

Efficacy and safety of allergen immunotherapy in patients with allergy to molds: A systematic review

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Abstract

Background: Allergen immunotherapy (AIT) with mould extracts has been performed for many years but the final demonstration of its clinical efficacy is still missing, due to the small number of studies and their inconsistent results.

Objective: To systematically review efficacy and safety of AIT for the treatment of respiratory allergies to moulds.

Design: The primary outcomes were safety and reduction of symptoms (Symptom Score, SS) and medication use (Medication Score, MS) in patients treated with AIT compared to controls. 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.

Data sources: Medline, Web of Science and the Cochrane Library (through September 2017) supplemented with manual searches of reference lists.

Eligibility criteria: Randomized studies of intervention comparing AIT to placebo/pharmacotherapy. Studies not reporting on our outcome of interest or without a control population were excluded.

Results: Nine studies (168 children, 99 adults; median sample size, 27) met the inclusion criteria. The risk of bias was moderate-to-high in all but one study. Low strength evidence supports the assumption that AIT is effective in reducing symptoms and medication use, with only four of nine studies reporting higher benefit of AIT vs. comparators. The highest benefit of AIT compared to pharmacotherapy/placebo was reported in studies with a longer follow-up (SMD for MS from -3.96 to -3.97 in favour of AIT) and low risk of bias (VAS for SS: 66.3 ± 13 in AIT group; 186.6 ± 39 in comparators; $P < 0.05$). No difference was reported with respect to study sample size, route of administration, age of participants. Generalised adverse reactions were reported in 12.5% of participants treated with sublingual immunotherapy, and 37.2% of participants treated with subcutaneous immunotherapy.

Conclusions: Low strength evidence suggests that mould AIT is efficacious for the treatment of respiratory allergies. High-quality studies with an adequate sample size are needed.

KEYWORDS

allergen immunotherapy, *Alternaria*, asthma, *Cladosporium*, development and evaluation, grading of recommendations assessment, rhino-conjunctivitis

1 | INTRODUCTION

Mould allergy is a relatively common condition with a prevalence estimated about 5% of European patients with respiratory allergy, as determined by skin prick tests.^{1,2} There is a large geographical variation in mould sensitization among countries, with a prevalence ranging from 2% in Nordic countries to more than 20% in some Mediterranean areas.¹⁻⁴ *Alternaria*, one of the most important outdoor mould together with *Cladosporium*, is the fifth most common sensitizing agent in Europe, after *Dermatophagoides*, *Graminaceae*, cat and birch. Notably, *Alternaria* sensitization appears to be overall more common than sensitization to olive pollen and *Parietaria* pollen.¹⁻⁴

Alternaria and *Cladosporium* are responsible for seasonal respiratory allergy, in the form of both rhinitis and asthma.

Subjects with allergic rhinitis or asthma resistant to usual pharmacotherapy can be treated by allergen immunotherapy (AIT), which is the only causal treatment for allergic diseases.^{5,6} AIT has been proven effective, with a variable clinical benefit, in reducing symptoms and the use of anti-symptomatic medications in patients with allergic rhino-conjunctivitis and asthma due to pollen exposure.⁵⁻⁸

Although AIT with mould extracts has been performed for many years,^{9,10} the final demonstration of its clinical efficacy is still missing, due to the small number of studies performed to date and the inconsistency of their results, hindering the overall assessment of treatment efficacy. Possible explanations for the inconsistency among the results of the existing individual studies are the heterogeneity of their design and conduct and the heterogeneity of the enrolled population.

In this study, we evaluated the strength of the evidence of the efficacy and safety of AIT with mould extracts for the treatment of allergic asthma and rhinitis in children and adults. We reviewed the quality of the available studies using the GRADE (Grading of Recommendations Assessment, Development and Evaluation) approach to collect evidence on the effect of AIT on symptom improvement and reduction of medication use.¹¹

2 | METHODS

2.1 | Data sources and searches

The primary sources of the reviewed studies were Medline, Web of Science and the Cochrane Library (inception to 18 September 2017) with a specific search strategy based on the following medical subject headings: rhinitis, rhino-sinusitis, rhino-conjunctivitis, conjunctivitis, asthma, allergen immunotherapy, immunotherapy, moulds, fungus, fungi, *Alternaria*, *Cladosporium*. We also searched in ClinicalTrials.gov and the EU Clinical Trial Register. The computer search

was supplemented with manual searches of reference lists for all available review articles, primary studies and abstracts from meetings (see Appendix S1). There was no language restriction.

2.2 | Study selection

We required that studies: (a) were randomized studies of intervention of subcutaneous immunotherapy (SCIT), sublingual immunotherapy (SLIT) or oral immunotherapy (OIT) comparing subjects treated with AIT to subjects who received placebo or conventional treatment (pharmacotherapy) without placebo; (b) included mono-sensitized or poly-sensitized patients with allergic rhinitis/rhinosinusitis/conjunctivitis and/or asthma with a positive allergen-specific skin prick test and/or elevated serum allergen-specific IgE to *Alternaria alternata/tenuis* or *Cladosporium*. Studies were excluded if they did not include a control population.

2.3 | Data extraction and quality assessment

Two separate reviewers (DDB, FF) independently extracted detailed information on the study characteristics, study participants, interventions, outcomes and their methods of ascertainment. Disagreements were solved by consensus adjudication.

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.¹² We assessed the following six categories of potential bias: (a) lack of randomization, (b) lack of allocation concealment, (c) inadequate blinding, (d) incomplete data reporting, (e) selective outcome reporting and (f) other sources of bias, such as participation of sponsor company in the study design and interpretation of data. Trials assessed as having low risk of bias in all of the above-specified domains were considered trials at low risk of bias. Trials assessed as having uncertain risk of bias or high risk of bias in one or more of the above specified domains were considered trials at moderate to high risk of bias.

The GRADE approach was used to rate the overall quality of evidence.¹¹ 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 study data to the question of interest (directness), the imprecision, the publication bias and the magnitude of the effect. The evidence for the established outcome was graded as: (a) high grade, that is high confidence that the evidence reflects the true effect; (b) moderate grade, that is 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); (c) low grade, that is the confidence in the effect estimate is limited (the true effect may be substantially different from the estimate of the effect); (d) insufficient evidence. Observational studies without special strengths (ie, large magnitude of an effect; dose-response gradient; effects of all plausible residual confounding accounted for) or with important limitations (ie, study design or execution; publication bias; imprecision; inconsistency) were considered to provide low quality of evidence.

