REVIEW ARTICLE

Efficacy of allergen immunotherapy in reducing the likelihood of developing new allergen sensitizations: a systematic review

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Abstract

Background: Guidelines and position papers indicate that allergen immunotherapy (AIT) is the only disease-modifying treatment, including prevention of the onset of new allergen sensitizations. However, this preventive effect was shown by only a few observational studies. Our aim was to systematically review the efficacy of AIT in preventing the onset of new allergen sensitizations.
Methods: Computerized bibliographic searches of Medline, EMBASE, and the Cochrane Library (through June 2015) were supplemented with manual searches of reference lists. Observational studies or randomized controlled trials with a long-term observation period were included. Paired reviewers extracted data about study characteristics and assessed biases. The end point was the risk difference in the onset of new allergen sensitizations between patients treated with AIT and pharmacotherapy. The strength of the evidence was graded based on the risk of bias, consistency, and magnitude of effect, according to the GRADE Working Group's guide.
Results: Eighteen studies (1049 children, 10 057 adults) met the inclusion criteria. The risk of bias was high in all but one study. Low evidence supports the position that AIT prevents the onset of new allergen sensitizations, with 10 of 18 studies reporting a reduction in the onset of new sensitizations in patients treated with AIT *vs* placebo. Small studies and studies with a shorter follow-up showed the highest benefit of AIT.
Conclusions: The overall evidence provides a low-grade level of the evidence supporting the efficacy of AIT in preventing the onset of new allergen sensitizations, but high-quality studies could change this estimate.

Allergen immunotherapy (AIT) has been proven effective, with variable clinical benefit, in reducing symptoms and the use of antisymptomatic medications in patients with allergic rhinoconjunctivitis and asthma (1–3). In contrast to antisymptomatic medications, AIT, administered by the subcutaneous (SCIT) or sublingual (SLIT) route in clinical practice, may have persisting effects after its discontinuation, as it acts through a modification of the immune response. Among these persisting effects, the ability to reduce the likelihood of developing new allergen sensitizations in mono- or polysensitized patients has been shown (2).

The preventive effect of AIT on the onset of new allergen sensitizations was reported in many reviews, position papers, and consensus conference (4–13). However, to support this conclusion the findings from only a few observational studies performed on children and adults were examined in these reports, and an extensive review of the literature has not been conducted, yet (14). Therefore, the primary objective of this study was to evaluate the strength of the evidence of the effect of AIT on prevention of new allergen sensitizations in children and adults. We reviewed the quality of the available studies using the GRADE (Grading of Recommendations Assessment, Development, and Evaluation) approach and the Cochrane Risk of Bias Assessment Tool for Non-randomized and Randomized Studies (15–17).

Methods

Data sources and searches

The primary sources of the reviewed studies were Medline, EMBASE, and the Cochrane Library (inception to June 30, 2015) with a specific search strategy with the following medical subject headings: rhinitis, hay fever, rhinosinusitis, rhinoconjunctivitis, conjunctivitis, asthma, allergen-specific immunotherapy, immunotherapy, new sensitizations, long term, follow-up. The computer search was supplemented with manual searches of reference lists for review articles, primary studies and abstracts from meetings, with no language restriction.

Study selection

We required that studies: (i) were prospective or retrospective observational studies with a long-term observation period (here defined as at least three years including treatment and follow-up) or long-term follow-up of randomized control trials (RCTs) of SCIT or SLIT comparing subjects treated with AIT to subjects who did not receive AIT; (ii) included monosensitized or polysensitized patients with allergic rhinitis and/or asthma with positive allergen-specific skin prick tests, and/or elevated serum allergen-specific IgE; and (iii) reported new sensitization onset as outcome measure of the treatment effect, regardless of whether this was the primary end point.

Studies were excluded if they did not report on our outcome of interest or if they did not include a control population.

Data extraction and quality assessment

Two separate reviewers (MSLB, SLP) independently extracted the study data. The accuracy of data extraction was confirmed by two other reviewers (DDB, GDL). Disagreements were solved by consensus adjudication.

We used the Cochrane Risk of Bias Assessment Tool for Non-randomized Studies of Interventions (ACROBAT-NRSI) to evaluate the risk of bias in observational nonrandomized studies (16). The scale evaluates seven domains through which bias might be introduced: bias due to confounding, selection bias, bias in measurement of interventions, bias due to departures from intended intervention, bias due to missing data, bias in measurement of outcome, and bias in selection of the reported results. Studies were categorized as having a low, moderate, serious, or critical risk of bias depending on their performance across these seven domains (16).

