCOPUS®, the total citations

TABLE 7. Citations, children treated with AIT or with pharmacotherapy, percentages of new sensitizations

Studies	Citations (n)	Children treated with AIT (n)	New sensitizations in AIT (n)	Children treated with pharmacotherapy (n)	New sensitizations in pharmacotherapy (n)	Total children studied (n)	Total new sensitizations (%)	AIT new sensitizations (%)	Pharmacotherapy new sensitizations (%)
Des Roches et al. ²²	487	22	12	22	22	44	77.3	55.4	100
Pajno et al. ²³	441	69	17	54	36	126	43.1	24.6	66.7
Pifferi et al. ²⁴	57	15	0	10	5	25	20	0	50.0
Di Rienzo et al. ²⁵	276	35	3	25	2	60	8.3	8.6	8.0
Gulen et al. ²⁶	7	68	31	55	10	123	33.3	45.6	18.2
Inal et al. ²⁷	68	85	64	62	29	147	63.3	75.3	46.8
Reha and Ebru ²⁸	26	43	9	51	20	94	30.8	20.9	39.2
Harmanci ²⁹	6	53	17	52	14	105	29.5	32.1	26.9

AIT = allergen immunotherapy.

for each study are reported in Table 7. The sensitivity analyses with assumptions describing all the possible scenarios (best to worst), depending on the variability of the event rate in loss to follow-up participants, that we performed for all the studies with missing data to test the robustness of their results, showed a huge variability in the estimate of the risk difference between AIT and pharmacotherapy alone, leading to overestimates of the treatment effects in most cases.

In 2 of 8 studies there is a potential conflict of interest of 1 or more authors of the studies.^{23,25} However, only 1 of these reporting an AIT preventive effect on the onset of new sensitizations has among its authors of the studies a pharmaceutical company employee, suggesting that the potential conflict of interest did not significantly bias the results.

The present study has some limitations. Although we conducted the search using 3 different electronic databases, and the reference lists of the retrieved articles, including position papers, consensus documents, and also a gray-literature search, we encountered several challenges during our review process, since this specific outcome is not always the primary study outcome and therefore often it is not reported in the title and the abstract. However, the overall low strength of the evidence, and the inclusion of all the studies that were analyzed for the evaluation of an AIT preventive effect in the consensus conference reports makes us confident that no potentially relevant study overlooked would significantly change the general assessment of the results of this review.

Moreover, the variability in the dosing, further reported in a variety of units biological units, standardized quality units, micrograms, index of reactivity, therapeutic units, protein nitrogen units, etc., and in the therapeutic schedule made it impossible to compare dose and effect among studies. The main strengths of this work are that it is the first comprehensive review of this aspect of AIT, although the presence of publication bias, with studies reporting positive results being more likely to be published than studies reporting negative results, was not formally excluded by specific tests.

Conclusion

In conclusion, our review found low strength evidence to support the preventive effect of AIT in the onset of new allergen sensitizations. This indicates that the confidence in the effect estimate is limited. Ideally, long-term follow-up studies of RCTs including monosensitized children or adolescents to ensure a long-term observation period could eventually provide more reliable estimates, avoiding most of the bias of the available observational studies. ③

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References

1. Abramson MJ, Puy RM, Weiner JM. Is allergen immunotherapy effective in asthma? A meta-analysis of randomized controlled trials. *Am J Respir Crit Care Med.* 1995;151:969–974.
2. Compalati E, Passalacqua G, Bonini M, Canonica GW. The efficacy of sublingual immunotherapy for house dust mites respiratory allergy: results of a GA2LEN meta-analysis. *Allergy.* 2009; 64:1570–1579.
3. Bousquet J, Van Cauwenberge P, Khaltaev N; Aria Workshop Group; World Health Organization. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol.* 2001;108(5 Suppl): S147–S334.
4. Bousquet J, Khaltaev N, Cruz AA, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update [in collaboration with the World Health Organization, GA2LEN and AllerGen]. *Allergy.* 2008;63(Suppl 86):8–160.
5. Silvestri M, Rossi GA, Cozzani S, et al. Age-dependent tendency to become sensitized to other classes of