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 of effect plus one study with medium risk of bias or strong magnitude of effect in the context of consistent overall evidence. Evidence was low grade if it did not meet any of these categories. Insufficient evidence was assigned if there were no relevant studies.

There was a wide range of clinical diversity in the type of the participants, their allergy, the specific allergen treated and geographic study location. Methodological diversity was also evident in the study design. Despite these critical limitations, an attempt to meta-analyse the data was made by including studies consistently reporting on the same outcome (see Appendix S1).

3 | RESULTS

Our search strategy identified 691 publications, from inception to 18 September 2017, the titles and abstracts of which were screened for inclusion. The full text of 14 studies was finally retrieved, of which nine met the inclusion criteria,¹³⁻²¹ after applying triage and exclusion to other topics (Figure 1). We excluded the Cantani study because this study had a case-control design.²²

We excluded the Zapatero study²³ and the Meltzer study,²⁴ because they did not include a control population, and the Bernardis study,²⁵ as it compared SCIT to SLIT (Figure 1). Finally, we identified another RCT, started in 2011, whose results are still unpublished (EUCTR2010-024440-15-ES).²⁶

Table 1 shows descriptive data for the 9 systematically reviewed studies.¹³⁻²¹ The six child^{13,15-17,20,21} and three adult studies^{14,18,19} included a total of 268 patients (169 children, 99 adults). All the studies were performed in Europe. Nine studies were randomized,¹³⁻²¹ two of which had an open design^{16,21}; one study was single blinded.¹⁹

The comparator groups of the studies were either placebo or conventional treatment (pharmacotherapy) without placebo. Two studies were performed on patients sensitized to *Cladosporium*,^{13,14} and the remaining seven studies on patients sensitized either to *A. alternata* or *A. tenuis*.¹⁵⁻²¹ Immunotherapy was administered by subcutaneous route in six studies,^{13-15,17,20,21} by sublingual route in two studies^{18,19} and by oral route in one study.¹⁶ The sample size of the studies was small (median sample size, 27 participants),

ranging from 16²¹ to 51 patients.¹⁹ The median of the mean age of patients at inclusion was 12 years (range 10.1²¹-12.9¹⁷) in the child studies, and 23.5 years (range 18.5¹⁹-28¹⁴) in the adult studies. Five studies enrolled mainly patients with bronchial asthma.^{13,14,16,20,21} Three studies did not report the percentage of patients with allergic rhinitis.^{13,14,21} The severity of the main disease at baseline (asthma or rhinitis) or comorbidities (asthma, rhinitis, other diseases) was not clearly reported in the majority of the studies. The duration of treatment ranged from 10¹⁸ to 36 months.^{19,20} There was large heterogeneity in reporting the maintenance or cumulative AIT dose delivered and a variety of units to report dosing (Table 1). The outcome reporting was heterogeneous, with diverse systems and scores for evaluating both primary and secondary outcomes (SS, MS, VAS, Quality of Life, bronchial/nasal/conjunctival provocation test results, peak expiratory flow, forced expiratory volume in 1 s, changes in serum specific IgE levels, changes in skin prick test results).

The risk of bias was estimated as high in four studies^{14,16,19,21} moderate in four studies^{13,15,17,18} and low in one study²⁰ (Table 2).

We found low strength evidence to support the use of AIT in improving the clinical outcomes, that is symptoms and medication use. See Table 3.

SS and MS were the primary outcomes for the treatment efficacy. Safety was also assessed by reporting the total number of adverse events, both local and general.

No secondary outcomes were assessed due their possibly modest clinical relevance. Moreover, they were reported only in a subgroup of the selected studies, with a small sample size, insufficient to allow drawing reliable conclusions.

3.1 | Asthma and rhinitis symptoms

SS was reported in eight studies.^{13-18,20,21} The Pozzan and the Kuna studies reported VAS as measure of efficacy.¹⁹ Three out of the eight studies using SS as the primary outcome demonstrated a statistically significant improvement in asthma or rhino-conjunctivitis symptoms in the AIT group, relative to placebo or pharmacotherapy.^{15,18,20} Also the Pozzan study reported a greater benefit of AIT compared to pharmacotherapy.¹⁹ The remaining five studies showed no statistically significant difference between the groups (Table 3).^{13,14,17,21} We performed subgroup analyses to understand whether inconsistency of the results could be explained on the basis of differences in populations, interventions or study design. The comparisons of groups of studies sharing similar characteristics showed that studies with a longer follow-up showed the highest benefit of AIT (Table 4).^{19,20} No difference was reported with respect to the study sample size, route of administration (SCIT vs. SLIT) and age of participants (Table 4). The two studies with *Cladosporium* did not report any difference between AIT and pharmacotherapy,^{13,14} while four^{15,18-20} of the eight studies with *Alternaria* reported a greater efficacy of AIT.

An attempt to meta-analyse these data was made for studies consistently assessing the same outcomes. The effect of mould AIT on symptom score, in which AIT is compared with placebo/

