

Review article

The efficacy of sublingual immunotherapy for house dust mites respiratory allergy: results of a GA²LEN meta-analysis

Recent meta-analyses documented the efficacy and safety of sublingual immunotherapy (SLIT) in patients with allergic rhinitis (AR) and asthma (AA). Although SLIT appeared globally effective, the sub-analyses for single allergens provided uncertain results. This study is aimed to investigate the efficacy of SLIT with house dust mite (HDM) extracts in AR and AA through an updated reassessment of randomized controlled trials. Electronic databases were searched up to March 31, 2008, for randomized DBPC trials, assessing the efficacy of SLIT in AR and AA due to HDM sensitization. Outcomes were symptom scores and rescue medications use. For AR, eight studies fulfilled the selection criteria. A significant reduction in symptoms of AR (SMD -0.95; CI 95% -1.77 to -0.14 $P = 0.02$) was found in 194 patients (adults and children) receiving SLIT compared to 188 receiving placebo. For AA, with nine studies, similar results were found for symptoms (SMD -0.95; CI 95% -1.74 to -0.15 $P = 0.02$) in 243 patients (adults and children) receiving SLIT compared to 209 receiving placebo. A reduction in rescue medication use was found for AR (SMD -1.88; CI 95% -3.65 to -0.12 $P = 0.04$) in 89 patients, and AA (SMD -1.48; CI 95% -2.70 to -0.26 $P = 0.02$) in 202 patients. A relevant inter-study heterogeneity was detected. Promising evidence of efficacy for SLIT, using mite extract in allergic patients suffering from AR and AA, are herein shown. These findings suggest that more data are needed, derived from large-population-based high quality studies, and corroborated by objective outcomes, mainly for AA.

E. Compalati, G. Passalacqua, M. Bonini, G. W. Canonica

Allergy & Respiratory Disease Clinic, Department of Internal Medicine, University of Genoa, Genoa, Italy

Key words: efficacy; house dust mites; meta-analysis; specific immunotherapy.

G. W. Canonica
Allergy & Respiratory Diseases Clinic, Department of Internal Medicine
University of Genoa
Padiglione Maragliano, L.go R. Benzi 10
16132 Genoa
Italy

Accepted for publication 13 May 2009

Background

According to evidence-based medicine (EBM) criteria, conclusions from meta-analyses and randomized controlled trials (RCT) represent the most robust proof of the efficacy of an intervention (1). The meta-analysis is a statistical technique that elaborates the results of independent single studies, and provides a quantitative estimation of the global effect of an intervention, by using particular solutions to reduce the potential biases and the effects of heterogeneity between the sources. Systematic reviews performed according to Cochrane group and QUOROM statements recommendations take advantage of the best methodological quality (2).

Allergen specific immunotherapy (SIT) was introduced in practice about one century ago. In the last 20 years, the sublingual route of immunotherapy (SLIT) was gradually accepted as a viable alternative to injection SIT (3). In this context the need for an unequivocal assessment of the efficacy of SLIT in respiratory allergy has come out, and some meta-analyses of RCT have been published (4–8).

These systematic reviews, conducted for allergic rhinitis (AR) and asthma in adults and children, suggested a significant effect of SLIT in reducing symptoms and rescue medications usage. Otherwise the subgroup analyses for seasonal and perennial allergens provided controversial results.

In pediatric patients affected by AR, SLIT was found to be clinically effective with pollens but not with mites (4). Conversely, when administered to children suffering from AA, the sub-analysis showed that SLIT with mites extracts has a significantly greater efficacy as compared to SLIT with pollens (5). In adults with AR (6) and adults and children with AA (7), the attempt to identify a disproportionate benefit of treatment according to the administered allergen failed when assessing symptoms and medications reduction.

Although SLIT was found to be globally effective in the mentioned systematic reviews, performed according to Cochrane Collaboration and QUOROM methods, the sub-analyses for single allergens provided overall uncertain results, mainly due to the low number of patients

included. During the last 4 years, new trials have been published, thus new data for meta-analysis have become available. This study is aimed to investigate the efficacy of SLIT using HDM extracts in both AR and AA in order to solve the controversial results regarding the efficacy of desensitization to this allergen.

Methods

Objectives

To evaluate the efficacy of SLIT with HDM extract compared to placebo in reducing symptoms and medication requirement in AR and AA.

Search strategy

A comprehensive search of the MEDLINE, EMBASE, LILACS and SCOPUS databases was performed to identify all randomized double blinded placebo controlled clinical trials of SLIT with HDM extracts, published up to March 1, 2008. The search was conducted using the following keywords: *sublingual immunotherapy, allergic rhinitis, allergic asthma, swallow, drops, oral, oromucosal, desensitization, house dust mites, randomized clinical trials, and meta-analysis*. Reference lists of recent reviews and published trials were searched as well as abstracts of relevant meetings.

Patients

Patients of any age, with a history of AA alone, AR with or without allergic conjunctivitis and/or asthma, in whom the causal allergen was identified, and IgE sensitization was ascertained by prick test and/or specific IgE assays.