- aeroallergens in atopic asthmatic children. *Ann Allergy Asthma Immunol.* 1999;83:335–340.
6. Peternel R, Milanovic SM, Hrga I, et al. Incidence of Betulaceae pollen and pollinosis in Zagreb, Croatia, 2002–2005. *Ann Agric Environ Med.* 2007;14:87–91.
 7. Ciprandi G, Alesina R, Ariano R, et al. Characteristics of patients with allergic polysensitization: the POLISMAIL study. *Eur Ann Allergy Clin Immunol.* 2008;40:77–83.
 8. Alvarez-Cuesta E, Bousquet J, Canonica GW, Durham SR, Malling HJ, Valovirta E. Standards for practical allergen-specific immunotherapy. *Allergy.* 2006;61(Suppl 82):1–20.
 9. Cox L, Li JT, Nelson H, Lockley R. Allergen immunotherapy: a practice parameter second update. *J Allergy Clin Immunol.* 2007;120(Suppl):S25–S85.
 10. Canonica GW, Bousquet J, Casale T, et al. Sublingual immunotherapy: World Allergy Organization Position Paper 2009. *Allergy.* 2009;64:1–59.
 11. Saranz RJ, Lozano A, Caceres ME, et al. Allergen immunotherapy for prevention and treatment of respiratory allergy in childhood. *Arch Argent Pediatr.* 2010;108:258–265.
 12. Zuberbier T, Bachert C, Bousquet PJ, et al. GA[2]LEN/EAACI pocket guide for allergen-specific immunotherapy for allergic rhinitis and asthma. *Allergy.* 2010;65:1525–1530.
 13. Cox L, Nelson H, Lockey R, et al. Allergen immunotherapy: a practice parameter third update. *J Allergy Clin Immunol.* 2011;127(Suppl):S1–S55.
 14. Walker SM, Durham SR, Till SJ, et al. Immunotherapy for allergic rhinitis. *Clin Exp Allergy.* 2011;41:1177–1200.
 15. Canonica GW, Cox L, Pawankar R, et al. Sublingual immunotherapy: World Allergy Organization position paper 2013 update. *World Allergy Organ J.* 2014;7:6.
 16. Pfaar O, Bachert C, Bufe A, et al. Guideline on allergen-specific immunotherapy in IgE-mediated allergic diseases: S2k Guideline of the German Society for Allergology and Clinical Immunology (DGAKI), the Society for Pediatric Allergy and Environmental Medicine (GPA), the Medical Association of German Allergologists (AeDA), the Austrian Society for Allergy and Immunology (ÖGAI), the Swiss Society for Allergy and Immunology (SGAI), the German Society of Dermatology (DDG), the German Society of Oto-Rhino-Laryngology, Head and Neck Surgery (DGHNO-KHC), the German Society of Pediatrics and Adolescent Medicine (DGKJ), the Society for Pediatric Pneumology (GPP), the German Respiratory Society (DGP), the German Association of ENT Surgeons (BV-HNO), the Professional Federation of Paediatricians and Youth Doctors (BVKJ), the Federal Association of Pulmonologists (BDP) and the German Dermatologists Association (BVDD). *Allergo J Int.* 2014;23:282–319.
 17. Jutel M, Agache I, Bonini S, et al. International consensus on allergy immunotherapy. *J Allergy Clin Immunol.* 2015;136:556–568.
 18. Pawankar R, Canonica GW, Holgate ST, Lockey SF, Blaiss MS. *The WAO White Book on Allergy.* Milwaukee, WI: World Allergy Organization; 2013.
 19. Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA.* 2000;283:2008–12.
 20. Sterne JAC, Higgins JPT, Reeves BC on behalf of the development group for ACROBAT-NRSI. A Cochrane Risk Of Bias Assessment Tool: for Non-Randomized Studies of Interventions (ACROBAT-NRSI), Version 1.0.0, 24 September 2014. Available from <http://www.riskofbias.info>. Accessed July 8, 2015.
 21. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials.* 1986;7:177–188.