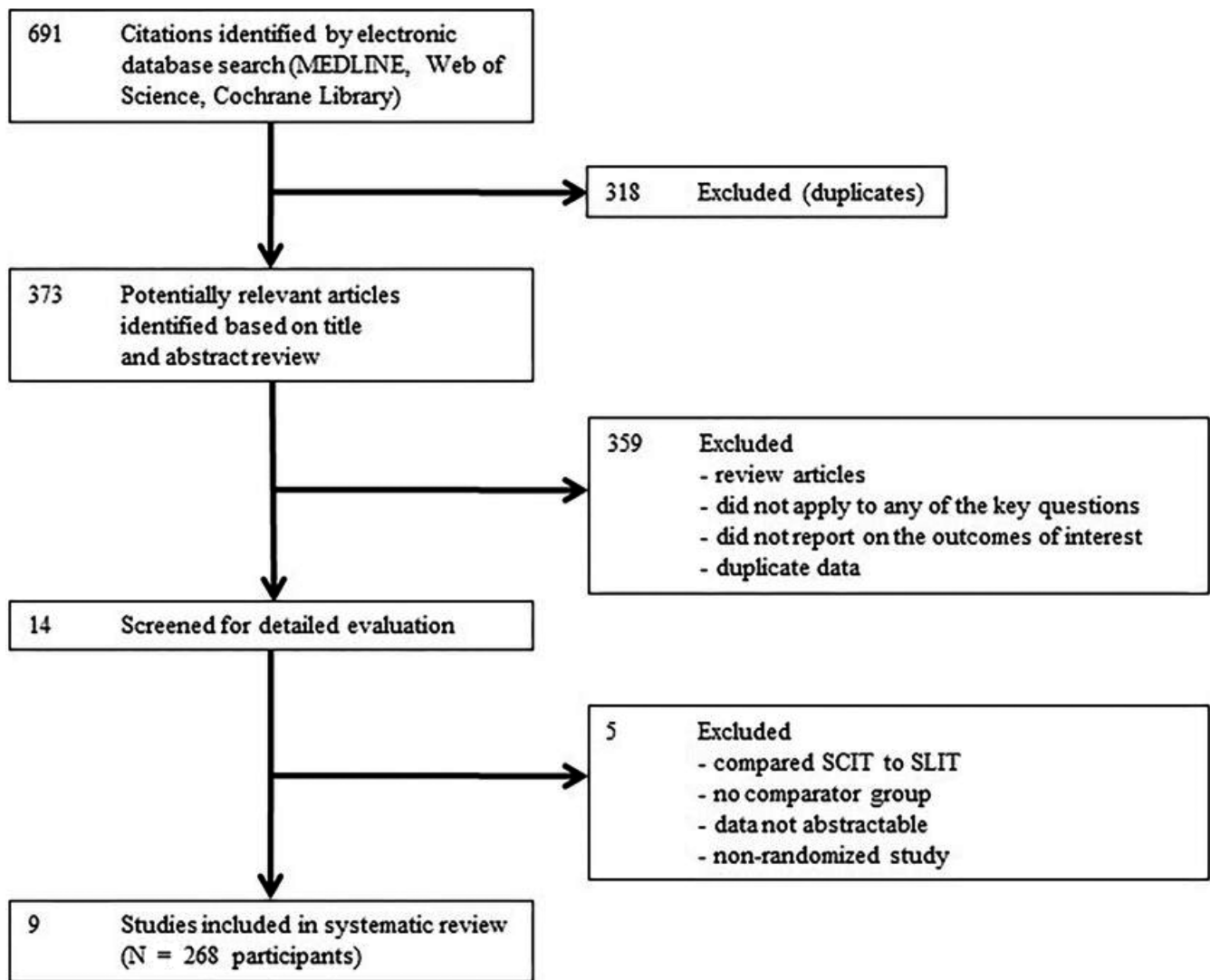


FIGURE 1 Flow diagram of AIT studies

pharmacotherapy, is reported in Figure S1A. Two studies showed a statistically significant reduction of symptom score in actively treated patients compared with controls,^{15,18} while one study was in favour of the control group.¹⁷ The pooled Standardized Mean Difference (SMD) for the treatment effect was -0.90 (95% CI, -2.19 to 0.40 ; $P = 0.18$), showing a not statistically significant association between AIT and symptom reduction. A remarkable degree of heterogeneity between the results of individual studies was calculated ($I^2 = 84\%$).

Analytical data from the studies not included in the meta-analysis are reported in Figure S1B. Two studies were in favour of AIT and reported a statistically significant difference between the two groups.^{21,22} All remaining four studies did not show a statistically significant difference between the groups.^{13,14,16,21}

Considering the inconsistency in study results (which remained unexplained), the small study sample size and that the risk of bias was moderate to high in almost all the studies (eight out of nine), we graded the strength of evidence as low in supporting AIT with fungal extracts for the treatment of allergic asthma and/or rhinoconjunctivitis.

3.2 | Medication use

MS was reported in nine studies.¹³⁻²¹ Four studies demonstrated greater improvement in MS in the AIT group vs. the comparator group.^{15,18-20} The remaining five studies reported no significant difference (Table 3).^{13,14,16,17,21}

The effect of mould AIT on medication score was estimated by meta-analysis, comparing AIT with placebo/pharmacotherapy (Figure S2A). Five studies were suitable for meta-analysis.^{15,17-20} Four studies showed a statistically significant reduction of medication score in actively treated patients compared with controls,^{15,18-20} while one study was in favour of the control group.¹⁷ The pooled SMD for the treatment effect was -2.11 (95% CI, -3.84 to -0.39 ; $P = 0.02$), showing a statistically significant association between AIT and medication use reduction. However, a remarkable degree of heterogeneity between the results of individual studies was calculated also in this case ($I^2 = 94\%$).

TABLE 1 Characteristics of the studies

Study	Country	Study design	Allergen	AIT type	Patients (n)	Male (%)	Mean age (mean $\pm$ SD)	ARC (%)	Asthma (%)	Treatment duration (months)	Major allergen	Cumulative dose
Dreborg (1986) ¹³	Sweden Finland	DBRPC	Cladosporium herbarum	SCIT	AIT: 16 > 16 Pla: 14 > 14	46.6	11	NR	100	12	NR	508 880 BU
Malling (1986) ¹⁴	Denmark Sweden	DBRPC	Cladosporium herbarum	SCIT	AIT: 11 > 11 Pla: 11 > 11	72.7	25 31	NR	100	12	NR	557 260 BU
Horst (1990) ¹⁵	France	DBRPC	Alternaria alternata	SCIT	AIT: 13 > 13 Pla: 11 > 11	70.8	12.1 $\pm$ 4.7 13.3 $\pm$ 14.6	100	41.7	12	Alt a 1	58 850 BU (47.1 μ g)
Criado Molina (2002) ¹⁶	Spain	Randomized (no placebo)	Alternaria alternata	OralIT	AIT: 22 > 16 PT: 22 > 16	59.4	12.4 $\pm$ 3.3 12.3 $\pm$ 3.2	84.4	90.6	12	Alt a 1	288 000 PNU
Tabar (2008) ¹⁷	Spain	DBRPT	Alternaria alternata	SCIT	AIT: 14 > 13 Pla: 14 > 10	83.3	12.8 $\pm$ 4.1 14.9 $\pm$ 6.5	100	89.3	12	Alt a 1	20 483 UBE (1.23 μ g)
Cortellini (2010) ¹⁸	Italy	DBRPT	Alternaria alternata	SLIT	AIT: 15 > 15 Pla: 12 > 11	55.5	18.8 $\pm$ 7.2 24.0 $\pm$ 8.8	100	14.8	10	Alt a 1	60 μ g
Pozzan (2010) ¹⁹	Italy	SB ^a , randomized (no placebo)	Alternaria alternata	SLIT	AIT: 34 > 33 PT: 18 > 18	63.5	18 $\pm$ 9 19 $\pm$ 10	73.1	32.7	36	Alt a 1	129.6 μ g 43.2 μ g/yr
Kuna (2011) ²⁰	Poland	DBRPT	Alternaria alternata	SCIT	AIT: 30 > 25 Pla: 20 > 19	50	11.7 $\pm$ 3.9 11.2 $\pm$ 4.1	92	76	36	Alt a 1	123 000 TU (196.8 μ g:65.6 μ g/yr)
Kilic (2011) ²¹	Turkey	Randomized (no placebo)	Alternaria alternata	SCIT	AIT: 12 > 9 PT: 12 > 7	NR	10.2 $\pm$ 2.2 10.1 $\pm$ 2.1	NR	100	12	NR	NR