We used 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 (17). We assessed the following six categories of potential bias: (i) lack of randomization, (ii) lack of allocation concealment, (iii) inadequate blinding, (iv) incomplete data reporting, (v) other sources of bias, and (vi) participation of sponsor company in the study design and interpretation of data.

The GRADE was used to rate the overall quality of evidence (15). The grading incorporated the risk of biases, the consistency of direction of the effect across studies for a given comparison and outcome, the relevance of the collection of studies to the question of interest (directness), the imprecision, the publication bias, and the magnitude of the effect (15). The evidence for the established outcome was graded as (i) high grade: high confidence that the evidence reflects the true effect; (ii) moderate grade: moderate confidence that the evidence reflects the true effect: the true effect is likely to be close to the estimate of the effect, but there is the possibility that it is substantially different; (iii) low grade: the confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect; (iv) insufficient evidence.

According to the GRADE, all the observational studies included in this systematic review without special strengths (such as large magnitude of an effect, dose–response gradient, or effects of all plausible residual confoundings accounted for) were considered to provide low quality of evidence, owing to their significant limitations (such as study design or execution, publication bias, imprecision, inconsistency). The randomized observational studies, potentially providing high-quality evidence, were automatically downgraded for limitations in design (risk of bias).

To upgrade the overall evidence, we required a minimum of two or more studies with low risk of bias, and at least one strong magnitude of effect in the context of largely consistent overall evidence. Moderate-grade evidence required one or more studies with low risk of bias or strong magnitude of effect or one study with low risk of bias or moderate magnitude plus one study with medium risk of bias or strong magnitude. Evidence was low grade if it did not meet any of these categories. Insufficient evidence was assigned if there was no relevant study.

A meta-analysis was not performed due to the extreme heterogeneity of the included studies.

To assess the potential impact of loss to follow-up, we evaluated the effect of several assumptions about the outcomes of participants lost to follow-up on the estimate of effect for the outcome (the onset of new sensitizations) (18). Four assumptions were evaluated: (i) All the participants lost to follow-up had the event (the onset of new sensitizations); (ii) none of those lost to follow-up in the treatment group had the event and all those lost to follow-up in the control group did (best case scenario); (iii) all participants lost to follow-up in the treatment group had the event and none of those in the control group did (worst case scenario); (iv) none of the participants lost to follow-up had the event.

Statistical analysis

All our analyses were performed by R 3.3.2 (R Core Team, 2016) (19).

Results

Our search strategy identified 1378 unique publications, including over 338 peer-reviewed studies published from inception to June 30, 2015. The full text of 24 studies was

finally retrieved (20–43), of which 18 met the inclusion criteria (20–37). We excluded the Jacobsen study (38), because the outcome was reported in a subgroup of patients regardless of the treatment received, the Eifan (39) Acquistapace (40), Szepfalusi (41), and Shao (42) studies because they were not long-term studies (<3 years of treatment/follow-up).

The Johnstone study (43) was excluded because the outcome was not assessed by skin prick tests or dosage of specific serum IgE. The Novembre (25) and Durham (37) studies reported on the outcome, but did not report the crude data, and were only qualitatively assessed.

Table 1 shows descriptive data for the 18 systematically reviewed studies (20–37). The 11 child (20–30) and seven adult studies (31–37) included a total of 11 106 patients (1049 children, 10 057 adults), of which 8369 (75.6%) were included only in the Purello D'Ambrosio study (31). Nine of 18 studies were conducted in Italy (21, 22, 24, 25, 29, 31, 33–35), four in Turkey, and (26–28, 30) the remaining five in other European countries (20, 23, 32, 36, 37). The Durham study is the only long-term follow-up of a previous RCT (37). All the other studies are observational studies with a case–control/cohort design (20–36). In the Pifferi (22), Novembre (25), and Marogna (29, 33) studies, the participants were allocated to the AIT or pharmacotherapy groups after randomization. In the remaining observational studies, patient's preference or parent's preference, in child studies, was taken into account for inclusion of participants in each group.

The Dominicus study included as active cases a subgroup of subjects who underwent AIT in a previous RCT (44).

The sample size of the studies varied greatly, from 28 (23) to 8369 patients (31) (median, 106).