 22. Des Roches A, Paradis L, Menardo JL, Bouges S, Daurès JP, Bousquet J. Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. VI. Specific immunotherapy prevents the onset of new sensitizations in children. *J Allergy Clin Immunol.* 1997;99:450–453.
 23. Pajno GB, Barberio G, De Luca F, Morabito L, Parmiani S. Prevention of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy. A six-year follow-up study. *Clin Exp Allergy.* 2001;31:1392–1397.
 24. Pifferi M, Baldini G, Marrazzini G, et al. Benefits of immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract in asthmatic children: a three-year prospective study. *Allergy.* 2002;57:785–790.
 25. Di Rienzo V, Marcucci F, Puccinelli P, et al. Long-lasting effect of sublingual immunotherapy in children with asthma due to house dust mite: a 10-year prospective study. *Clin Exp Allergy.* 2003;33:206–210.
 26. Gulen F, Zeyrek D, Can D, et al. Development of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy. *Asian Pac J Allergy Immunol.* 2007;25:7–11.
 27. Inal A, Altintas DU, Yilmaz M, Karakoc GB, Kendirli SG, Sertdemir Y. Prevention of new sensitizations by specific immunotherapy in children with rhinitis and/or asthma monosensitized to house dust mite. *J Invest Allergol Clin Immunol.* 2007;17:85–91.
 28. Reha CM, Ebru A. Specific immunotherapy is effective in the prevention of new sensitivities. *Allergol Immunopathol (Madr).* 2007;35:44–51.
 29. Harmanci K, Razi CH, Toyran M, Kanmaz G, Cengizlier MR. Evaluation of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy. *Asian Pac J Allergy Immunol.* 2010;28:7–13.
 30. Acquistapace F, Agostinis F, Castella V, et al. Efficacy of sublingual specific immunotherapy in intermittent and persistent allergic rhinitis in children: an observational case-control study on 171 patients. The EFESO-Children Multicenter trial. *Pediatr Allergy Immunol.* 2009;20:660–664.
 31. Szépfalusi Z, Bannert C, Ronceray L, et al. Preventive sublingual immunotherapy in preschool children: first evidence for safety and pro-tolerogenic effects. *Pediatr Allergy Immunol.* 2014;25:788–795.
 32. Shao J, Cui YX, Zheng YF, et al. Efficacy and safety of sublingual immunotherapy in children aged 3–13 years with allergic rhinitis. *Am J Rhinol Allergy.* 2014;28:131–139.
 33. Di Bona D, Plaia A, Leto-Barone MS, La Piana S, Di Lorenzo G. Efficacy of grass pollen allergen sublingual immunotherapy tablets for seasonal allergic rhinoconjunctivitis: a systematic review and meta-analysis. *JAMA Intern Med.* 2015;175:1301–1309.
 34. Di Bona D, Plaia A, Scafidi V, Leto-Barone MS, Di Lorenzo G. Efficacy of sublingual immunotherapy with grass allergens for seasonal allergic rhinitis: a systematic review and meta-analysis. *J Allergy Clin Immunol.* 2010;126:558–566.
 35. Di Bona D, Plaia A, Leto-Barone MS, La Piana S, Di Lorenzo G. Efficacy of subcutaneous and sublingual immunotherapy with grass allergens for seasonal allergic rhinitis: a meta-analysis-based comparison. *J Allergy Clin Immunol.* 2012;130:1097–1107.e2.
 36. Haddaway NR. Maximizing legacy and impact of primary research: a call for better reporting of results. *Ambio.* 2014;43:703–706.
 37. Hirsh J, Guyatt G. Clinical experts or methodologists to write clinical guidelines? *Lancet.* 2009;374:273–275.
 38. Sanderson S, Tatt ID, Higgins JP. Tools for assessing quality and susceptibility to bias in observational studies in epidemiology: a systematic review and annotated bibliography. *Int J Epidemiol.* 2007;36:666–676.