AIT, allergen immunotherapy; ARC, allergic rhino-conjunctivitis; BU, biological units; DB, double blind; IT, immunotherapy; NR, not reported; PC, placebo controlled; Pla, placebo; PNU, Protein Nitrogen Units; PT, pharmacotherapy; RCT, randomized controlled trial; SB, single blind; SCIT, subcutaneous immunotherapy; SD, standard deviation; SLIT, sublingual immunotherapy; TU, total units; UBE, unidades biológicas equivalentes; range yr, year.

^aAssessor blinded. The symbol ">" separated the patients randomized (left) from the patients analysed (right).

TABLE 2 Risk of bias in randomized studies

Study	Sequence generation	Allocation concealment	Blinding ^a	Incomplete data outcome	Selective outcome reporting	Tie with study sponsor	Risk of bias
Dreborg, (1986) ¹³	Not described	Not described	Double blind	No	No	No	Moderate
Malling, (1986) ¹⁴	Not described	Not described	Double blind	No	No	Yes	High
Horst, (1990) ¹⁵	Not described	Adequate	Double blind	No	No	No	Moderate
Criado-Molina, (2002) ¹⁶	Not described	No	No blinding	Yes	No	No	High
Tabar, (2008) ¹⁷	Adequate	Adequate	Double blind	No	No	Yes	Moderate
Cortellini, (2010) ¹⁸	Adequate	Adequate	Double blind	No	No	Yes	Moderate
Pozzan, (2010) ¹⁹	Adequate	No	Assessor blinded	No	No	Yes	High
Kuna, (2011) ²⁰	Adequate	Adequate	Double blind	No	No	No	Low
Kilic, (2011) ²¹	Not described ^b	No	No blinding	Yes	No	No	High

^aBlinding of participants, personnel and outcome assessors.

^bSignificant differences in patient characteristics between the groups at baseline.

Analytical data from the studies not included in the meta-analysis are reported in Figure S2B. These four studies did not show a statistically significant difference between the groups.^{13,14,16,21}

We graded the strength of evidence as low in demonstrating AIT efficacy in reducing medication use, due to inconsistency, low sample size and study quality (Table 3). Subgroup analysis showed results similar to those reported for SS (data not shown).

3.3 | Safety

Local and general reactions were not reported in all studies. Local reactions were reported in a total of 26 patients (20.6% of the 126 patients available for the analysis). The majority of these reactions were mild and easily controlled by symptomatic treatment (Table 5). A total of 37 patients (26.1% of the 142 patients available for the analysis) reported systemic reactions, including asthma and anaphylaxis (three patients in the Malling study).¹⁴ Notably, 24 of 37 systemic reactions were reported in the only two studies on *Cladosporium* AIT.^{13,14} Notably, in the Dreborg study,¹³ using an accelerated cluster schedule with high allergen dose, systemic reactions were reported in all the subjects who experienced large local reactions (>10 cm in diameter). In contrast AIT with *Alternaria* extracts caused systemic side effects in a smaller proportion of patients (13 of 115; 11.3% of patients) (Table 5). Overall, generalised adverse reactions were reported in eight of 64 (12.5%) participants treated with sublingual immunotherapy (reported in three studies), and 29 of 78 (37.2%) participants treated with subcutaneous immunotherapy (reported in five studies), compared with three of 65 (4.6%) participants treated with subcutaneous placebo injections (reported in five studies).

4 | DISCUSSION

To our knowledge, this work is the first systematically reporting on efficacy and safety of AIT for outdoor moulds, through a

comprehensive review of the literature and grading of evidence for clinical outcomes. The results provided here are applicable to patients with both allergic rhinitis and asthma due to *Cladosporium* and *Alternaria*. There is very limited evidence for the use of immunotherapy to treat allergy caused by other mould species, including *Aspergillus*. We found that the collected evidence is of low strength overall, and it is not sufficient to support the opinion that AIT with mould extracts is effective in reducing symptoms and the medication use in patients with respiratory allergy to moulds. The strength of the evidence was graded as low, considering the inconsistency of study results, the small study sample size and the moderate-to-high risk of bias of almost all the studies, according to the GRADE criteria.¹¹

Our conclusions are in accordance with those of a recently published review by Larenas-Linnemann, showing that low-quality evidence exists supporting the use of subcutaneous immunotherapy for *Alternaria* to treat allergic rhinitis and asthma and very low quality evidence supports the use of subcutaneous immunotherapy for *Cladosporium* and sublingual immunotherapy for *Alternaria*.²⁷

Overall, an AIT benefit was observed in only 4 of 9 studies, the remaining studies being negative or null. This high level of inconsistency was not explained by subgroup analyses comparing studies sharing similar characteristics. In fact, no difference between AIT and comparator groups was reported with respect to age of participants, allergen used (*Alternaria/Cladosporium*), route of administration, study sample size.

The only exception was represented by the observation that the studies with longer duration of treatment reported the highest benefit from AIT. This could suggest that adequately longer treatment duration (as recommended by the Guidelines) would perhaps lead to a significant clinical response.^{5,6} However, the overall small number of studies and their small sample size makes it impossible to draw any meaningful conclusion based on these latter findings.