A high rate of loss to follow-up (>20%) (45, 46) was reported in the Marogna (35), and Durham (37) studies. The median of the mean age of patients at inclusion was 9.3 years [range 4 (20)–14.4 (27)] in the child studies, and 27 years [range 21.5 (33)–41.7 (36)] in the adult studies. The majority of the child studies [8 (20–24, 26–28) of 11] enrolled patients with bronchial asthma, while the adult studies were performed mainly in patients with allergic rhinitis [5 (31, 33, 35–37) of 7]. Eight of the 11 child studies were performed on patients monosensitized to house dust mites (HDMs) (20–22, 24, 26–28, 30). The remaining three studies enrolled patients sensitized to different allergens, including HDM (23, 25, 29). In contrast, the adult studies were mainly performed on patients using AIT with various different allergens, except the Marogna (35) and Durham (37) studies. Monosensitized patients were enrolled in four of seven adult studies (31, 32, 34, 35). The severity of the main disease at baseline or comorbidities was not clearly reported in the majority of the studies. Eight of 11 child studies (20–23, 26–28, 30) and four of seven adult studies (31, 32, 34, 36) used SCIT. The duration of treatment ranged from 3 (20–23, 25, 29, 33, 34, 36, 37) to 5 years (24, 27, 32, 35). Nine of 18 studies evaluated patients for the onset of new sensitizations at the end of treatment (20, 22, 25, 27–30, 32, 33), while the remaining studies evaluated patients from 2 (26) to 10 years (35) after the end of treatment. There was great heterogeneity in the reporting of the maintenance or cumulative dose delivered, and a variety of units to report dosing (Table 1).

The risk of bias was estimated as serious in the 13 observational studies with a nonrandomized design (20, 21, 23, 24, 26–28, 30–32, 34–36), mainly because important confounding domains were not appropriately assessed and adjusted (Table 2), and in three of the five randomized studies, owing to the presence of bias such as the limitation of the study design (22, 29, 33) or inclusion of the sponsor (15, 45–49) among the authors (Table 2).

We found low evidence for the outcome 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 $\leq$ 0.5 or >2) in four of 11 child studies (36%) (22, 26, 28, 29), but in three of them the direction of the association was in favor of AIT (22, 28, 29) while in the remaining study was in favor of pharmacotherapy (26). The magnitude of association was strong and in favor of AIT in four of the seven adult studies (31, 33, 35, 36), but the only adult follow-up study of a previous RCT did not find any difference between AIT and pharmacotherapy (37). Overall, the association between AIT and the reduction in onset of new allergen sensitizations was reported in six (20–23, 28, 29) of 11 child studies (the remaining five were null (24, 25, 30) or negative (26, 27)] and four (31, 33, 35, 36) of seven adult studies [the remaining three were null (32, 37) or negative (34)]. The comparisons of groups of studies sharing similar characteristics evidenced that small studies (20, 22, 23, 35, 36) and studies with a shorter follow-up (20, 22, 29, 33) showed the highest benefit of AIT (Table 4).

No difference was reported with respect to allergen used, route of administration (SCIT *vs* SLIT), and age of participants (Table 4). The comparison limited to the randomized studies showed that three (22, 29, 33) of five (22, 25, 29, 33, 37) studies reported a benefit of AIT.

Considering the inconsistent study results and that the risk of bias was serious in almost all the studies, we graded the strength of evidence as low in support of AIT for reducing the likelihood of onset of new allergen sensitizations.

To assess the potential impact of the missing data in the studies owing to loss to follow-up, we calculated the variations of the estimates under a number of assumptions about the outcomes of participants lost to follow-up. Eight (20, 24, 27, 28, 31, 32, 34, 36) of the 16 (20–24, 26–36) studies providing the analytical data did not report loss to follow-up or did not report whether or not loss to follow-up occurred (21–23, 26, 29, 30, 33, 35). In the eight reports that gave the relevant information, the median percentage of participants lost to follow-up was 14%, higher in controls (up to 43%) (35) than in cases (up to 18%) (35). When we varied assumptions about loss to follow-up, referring to scenarios three (worst scenario) and four, one of the eight studies was no longer significant and the others showed reduced differences between the groups, suggesting that loss to follow-up led to an overestimation of the AIT effect on the outcome (Fig. 1); only scenario 2 (best scenario) showed increased differences between the groups.