Interventions

SLIT with HDM extract, whether or not the allergen was subsequently swallowed. Allergen was considered at all doses and for all durations of the treatment.

Outcome measures

Primary outcomes. Reductions in symptoms of AR and/or AA and in medication intake however recorded (e.g. daily or weekly symptom diaries, Visual Analogue Scores, overall assessments).
Secondary outcomes. Reductions in symptoms of AR and AA and in medication intake in the independent populations of children and adults.

Study selection

Two investigators (Compalati Enrico, Bonini Matteo) independently evaluated studies for inclusion. Clinical trials were included if they were randomized, placebo controlled, and double-blind (DBRPC). Trial eligibility was determined on full-text format by two authors (Compalati Enrico, Passalacqua Giovanni), and subsequently checked by the principal investigator (Canonica Giorgio Walter). The observed percentage agreement between the investigators for the evaluation of inclusion was calculated by using the κ test (9, 10).

Quality assessment and evaluation of validity

Trials were rated for their methodological quality in duplicate using the Jadad scale and scored out of a maximum of 5 (11, 12). An inter-rater agreement was also calculated by using the κ statistic (9, 13). The quality assessment was based on study type and methodology; number and description of subjects; details of type, dosage and time schedule/duration of intervention; type, timing and measurement method of outcomes. Concealment of allocation and blinding of study participants and investigators (detection bias) was assessed according to the guidelines of the Cochrane Collaboration (14).

Concealment of allocation: A – Adequate (centralized randomization by a central office); B – Unclear (list or table or apparently adequate concealment but no other information in trial); C – Unmet (alternation, days of the week, any allocation that is potentially transparent).

Blinding: A – Adequate (trials in which blinding of investigators assessing outcomes was adequate); B – Unclear (trials in which blinding of investigators was not described adequately); C – Unmet (trials in which blinding of investigators was clearly not performed).

Attrition bias was considered adequate (trials where an intention to treat analysis is possible and drop out rate was less than 20% after 1 year in all groups), unclear (trials where drop out rate was more than 20% after 1 year or large differences in drop out rates between groups were observed), unmet (no reporting on drop out rates was available and intention to treat analysis not possible).

Trials, which unmet allocation concealment, were excluded from the analysis; an overall quality score was attributed considering Low risk of bias (where all criteria were 'Adequate') – Medium risk of bias (where one or more criteria were 'Unclear' and the rest were 'Adequate') – High risk of bias (where one or more criteria were unmet).

Data Extraction and synthesis

Two independent reviewers extracted data from the selected articles, reconciling differences by consensus. All but one articles (25) reported eligible data. The investigators responsible for the included studies were contacted to confirm/clarify results of data extraction.

Outcome data analyzed was quantitative and continuous (symptom scores, medication use). Since the authors of the original studies used a wide variety of scoring systems and scales we calculated the standardized mean difference (SMD). This measure provides the effect size of the intervention in SD units, and its value does not depend on the measurement scale. Heterogeneity was calculated with the χ^2 and I^2 test. All results are reported with 95% confidence intervals (CIs) and all *P* values are two-tailed (15, 16). For the significant inter-study heterogeneity we used the random-effects model (REM). Analysis was performed with the REVMAN 5 program (17).

Results

Search results

The initial search identified 26 randomized studies on SLIT with HDM extracts, of which 12 were potentially relevant (18–30), nine in patients with AR (18–25, 30) and nine (18, 19, 22, 23, 26–30) in patients with AA. Among them, 6 studies for AR (18–23) and seven for AA (18, 19,

22, 23, 26–28) had been included in previous meta-analyses as well; three studies for AR (24–30) and two for AA (29, 30) were not, because published afterwards. One of the new studies in AR (24) was excluded as reporting results with median and quartiles; the trial by Pham-Thi et al. (30) was conducted with a combined design, involving SLIT associated to a drug step down approach. Nevertheless, this study was included because however comparable with the others (see comments in discussion section). Finally eight studies for AR and nine for AA were pooled together (Fig. 1).

Trial characteristics

The trials globally enrolled 382 patients for AR and 476 for AA and could therefore be analyzed. The age range of participants was 5–56 years. Each trial included a median of 44 participants (range, 14–72) for AR and 50 for AA (range, 14–109) (Table 1). Nine studies used an extract in drops and three in tablets. The treatments were administered for a median of 18 months (range, 6–24 months) (Table 2).

Methodological quality of included studies

All of the trials were DBRPC. Informed consent was required by all authors. Each trial reported dropouts and withdrawals and analyzed patients who completed the trial; the dropout rate ranged from 0% to 23%.

According to the Jadad scale, four studies received a score of 5/5 and eight studies received a score of 4/5. The score for inter-rater agreement on methodological quality scores was 0.90 (95% CI 0.80–1.0).

The overall assessment for risk of bias, coming from the analysis of allocation concealment, attrition and detection bias, resulted in medium level (Table 1).