In fact, the main limitation of the studies included in this review was the small sample size (median = 27 participants per study),

TABLE 3 AIT evidence summary

Outcome	Number of participants	Number of studies	Allergens	Summary of grading data	Findings	Strength of the evidence	Comment
Symptoms	268 - AIT, 151 - PT, 117	9	- <i>Cladosporium</i> ^{13, 4} - <i>Alternaria</i> ¹⁵⁻²¹	- Four studies had a high risk of bias. ^{14,16,19,21} Four studies had a moderate risk of bias. ^{13,15,17,18} One study had a low risk of bias. ²⁰ The sample size was small in all the studies, leading to imprecision in the estimates of the treatment effect and to inconsistency of results.	Four studies were in favour of AIT. ^{15,18-20a} , 2 of which showing a large magnitude of effect. ^{15,18} Five studies reported no statistically significant differences. ^{13,14,16,17,21}	Low	The quality of evidence was downgraded by 2 levels due to high level of inconsistency: 4 studies in favour of AIT; 5 negative or null. Meta-analysis including 3 out of the 9 studies showed a considerable (i.e. $I^2 > 75\%$) level of heterogeneity ($I^2 = 84\%$) between the study results (Figure S1). The quality of evidence can also be downgraded by 2 levels due to imprecision, since CIs around estimates of effect are wide, including both appreciable benefit and appreciable harm (Figure S1). Moreover, the studies include relatively few patients.
Medication use	268 - AIT, 151 - PT, 117	9	- <i>Cladosporium</i> ^{13,14} - <i>Alternaria</i> ¹⁵⁻²¹	- Four studies had a high risk of bias. ^{14,16,19,21} Four studies had a moderate risk of bias. ^{13,15,17,18} One study had a low risk of bias. ²⁰ The sample size was small in all the studies, leading to imprecision in the estimates of the treatment effect and to inconsistency of results.	Four studies were in favour of AIT. ^{15,18-20} 2 of which showing a large magnitude of effect. ^{15,18} Five studies reported no statistically significant differences. ^{13,14,16,17,21}	Low	The quality of evidence was downgraded by 2 levels due to high level of inconsistency: 4 studies in favour of AIT; 5 negative or null. Meta-analysis including 6 out of the 10 studies showed a considerable (i.e. $I^2 > 75\%$) level of heterogeneity ($I^2 > 94\%$) between the study results (Figure S2). The quality of evidence can also be downgraded by 2 levels due to imprecision, since CIs around estimates of effect are wide (Figure S2). Moreover, the studies include relatively few patients.

^aThe Pozzan study was in favour of AIT, but used VAS as the measure of efficacy, instead of SS. AIT, allergen specific immunotherapy; PT, pharmacotherapy; RCT, randomized controlled trial.

TABLE 4 AIT for moulds: comparisons of subgroups for symptom improvement

Subgroup	Number of participants	Number of studies	Favours AIT (number of studies)	Favours pharmacotherapy or null (Number of studies)	Comment
Age					
Children	168	6	2 ^{15,20}	4 ^{13,16,17,21}	A two-study-difference in favour of pharmacotherapy in adult studies; a one-study-difference in favour of AIT in child studies
Adults	99	3	2 ^{18,19}	1 ¹⁴	
Sample size					
>28	156	4	2 ^{19,20}	2 ^{13,16}	The effect of AIT was not influenced by the sample size. Notably, all the available studies had a small sample size.
≤28	112	5	2 ^{15,18}	3 ^{14,17,21}	
Type of treatment					
SCIT	158	6	2 ^{15,20}	4 ^{13,14,17,21}	A one-study-difference in favour of AIT in SLIT studies; a two-study-difference in favour of pharmacotherapy in SCIT studies.
SLIT/oral immunotherapy	110	3	2 ^{18,19}	1 ¹⁶	
Type of allergen					
<i>Cladosporium</i>	52	2	0	2 ^{13,14}	The only 2 studies on <i>Cladosporium</i> did not show any benefits of AIT. There was a slight prevalence of studies in favour of AIT in the <i>Alternaria</i> subgroup.
<i>Alternaria</i>	216	7	4 ^{15,18-20}	3 ^{16,17,21}	
Length of follow-up					
≤1 year	173	7	2 ^{15,18}	5 ^{13,14,16,17,21}	The benefit of AIT was more evident in studies with a longer follow-up.
>1 years	95	2	2 ^{19,20}	0	

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

leading to imprecision in the estimates. A preliminary calculation of sample size was made only in few studies. However, as suggested by the GRADE approach,¹¹ whenever the sample size is smaller than 400, approximately, this should not be considered sufficient to prevent prognostic imbalance. Therefore, in these cases, one should consider rating down the evidence for imprecision. The total number of patients included from the 9 studies in our analysis was 268, below the above-mentioned threshold for a single adequately powered trial.²⁸ This limit increases the risk of imprecision of estimates, reducing the confidence in the study results.

The overall risk of bias was moderate-to-high overall. The unclear description of the random sequence generation^{13-16,21} and allocation concealment,^{13,14,16,19,21} and the lack of blinding^{16,19,21} were the most frequent biases for the randomized studies. Furthermore, in 4 studies, there was the participation of sponsor companies in the study design and the interpretation of data.^{14,17-19} As it was repeatedly shown, these studies are more likely to report results that favour the sponsor's product than studies supported by independent sources.^{29,30}

There were many sources of heterogeneity that limited the comparability of the study results. The studies were heterogeneous in design and conduct (double or single blinded, open parallel design, non-paired controls, evaluation at different time points, per protocol or intention to treat evaluation, etc.). Moreover, different scoring and reporting systems were used for clinical outcomes and safety data.

Furthermore, some studies used combined symptom scores (asthma plus rhinitis) or combined symptom-medication scores. The use of asthma plus rhinitis combined score may not accurately reflect the degree of control of each disease. The use of symptom plus medication score, besides being methodologically incorrect,^{7,31} does not allow estimating the real benefit in each individual outcome. In any case, it is difficult to compare studies using combined scores to studies using separate scores for each outcome.

Asthma and rhinitis severity was not clearly reported in the majority of the studies and some of them compared groups with different characteristics at baseline. This led to possible serious bias such as inception or selection bias.

There was a great variability in the dosing and treatment schedules among the studies. Different units were used to report the doses (biological units, protein nitrogen units, total units, micrograms). The major allergen content was reported only in half of the SCIT studies and in the two SLIT studies. When considering the dosing for *Alternaria* in micrograms per year, the highest dose used in SCIT studies was more than 50 times greater²⁰ than the lowest¹⁷ (Table 1). Moreover, some SLIT studies used a dose lower than certain SCIT studies, suggesting that higher doses would possibly lead to higher SLIT efficacy.