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

Study (years)	Country	Study type	Groups	Participants	Male (%)	Mean age, years (range)	Sensitization allergen (s)	Asthma (%)	Rhinitis (%)	Type of AIT	Allergen, maintenance dose, and manufacturer	Treatment duration (years)‡	Evaluation period
Children													
Des Roches 1987 (20)	France	Open, prospective	AIT C	22 → 22 22 → 22	68 64	5* (4–6) 4* (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 2001 (21)	Italy	Open, prospective	AIT C	75 → 69 63 → 54	56** 51**	7.1 (6–8) 6.4 (5–7)	HDM Mono-S	100 100	37.7 42.6	SCIT	D. pt/D. fa, 50/50% 50 000; once/month Alutard SQ, Alk	3	3 years after EOT
Pifferi 2002 (22)	Italy	Open, prospective randomized	AIT C	15 → 15 14 → 10	55§	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
Eng 2002 (23)	Switzerland	Open, prospective	AIT C	14 → 13 14 → 10	77¶ 80¶	9.6 (5–16)¶ 8.8 (7–13)¶	Grass Mono/Poly-S	69¶ 80¶	100¶ 100¶	SCIT	Grass pollen depot allergoid, Allergovit, Allergopharma Dose, n.r.	3	6 years after EOT
Di Rienzo 2003 (24)	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. fa, 50/50% 1.12 μg group 1 and 0.56 μg group 2, twice weekly ALK-Abellò	4–5	4–5 years after EOT
Novembre 2004 (25)	Italy	Open, prospective randomized	AIT C	54 → 48 59 → 49	70.2 70	8.96 (5–14) 7.74 (4–16)	Grass Mono-S	0 0	100 100	SLIT (drops)	0.5 μg group five major allergen once daily, 5 days/week. ALK-Abellò	3	EOT*
Gulen 2007 (26)	Turkey	Open, prospective	AIT C	70 → 68 59 → 55	58** 54**	8.4 (6–10) 8.7 (6–10)	HDM Mono-S	100 100	n.r.	SCIT	D. pt/D. fa, 50/50% 50 000 SQU once a month Stallergenes	4	2 years after EOT
Inal 2007 (27)	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 2007 (28)	Turkey	Open, prospective	AIT C	56 → 56 51 → 51	71 65	10.3 (4–15) 10.9 (4–11)	HDM 77%†† Grass 23%†† Mono-S	95 100	5 12	SCIT	D. pt/D. fa/Grass mix 800 U/ml every 4–6 weeks Phostal, Stallergenes	4	EOT

Table 1 (Continued)

Study (years)	Country	Study type	Groups	Participants	Male (%)	Mean age, years (range)	Sensitization allergen (s)	Asthma (%)	Rhinitis (%)	Type of AIT	Allergen, maintenance dose, and manufacturer	Treatment duration (years)‡	Evaluation period
Marogna 2008 (29)	Italy	Open, prospective randomized	AIT C	144 → 130 72 → 66	72.2 59.7	10.7 (5–17) 10.0 (5–17)	HDM, grass, trees, weeds Mono/Poly-S	57.9 62.5	100 100	SLIT (drops)	Mean cumulative dose per year: 480 μg Der p 1/Der p 2, 40 μg Phl p 1, and Par j 1 and 100 μg Bet v 1. Anallergo, Italy	3	EOT
Harmanci 2010 (30)	Turkey	Open, prospective	AIT C	62 → 53 60 → 52	65** 46**	12.7 (8–18) 12 (8–18)	HDM Mono-S	46 42	100 100	SCIT	D. pt/D. fa 50/50% (IR 10) 0.8 ml every 4–8 weeks APSI retard, Stallergenes	4	EOT
Adults													
Purello D'Ambrosio 2001 (31)	Italy	Retrospective	AIT C	7182 1214	44 43	23.2 (>14) 22.4 (>14)	HDM, Grass, parietaria olive, birch compositae Mono-S	59 59	100 100	SCIT	Depot-injective Producers, n.r. Dose, n.r.	4	3 years after EOT
Tella 2003 (32)	Spain	Open, prospective	AIT C	66 → 66 34 → 34	47 41.2	28.8 (6–69) 26 (6–69)	HDM, parietaria, Grass Mono-S	78.8 53	72.7 83.6	SCIT	Producers, n.r., Dose n.r. (maximum tolerated) every 4 weeks	3–5	EOT
Marogna 2004 (33)	Italy	Open, prospective randomized	AIT C	319 → 271 192 → 170	56 63	22.8 (5–60) 21.5 (5–58)	HDM, grass birch, parietaria, compositae Mono/Poly-S	59.9 62.5	100 100	SLIT (drops)	Mean cumulative dose per year: 390 μg Der p 1/Der p 2, 70 μg Phl p 1 and Par j 1 and 100 μg Bet v 1. Anallergo, Italy	3	EOT
Asero 2004 (34)	Italy	Retrospective	AIT C	284 407	47 43	27 ± 12.4 27 ± 12.6	HDM, grass, birch, parietaria ragweed Mono-S.	n.r. n.r.	n.r. n.r.	SCIT	Depot aluminum hydroxide-adsorbed allergen extracts Allergopharma: 5000 TU Lofarma: 10 000 U Dome-Hollister/Stier: 10 000 PNU, every 3 weeks.	3	6–9 years
Marogna 2010 (35)	Italy	Open, prospective	AIT C	57 → 47 21 → 12	56 57	21.7 (14–36) 23.8 (15–38)	HDM Mono-S	53 43	100 100	SLIT	Der p1/p2, 390 μg per year. Anallergo, Italy	3, 4, or 5	Up to 15 years from inclusion