Another important source of between-study heterogeneity is likely related to the marked company-to-company difference, in source material and manufacturing procedures for the preparation of allergen extracts for AIT. This is due to the lack of a generally

TABLE 5 Safety data

Study	Type of reactions	AIT Number of patients	Placebo/PT Number of patients
Dreborg, (1986) ¹³	Local	4/16 (25%)	NR/14 ^a
	General	13/16 (81%)	0/14
Malling, (1986) ¹⁴	Local	8/11 (73%)	0/11
	General	11/11 (100%)	1/11 (9.1%)
Horst, (1990) ¹⁵	Local	0/13	0/11
	General	2/13 (15.4%)	0/11
Criado-Molina, (2002) ¹⁶	Local	NR/16	NR/16
	General	8/16 (50%)	NR/16
Tabar, (2008) ¹⁷	Local	0/13	0/10
	General	2/13 (15.4%)	0/10
Cortellini, (2010) ¹⁸	Local	1/15 (6.7%)	0/11
	General	0/15	0/11
Pozzan, (2010) ¹⁹	Local	6/34 (17.6%)	0/18
	General	0/33	0/18
Kuna, (2011) ²⁰	Local	7/25 (28%)	0/19
	General	1/25 (4%)	2/19 (10.5%)
Kilic, (2011) ²¹	Local	NR/9	NR/7
	General	NR/9	NR/7
Overall	Local	26/126 (20.6%)	0/80 (0%)
	General	37/142 (26.1%)	3/94 (3.2%)

PT, pharmacotherapy; NR, not reported.

^aOnly minor local reactions (not-quantified by the Authors).

accepted potency assay and the substantial variability of fungal species. In the most recent studies, the major allergen content (Alt a1) was reported in µg. However, the lack of standardization does not ensure full consistency of allergen products.

The clinical-methodological heterogeneity is also evident in the meta-analyses including the studies assessing the same outcomes. See Appendix S1. We observed a considerable degree of between-study heterogeneity in the meta-analyses of SS and MS (as shown by the I^2 statistics: 84% to 94%). The main reason of heterogeneity was likely the small sample size of all the studies leading to imprecision in the estimates of the treatment effect. Furthermore, the studies were heterogeneous with respect to age of participants, comorbidities (asthma), route of administration (SLIT and SCIT), allergen dose used, length of treatment. Therefore, the pooled estimates should be considered with caution, as the overall benefit may not be generalized.

Publication bias, with studies reporting positive results being more likely to be published than studies reporting negative results, could not be assessed with this set of data. In fact, due to the incomplete reporting on relevant statistical information, such as standard deviation, standard error or confidence interval, we were unable to prepare a meaningful funnel plot. We tried to overcome publication bias through an extensive search of the literature, both performing electronic database searches and hand searches of reference lists of original publications or review articles. However, this

bias still cannot be excluded. In any case, given the overall result inconsistency, we are confident that a publication bias, if any, would be unlikely to influence the findings of this analysis.

Regarding safety, although all, but one²¹ studies included in this analysis reported on this subject, they did not report on safety data in a uniform and consistent manner. For this reason, we reported in our review the number of patients affected by adverse events, rather than the number of total adverse events. There was no report of life-threatening adverse events, in this review. Overall, both local and general adverse events were more frequent in subjects treated with AIT than in the comparator groups. AIT with *Alternaria* was apparently better tolerated than AIT with *Cladosporium*, which was associated with a remarkable number of general adverse events (24 of 37 patients with general adverse events were treated with *Cladosporium* extracts). However, the allergen dose used in *Cladosporium* studies seems to be much higher than that used in *Alternaria* studies, although a clear comparison between the doses used in different studies is challenging, due to their difference in measure units. Moreover, safety data on *Cladosporium* were drawn from studies performed more than 30 years ago.^{13,14} More updated reports on this issue would be needed, as no other study on *Cladosporium* has been performed since then.

In conclusion, we found low strength in the evidence in supporting the use of AIT for moulds. However, it should be emphasized that our conclusions are based on a limited number of studies, with small sample size and of a low quality. Due to these limitations, further studies with more robust methodology would probably establish progress in this field. In fact, AIT with other allergens, such as *Graminaceae* or *Dermatophagoides*, was proven effective when modern RCTs with an adequate sample size were properly carried-out.³² Therefore, we recommend that future studies should be double-blinded placebo-controlled trials, assessing clinical outcomes, using common scores and transparently reporting on participant data and allergen content of AIT. The study sample size must be adequate and the study should possibly include subjects from different European and non-European countries, in order to increase the generalizability of the results. The option of using materials other than common allergenic extracts for immunotherapy, such as carrier-bound peptides without allergenic activity, should also be considered.³³ Thus, performing new adequate studies is particularly urgent since, in recent years, the requirements on the data that must be provided to successfully apply for marketing authorization have risen significantly and products with insufficient quality or limited data on clinical efficacy will hardly remain on the market,³⁴ to the detriment of patients who may need AIT.

AUTHOR CONTRIBUTION

DDB and LM developed the concept of this study and wrote the protocol. FF and DDB collated the data for the study. The first draft of the manuscript was written by DDB and thoroughly revised by LM. MFC, GDL and MA contributed with an exhaustive critical reading.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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REFERENCES

- Bousquet PJ, Chinn S, Janson C, Kogevinas M, Burney P, Jarvis D. Geographical variation in the prevalence of positive skin tests to environmental aeroallergens in the European Community Respiratory Health Survey I. *Allergy*. 2007;62:301-309.
- D'Amato G, Chatzigeorgiou G, Corsico R, et al. Evaluation of the prevalence of skin prick test positivity to *Alternaria* and *Cladosporium* in patients with suspected respiratory allergy. A European multicenter study promoted by the Subcommittee on Aerobiology and Environmental Aspects of Inhalant Allergens of the European Academy of Allergology and Clinical Immunology. *Allergy*. 1997;52:711-716.
- Mari A, Schneider P, Wally V, Breitenbach M, Simon-Nobbe B. Sensitization to fungi: epidemiology, comparative skin tests, and IgE reactivity of fungal extracts. *Clin Exp Allergy*. 2003;33:1429-1438.
- Calabria CW, Dice JP, Hagan LL. Prevalence of positive skin test responses to 53 allergens in patients with rhinitis symptoms. *Allergy Asthma Proc*. 2007;28:442-448.
- Devillier P, Dreyfus JF, Demoly P, Calderon MA. A meta-analysis of sublingual allergen immunotherapy and pharmacotherapy in pollen-induced seasonal allergic rhinoconjunctivitis. *BMC Med*. 2014;12:71.
- 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, GA(2)LEN and AllerGen). *Allergy*. 2008;63(Suppl 86):8-160.
- 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.
- 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.
- Domson JF. Review of therapeutic procedure for mould allergy. In: Al-Doory Y, Domson JF, eds. *Mould Allergy*. Philadelphia: Lea & Febiger; 1984:187-201.
- Reed CE. What we do and do not know about mold allergy and asthma. *J Allergy Clin Immunol*. 1985;76:773-775.
- Atkins D, Best D, Briss PA, et al. Grading quality of evidence and strength of recommendations. *BMJ*. 2004;328:1490.
- Chapter 8: Assessing risk of bias in included studies. In: Higgins JPT, Green S, eds. *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.1.0. Updated March, 2011. <http://www.cochrane.org/>. Accessed June 30, 2017.
- Dreborg S, Agrell B, Foucard T, Kjellman NI, Koivikko A, Nilsson S. A double-blind, multicenter immunotherapy trial in children, using a purified and standardized *Cladosporium herbarum* preparation. I. Clinical results. *Allergy*. 1986;41:131-140.
- Malling HJ, Dreborg S, Weeke B. Diagnosis and immunotherapy of mould allergy. V. Clinical efficacy and side effects of immunotherapy with *Cladosporium herbarum*. *Allergy*. 1986;41:507-519.
- Horst M, Hejjaoui A, Horst V, Michel FB, Bousquet J. Double-blind, placebo-controlled rush immunotherapy with a standardized *Alternaria* extract. *J Allergy Clin Immunol*. 1990;85:460-472.
- Criado Molina A, Guerra Pasadas F, Daza Muñoz JC, et al. Immunotherapy with an oral *Alternaria* extract in childhood asthma. Clinical safety and efficacy and effects on in vivo and in vitro parameters. *Allergol Immunopathol (Madr)*. 2002;30:319-330.
- Tabar AI, Lizaso MT, García BE, et al. Double-blind, placebo-controlled study of *Alternaria alternata* immunotherapy: clinical efficacy and safety. *Pediatr Allergy Immunol*. 2008;19:67-75.
- Cortellini G, Spadolini I, Patella V, et al. Sublingual immunotherapy for *Alternaria*-induced allergic rhinitis: a randomized placebo-controlled trial. *Ann Allergy Asthma Immunol*. 2010;105:382-386.
- Pozzan M, Milani M. Efficacy of sublingual specific immunotherapy in patients with respiratory allergy to *Alternaria alternata*: a randomised, assessor-blinded, patient-reported outcome, controlled 3-year trial. *Curr Med Res Opin*. 2010;26:2801-2806.
- Kuna P, Kaczmarek J, Kupczyk M. Efficacy and safety of immunotherapy for allergies to *Alternaria alternata* in children. *J Allergy Clin Immunol*. 2011;127:502-508.e1-6.
- Kiliç M, Altıntaş DU, Yılmaz M, Bingöl-Karakoç G, BURGUT R, Güneşer-Kendirli S. Evaluation of efficacy of immunotherapy in children with asthma monosensitized to *Alternaria*. *Turk J Pediatr*. 2011;53:285-294.
- Cantani A, Businco E, Maglio A. *Alternaria* allergy: a three-year controlled study in children treated with immunotherapy. *Allergol Immunopathol (Madr)*. 1988;16:1-4.
- Zapatero L, Martínez-Cañavate A, Lucas JM, et al. Clinical evolution of patients with respiratory allergic disease due to sensitisation to *Alternaria alternata* being treated with subcutaneous immunotherapy. *Allergol Immunopathol (Madr)*. 2011;39:79-84.
- Melzer JM, Driskill BR, Clenney TL, Gessler EM. Sublingual immunotherapy for allergic fungal sinusitis. *Ann Otol Rhinol Laryngol*. 2015;124:782-787.
- Bernardis P, Agnoletto M, Puccinelli P, Parmiani S, Pozzan M. Intranasal versus sublingual immunotherapy in *Alternaria tenuis* allergic patients. *J Invest Allergol Clin Immunol*. 1996;6:55-62.
- Multicentre, double-blind, randomized, placebo-controlled, parallel-group, clinical trial to evaluate the clinical efficacy and safety of immunotherapy with purified major allergen Alt a 1 in patients with allergic rhinoconjunctivitis with or without mild to moderate asthma, sensitized to the fungus *Alternaria alternata*. EUCTR2010-024440-15-ES. Accessed date: April 2017. <https://www.clinicaltrialsregister.eu/ctr-search/trial/2010-024440-15/ES/>
- Larenas-Linnemann D, Baxi S, Phipatanakul W, Portnoy JM; Environmental Allergens Workgroup. Clinical evaluation and management of patients with suspected fungus sensitivity. *J Allergy Clin Immunol Pract*. 2016;4:405-414.
- Guyatt GH, Oxman AD, Kunz R, et al. GRADE guidelines 6. Rating the quality of evidence—imprecision. *J Clin Epidemiol*. 2011;64:1283-1293.
- Lundh A, Sismondo S, Lexchin J, Busuioac OA, Bero L. Industry sponsorship and research outcome. *Cochrane Database Syst Rev*. 2012;12:MR000033.
- Drugs and devices look more effective in studies sponsored by industry. *BMJ*. 2012;345:e8386. <https://www.bmj.com/content/345/bmj.e8386.long>
- Caiaffa MF, Iudice A, Macchia L, et al. Multicenter double-blind comparative study of terfenadine and cetirizine in hay fever. *J Invest Allergol Clin Immunol*. 1992;2:162-166.
- Roberts G, Pfaar O, Akdis CA, et al. EAACI Guidelines on Allergen Immunotherapy: allergic rhinoconjunctivitis. *Allergy*. 2017;73:765-798.
- Twaroch TE, Focke M, Fleischmann K, et al. Carrier-bound Alt a 1 peptides without allergenic activity for vaccination against *Alternaria alternata* allergy. *Clin Exp Allergy*. 2012;42:966-975.

34. Bonertz A, Roberts GC, Hoefnagel M, et al. Challenges in the implementation of EAACI guidelines on allergen immunotherapy: a global perspective on the regulation of allergen products. *Allergy*. 2017;73:64-76.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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EFFICACY AND SAFETY OF ALLERGEN IMMUNOTHERAPY IN PATIENTS WITH ALLERGY TO MOLDS: A SYSTEMATIC REVIEW

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SUPPLEMENTS

Methods

Search method for the identification of the studies

The following search strategy was use to search MEDLINE:

#1 Mold
#2 Mould
#3 #1 OR #2
#4 Immunoth*
#5 Desensitiz*
#6 #3 #4 OR #5
#7 Alternaria
#8 Cladosporium
#9 #6 AND #7 OR #8
#10 Clinical study OR trial OR clinical trial OR groups OR random* OR placebo
#11 #9 AND #10

The date of the last search was September 2017.

All other search strategies were modelled on the MEDLINE version.