Table 1 (Continued)

Study (years)	Country	Study type	Groups	Participants	Male (%)	Mean age, years (range)	Sensitization allergen (s)	Asthma (%)	Rhinitis (%)	Type of AIT	Allergen, maintenance dose, and manufacturer	Treatment duration (years)‡	Evaluation period
Dominicus 2012 (36)	Germany	Observational follow-up study	AIT C	26 13	27 46	41.7 (24–63) 33.9 (19–47)	Grass w/wo other sensitizations Mono/Poly-S	n.r. n.r.	100 100	SCIT	6-grass pollens 6000 TU every 4 weeks. Allergovit	3	3 years after EOT
Durham 2012 (37)	Europe, multicenter	Long-term follow-up of a DB-PC-RCT	AIT C	282 → 137 286 → 104	57 61	33.8 34.5	Grass Mono/Poly-S	n.r. n.r.	100 100	SLIT (tablets)	Monthly dose: 450 μg Phl p 1 Grazax ALK-Abellò	3	4 years after EOT

n.r., not reported; R, randomized, PC, placebo-controlled; RCT, randomized controlled trial; AIT, allergen immunotherapy; HDM, house dust mite; C, controls, subjects not treated with AIT; EOT, end of treatment; →, patients analyzed at the end of the study; D. pt, *Dermatophagoides pteronissinus*; D. fa, *Dermatophagoides farinae*; Mono-S, monosensitized; Poly-S, polysensitized; TU, therapeutic units; PNU, protein nitrogen units; BU, biological units; SQU, standardized quality units; DB, double-blind.

*Median.

†AIT-ad, aluminum hydroxide or calcium phosphate adsorbed extracts.

‡AIT-aq, aqueous extracts.

§Percentage of males for both groups.

¶Data calculated on the subjects at the end of follow-up.

**Discrepancy between data in the text and in the tables in the original studies; data reported in the text are shown here.

††Data available only for AIT.

Table 2 Potential bias in non-randomized studies

Authors	Confounders (1)	Selection (2)	Measurement of intervention (3)	Missing data (4)	Overall	Comment
Children						
Des Roches 1997 (20)	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 2001 (21)	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)
Eng 2002 (23)	Serious	Low	Serious	Low	Serious	No assessment/adjustment of confounders (1): • severity of comorbidities (asthma) • socioeconomic status/education Disease duration assessed (2) Information bias (3) • house status (old/new)/location (city/country) • presence of animals • Low rate of loss to follow-up (4)
Di Rienzo 2003 (24)	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 2007 (26)	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 2007 (27)	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)

Table 2 (Continued)

Authors	Confounders (1)	Selection (2)	Measurement of intervention (3)	Missing data (4)	Overall	Comment
Reha 2007 (28)	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 2010 (30)	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)
Adults						
Purello D'Ambrosio 2001 (31)	Serious	Serious	Low	Low	Serious	No assessment/adjustment of confounders (1): • selection of cases and controls unclear • severity of disease (asthma or rhinitis) at baseline not precisely assessed • socioeconomic status/education No assessment of disease duration (2) Information bias (3) • house status/location (city/country) • presence of animals Rate of loss to follow-up not reported (retrospective study) (4)
Tella 2003 (32)	Serious	Serious	Serious	Low	Serious	No assessment/adjustment of confounders (1): • severity of disease (asthma or rhinitis) at baseline not precisely measured No assessment of disease duration (2) Information bias (3) • house status (old/new)/location (city/country) No missing data (4)
Asero 2004 (34)	Serious	Serious	Low	Serious	Serious	No assessment/adjustment of confounders (1): • severity of disease (asthma or rhinitis) at baseline not precisely measured • socioeconomic status/education No assessment of disease duration (2) Rate of loss to follow-up not reported (4)
Marogna 2010 (35)	Serious	Serious	Serious	Serious	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 High rate of loss to follow-up (4)

Table 2 (Continued)

Authors	Confounders (1)	Selection (2)	Measurement of intervention (3)	Missing data (4)	Overall	Comment
Dominicus 2012 (36)	Serious	Serious	Serious	Serious	Serious	No assessment/adjustment of confounders (1): • selection of controls unclear No assessment of disease duration, and age not matched (2) Information bias (3) • house status/location (city/country) • presence of animals Rate of loss to follow-up not reported (4)