Meta-analysis

The outcome data analyzed were continuous, but different scoring systems and scales for symptoms and medications were used by the Authors of the identified studies. Therefore, to compare the results, analyses were performed by the method of standardized mean difference (SMD), expressing the differences in means between AIT and placebo/pharmacotherapy in terms of units of the pooled SD. The overall SMD among patients treated with AIT and placebo was estimated with a model that was based on random-effects assumptions, as this model is considered the most conservative (S-1). The random-effects model applying the inverse variance method was used to estimate the SMD.

The outcome measures used for SMD calculations were symptom and medication scores. These measures were selected on the basis of their clinical relevance, compared to other outcome measures used in the individual studies, such as peak expiratory flow (PEF), forced expiratory volume in 1 second (FEV₁), conjunctival or nasal or bronchial provocation tests, circulating specific IgE levels. Some of these measures may be considered only as surrogate markers of clinical response. Furthermore, SS and MS were the only two outcome measures used in all the studies, except the Pozzan study which used VAS instead of SS (19).

The I^2 statistics, which describes the percentage of variability due to heterogeneity rather than sampling error, was used to quantify heterogeneity (S-2).

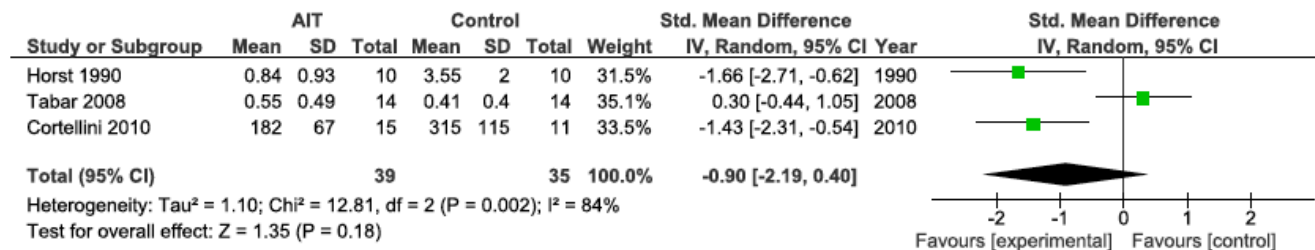
All our analyses were computed in Review Manager, Version 5.3. (S-3)

References

1. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Controlled Clin Trials* 1986;**7**:177-88.
2. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002;**21**:1539-58.
3. Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014.

Supplementary Fig. 1. AIT versus placebo/pharmacotherapy: effect on symptom score. A) Meta-analysis of 3 RTCs of AIT versus placebo/pharmacotherapy for allergic asthma/rhinosinusitis, using the random-effects model. The SMD and 95% CI for the effect of AIT on symptom score are shown as a forest plott. Studies of each group are arranged chronologically on the basis of the year of publication. The Horst study used a symptom-medication combined score. B) analytical data of the remaining 6 studies not suitable for meta-analysis.

A



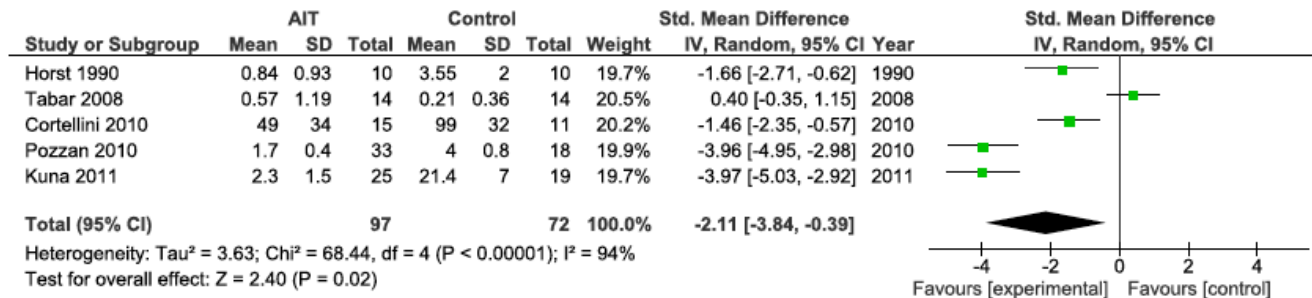
B

Study	AIT (n)	Control (n)	AIT findings	Control findings	P	Measures of position	Measures of outcome	Notes
Dreborg 1986	16	14	NR	NR	n.s.	-	SS	The two groups did not differ significantly regarding total symptom scores (no data provided)
Malling 1986*	11	11	1.83	1.5	n.s.	Median	SS	No statistically significant difference between the groups Favors control
Criado Molina 2002	16	16	0.31 (0.54)	0.56 (1.04)	0,43	Median (IR)	SS	No statistically significant difference between the groups Favors AIT
Pozzan 2010	34	18	4.7 ± 0.8	2 ± 1,6	0.0002	Mean (SD)	VAS	0=extreme worsening 6=extreme improvement Favors AIT
Kuna 2011*	25	19	66.3 (13)	186.6 (39)	0.04	Median (IR)	VAS	0=no symptoms 100=extremely bothersome Favors AIT
Kilic 2011	9	7	2 (1.38)	1,45 (0.39)	0.08	Median (IR)	SS	No statistically significant difference between the groups Favors control

*Data extracted from the graphs. NR, non reported; SS, symptom score; IR, interquartile range; VAS, visual analogue score.

Supplementary Fig. 2 AIT versus placebo/pharmacotherapy: effect on medication score.: A) Meta-analysis of 5 RTCs of AIT versus placebo/pharmacotherapy for allergic asthma/rhinosinusitis, using the random-effects model. The SMD and 95% CI for the effect of AIT on medication score are shown as a forest plot. Studies of each group are arranged chronologically on the basis of the year of publication. The Horst study used a symptom-medication combined score. B) analytical data of the remaining 4 studies not suitable for meta-analysis.

A



B

Study	AIT (n)	Control (n)	AIT findings	Control findings	P	Measures of position	Measures of outcome	Notes
Dreborg 1986	16	14	NR	NR	n.s.	-	MS	The two groups did not differ significantly regarding total medication scores (no data provided)
Malling 1986*	11	11	4.3	3.3	n.s.	Median	MS	No statistically significant difference between the groups Favors control
Criado Molina 2002	16	16	0.19 (0.47)	0.39 (1.75)	0.23	Median (IR)	MS	No statistically significant difference between the groups Favors AIT
Kilic 2011	9	7	2.51 (1.83)	1.38 (1.32)	0,2	Median (IR)	MS	No statistically significant difference between the groups Favors control

*Data extracted from the graphs. NR, non reported; MS, medication score; IR, interquartile range.