The table does not report three domains of The Cochrane Risk of Bias Assessment Tool for Non-randomized-Studies of Interventions (16) used for the risk of bias analysis: (i) Departure from intended intervention; (ii) Measurement of outcome; (iii) Selection of outcome, as 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 with 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), as many new sensitizations were due to animal dander and molds.
The Pifferi (22), Novembre (25), Marogna (29, 33), and Durham (37) studies were analyzed with a modification of the Cochrane Collaboration Tool for Assessing Risk of Bias from the Cochrane Handbook for Systematic Reviews of Interventions (17) as the subjects included in these studies were randomized. The randomized studies by Pifferi (22), Novembre (25), and Marogna (29, 33) potentially providing high-quality evidence were 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 (15). The Marogna studies (29, 33) were further downgraded to serious risk of bias because the sponsor authored the studies (15, 45–49). The randomized Pifferi study (22) was considered at serious risk of bias owing to its very small sample size and loss to follow-up. In contrast, the Durham study was considered at low risk of bias, despite the high loss to follow-up, because it represents the long-term follow-up of a previous RCT (37).

Discussion

We found that the available evidence supporting the position that AIT is effective in reducing the likelihood of developing new allergen sensitizations in allergic mono- or polysensitized patients is low in strength. By definition, low-grade indicates low confidence that the available evidence, showing in this review a slight prevalence of studies with higher magnitude of effect reporting a preventive effect of AIT, reflects a true effect (15). The strength of the evidence was graded as low considering the inconsistent study results and the serious risk of bias in almost all the studies, according to the GRADE criteria (15). To our knowledge, this work is the first one systematically reporting on this aspect of the preventive AIT effect, through a comprehensive review of the literature, and performing grading of evidence for this outcome (15). Overall, a benefit of AIT was reported only in 10 of 18 studies. 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, route of administration (SCIT *vs* SLIT), 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 (very small for SLIT), (3, 50, 51) that is, the reduction in symptom or medication scores, rendering any inference impossible as to the primary efficacy and long-term benefit of AIT.

The comparative analysis limited to the SLIT studies showed that only three of six studies reported a reduction in the onset of new sensitizations, but these three studies were performed by the same authors (29, 33, 35), while the remaining three negative or insignificant studies were performed by three different groups, raising some concerns about the generalizability of the results of the three positive studies. Note that one of the SLIT studies with insignificant benefit is the Durham study (37), which is a long-term follow-up of a previous RCT published six years before (52). Although analytical data about the new sensitization rate were not reported in this study, the authors clearly stated that no difference in the onset of new sensitizations was observed between AIT and placebo (37). The use of randomization and a double-blind placebo-controlled design, as well as a relatively big sample size, and a high-quality product for

Table 3 Allergen-specific immunotherapy for the prevention of new allergen sensitization: evidence summary

Outcome	No. of participants	No. of studies	Allergens	Summary of grading data	Findings	Strength of the evidence
New allergen sensitizations						
Children	1049 • AIT, 593 • Pharm., 456	11	• HDM (20–22, 24, 26–30) • Grass (23, 25*, 28, 29) • Trees (29) • Weeds (29)	• All the studies have high risk of bias (bias due to confounders, inconsistency, imprecision, high rate of loss to follow-up) except one (moderate risk of bias) (25*). • Four statistically significant studies have strong magnitude of effect, but with opposite directions (three with RR ≤ 0.5 (22, 28, 29) and one >2 (26).	Six studies were in favor of AIT; (20–23, 28, 29) three reported no statistically significant difference; (24, 25*, 30) two were in favor of pharmacotherapy (26, 27).	Low
Adults	10 057 • AIT, 8103 • Pharm., 1954	7	• HDM (31–35) • Grass (31–34, 36, 37)** • Parietaria (31–34) • Weeds (31, 34) • Birch (31, 33, 34) • Olive (31) • Compositae (31, 33)	• Six observational studies have high risk of bias (bias due to confounders, inconsistency, imprecision, high rate of loss at follow-up) (31–36). • Four studies (of the six with high risk of bias (31–36) are statistically significant with strong magnitude of effect (RR ≤ 0.5) (31, 33, 35, 36). • One long-term follow-up of a DB-RCT with low risk of bias reports no difference between the groups (37)**.	Four studies were in favor of AIT (31, 33, 35, 36); two report no statistically significant difference;(32, 37**) one was in favor of pharmacotherapy (34).	Low

Pharm., pharmacotherapy; AIT, allergen-specific immunotherapy; No, number; HDM, house dust mite; RR, risk ratio; DB, double-blind; RCT, randomized controlled trial.

*The Novembre study (25) did not report the crude data.

**The Durham study (37) did not report the crude data.

AIT make this evidence the most robust. Although many RCTs on SLIT and SCIT have been published to date, the only study reporting data on the onset of new sensitizations is the Durham study (37).

There are several sources of bias in the analyzed studies, including potentially unmeasured and unadjusted confounders, defined in this review as pre-intervention factors that predict whether an individual receives one or the other of two treatments. Socioeconomic status, education, severity of pre-existing 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 (47). We also included as potential source of bias the lack of investigation of the presence of domestic animals (taken in consideration by only one study) (32) 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, molds, pollens, and other environmental conditions (pollution) (47). The presence of these biases may explain the considerable heterogeneity in the analyzed studies. This raises the possibility of the presence of an 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. To illustrate this point, there is a huge variability in the percentage of patients with polysensitizations in the pharmacotherapy groups (controls) at the end of the observation period, ranging from 8% (24) to 100% (20, 23, 35) (median 43% for child studies, 53% for adult studies). Several epidemiological studies report a percentage of polysensitization among allergic patients from 50% to 80%, depending on age, populations, and regions (8, 53, 54). Therefore, the percentage of 100% of polysensitization reported in the control groups of the Des Roches (20), Eng (23), and Marogna (35) studies appears extremely high, in particular considering that two of these three studies (20, 23) were performed on children. These studies are among the studies featuring the smallest sample size, and thus, they are at the highest risk of imprecision. Note that the Des Roches (20) study is the most cited by the consensus on AIT (Table 5). Furthermore, in the Marogna (35) and the Eng (23) studies there is a high rate of loss to follow-up, and another serious limitation of these observational studies is precisely that dealing with of loss to follow-up and its potential impact on the estimates were not taken into consideration by the investigators, with a possible significant impact on the estimate of AIT effect. 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

Table 4 Allergen-specific immunotherapy for the prevention of new allergen sensitizations: comparisons of subgroups

Subgroup	Number of participants	Number of studies	Favors AIT (number of studies)	Favors pharmacotherapy or null (number of studies)	Comment
Age					
Children	1049	11	6 (20–23, 28, 29)	5 (24–27, 30)	Only a one study difference in favor of AIT in child and adult studies
Adults	10 057	7	4 (31, 33, 35, 36)	3 (32, 34, 37)	
Sample size					
>100	10 769	11	5 (21, 28, 29, 31, 33)	6 (25–27, 30, 34, 37)	The effect of AIT on the outcome is evident in the small studies, not in the big ones.
≤100	337	7	5 (20, 22, 23, 35, 36)	2 (24, 32)	
Type of treatment					
SCIT	10 022	12	7 (20–23, 28, 31, 36)	5 (26, 27, 30, 32, 34)	No difference reported in the SLIT studies. The three studies reporting a benefit of SLIT on the outcome are from the same authors, while the remaining three, including the follow-up study of the DB-PC-RCT, are from three different groups
SLIT	1084	6	3 (29, 33, 35)	3 (24, 25, 37)	
Type of allergen					
HDM	783	9	5 (20–22, 28, 35)	4 (24, 26, 27, 30)	Only a one study difference in favor of AIT in child and adult studies with respect to HDM
Others ± HDM	10 323	9	5 (23, 29, 31, 33, 36)	4 (25, 32, 34, 37)	
Length of follow-up					
3 years	844	5	4 (20, 22, 29, 33)	1 (25)	The benefit of AIT on the outcome is more evident in studies with a shorter follow-up
>3 years	10 265	13	6 (21, 23, 28, 31, 35, 36)	7 (24, 26, 27, 30, 32, 34, 37)	

AIT, allergen immunotherapy; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; HDM, house dust mite; DB-PC-RCT, double-blind, placebo-controlled, randomized controlled trial.

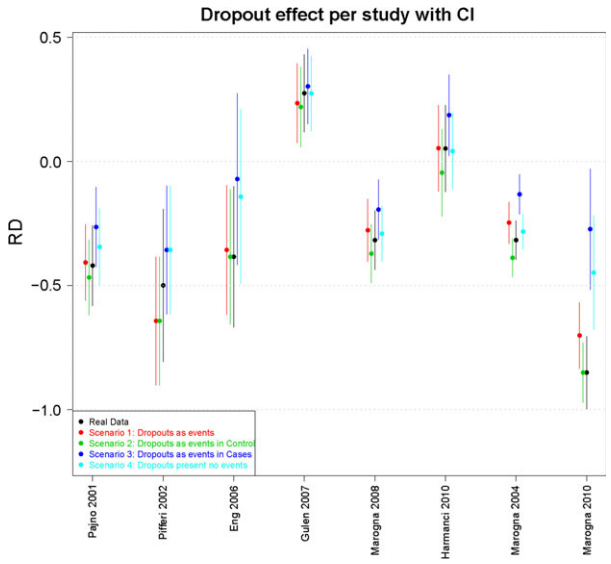

Figure 1 Analysis of the variations of the estimates under a number of assumptions about the outcomes of participants lost to follow-up. The point estimate (full circle) and the confidence intervals (vertical lines) for each assumption (colors) are plotted on the graph. CI, confidence interval; RD, risk difference.

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. High rate of loss to follow-up was also reported in the Durham long-term follow-up study (37), but the data reported after a shorter follow-up (2 years shorter) of the same population with a higher number of patients showed the same results (55). The information about loss to follow-up is missing in the two retrospective studies by Purello D'Ambrosio and Asero, (31, 34) rendering any adjustment of potential missing data impossible. These two studies that feature the biggest sample size report opposite results.

In the follow-up study by Dominicus, the loss to follow-up was reported only for cases, but not for controls, that were apparently selected from a different population, as about a 10-year difference was reported between the groups (36).

Finally, in six of 18 studies, there is potential conflict of interest of one or more authors of the studies (24, 25, 29, 31, 33, 37). However, only three (29, 31, 35) of these six studies reported an AIT preventive effect on the onset of new sensitizations, 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 three different electronic databases, and the reference lists of the retrieved articles, including

Table 5 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
Alvarez-Cuesta, 2006 (4)	EAACI SCIT	Des Roches (20) Pajno (21) Purello D'Ambrosio (31)	All studies in favor of AIT
Bousquet, 2008 (2)	ARIA	Des Roches (20) Pajno (21) Purello D'Ambrosio (31)	All studies in favor of AIT
Cox, 2009 (5)	AAAAI/ACAAI practice parameter	Des Roches (20) Pajno (21) Purello D'Ambrosio (31)	All studies in favor of AIT
Canonica, 2009 (6)	WAO SLIT Guidelines	Des Roches (20) Pajno (21) Purello D'Ambrosio (31)	All studies in favor of AIT
Saranz, 2010 (7)	Argentina Guidelines	Des Roches (20) Inal (27) Jacobsen (38)	One study in favor of AIT (20) Two insignificant studies (27, 38)
Zuberbier, 2010 (8)	GA^{2}LEN/EAACI Pocket guide for AIT	Jacobsen (38) Des Roches (20) Purello D'Ambrosio (31)	Two studies in favor of AIT (20, 31) One insignificant study (38)
Cox, 2011 (9)	AAAAI/ACAAI practice parameter third update	Des Roches (20) Pajno (21) Purello D'Ambrosio (31) Inal (27)	All studies in favor of AIT except the Inal study (27)
Walker, 2011 (10)	British Guidelines	Des Roches (20) Pajno (21)	All studies in favor of AIT
Canonica, 2014 (11)	WAO Position Paper	Des Roches (20) Marogna (35) Acquistapace (40)	All studies in favor of AIT
Pfaar, 2014 (12)	German, Austrian, and Swiss allergists' and specialists' consensus on AIT in patients with allergic airway diseases	Jacobsen (38) Eng (23) Purello D'Ambrosio (31) Pajno (21) Di Rienzo (24) Marogna (35)	Four studies in favor of AIT (21, 23, 31, 35) Two insignificant studies (24, 38)
Jutel, 2015 (13)	International consensus on allergy immunotherapy	Jacobsen (38) Des Roches (20) Purello D'Ambrosio (31)	Two studies in favor of AIT (20, 31) One insignificant study (38)

AIT, allergen immunotherapy; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; EAACI, European Academy of Allergy and Clinical Immunology; ARIA, Allergic Rhinitis And Its Impact On Asthma; AAAAI, American Academy of Allergy, Asthma & Immunology; ACAAI, American College of Allergy, Asthma & Immunology; GA^{2}LEN, Global Allergy and Asthma European Network; WAO, World Allergy Organization.

position papers and consensus documents, and also a gray literature search, we encountered several challenges during our review process, as this specific outcome is not always the primary study outcome and therefore it is often 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 AIT preventive effect in the consensus conference reports, makes us confident that no potentially relevant overlooked study may 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 effect among studies.

The main strengths of this work are that it is the first comprehensive review on 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.

In conclusion, our review found low strength evidence to support the preventive effect of AIT on 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.

Author contributions

DDB and GDL developed the concept of this study and wrote the protocol. MSLB and SLP collated the data for the study, and AP did the statistical analyses. The first draft of the manuscript was written by DDB and thoroughly revised by GDL and LM.

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Conflict of interest

We declare that we have no conflict of interest.

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