Primary outcomes results

Nasal symptoms scores. For this analysis eight trials were considered including 382 patients (194 SLIT and 188 placebo). SLIT induced a significant reduction in nasal symptoms scores compared with placebo (SMD -0.95 ; CI 95% -1.77 to -0.14 $P = 0.02$). A significant inter-study heterogeneity was found ($I^2 = 92.0\%$) (Fig. 2A).

Bronchial symptoms scores. Nine trials were included in this analysis, involving 476 patients (243 SLIT and 233 placebo). The results showed a significant reduction in symptom scores of AA (SMD -0.95 ; CI 95% -1.74 to -0.15 $P = 0.02$). A relevant heterogeneity was detected ($I^2 = 93.0\%$) (Fig. 3A).

Drug intake. Four trials for AR reported valid data on medication requirements, including 175 patients (89 SLIT and 86 placebo). The results showed a significant reduction of rescue drug use (SMD -1.88 ; CI 95% -3.65 to -0.12 $P = 0.04$). A relevant heterogeneity was detected ($I^2 = 95\%$) (Fig. 2B).

The same outcome could be evaluated for AA in seven trials, with 397 patients (202 receiving SLIT and 195 placebo). A significant reduction of drug use was found (SMD -1.48 ; CI 95% -2.70 to -0.26 $P = 0.02$). A relevant heterogeneity was detected ($I^2 = 96\%$) (Fig. 3B).

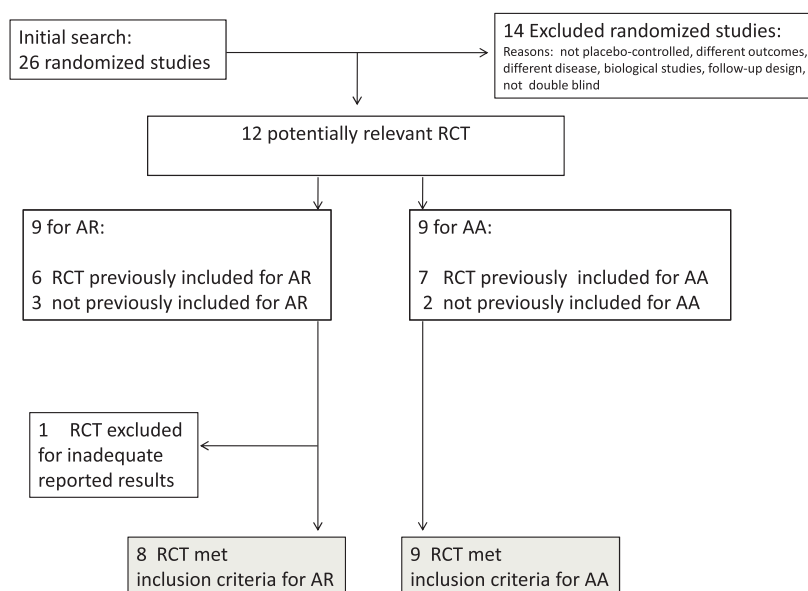


Figure 1. Retrieved articles from search strategy.

Table 1. Characteristics of studies and subjects

Source	Study design						Subjects					
	Study design	Concealment of allocation	Blinding	Quality score*	Informed consent	Overall quality assessment (Risk of bias)	Diagnosis	No.†	SLIT	Placebo	Age range (years)	Dropout rate (%)
Tari (18)	RCTDB	B	B	4/5	Yes	Medium	R, A	58	30	28	5–12	12
Hirsch (19)	RCTDB	A	A	5/5	Yes	Low	R, A	21	11	10	6–12	5
Passalacqua (20)	RCTDB	B	B	5/5	Yes	Medium	R	19	10	9	15–46	5
Guez (21)	RCTDB	B	B	4/5	Yes	Medium	R	72	36	36	17–42	21.6
Bahceciler (22)	RCTDB	B	B	4/5	Yes	Medium	R, A	14	7	7	7–18	0
Ippoliti (23)	RCTDB	B	B	4/5	Yes	Medium	R, A	33	18	15	5–12	0
Passalacqua (25)	RCTDB	B	B	5/5	Yes	Medium	R	56	28	28	14–56	18
Bousquet (26)	RCTDB	B	B	4/5	Yes	Medium	A	50	23	27	15–37	23
Pajno (27)	RCTDB	A	B	5/5	Yes	Medium	A	21	12	9	8–15	13
Niu (28)	RCTDB	B	B	4/5	Yes	Medium	A	97	49	48	6–12	12
Pham-Thi (30)	RCTDB	B	B	4/5	Yes	Medium	R, A	109	54	55	5–11	17
Lue (29)	RCTDB	B	B	4/5	Yes	Medium	A	20	10	10	6–12	0

A, asthma; R, rhinitis; RCTDB, randomized clinical trial, double-blind; SLIT, sublingual immunotherapy.

*Jadad scale.

†After dropout. Available data for analysis.

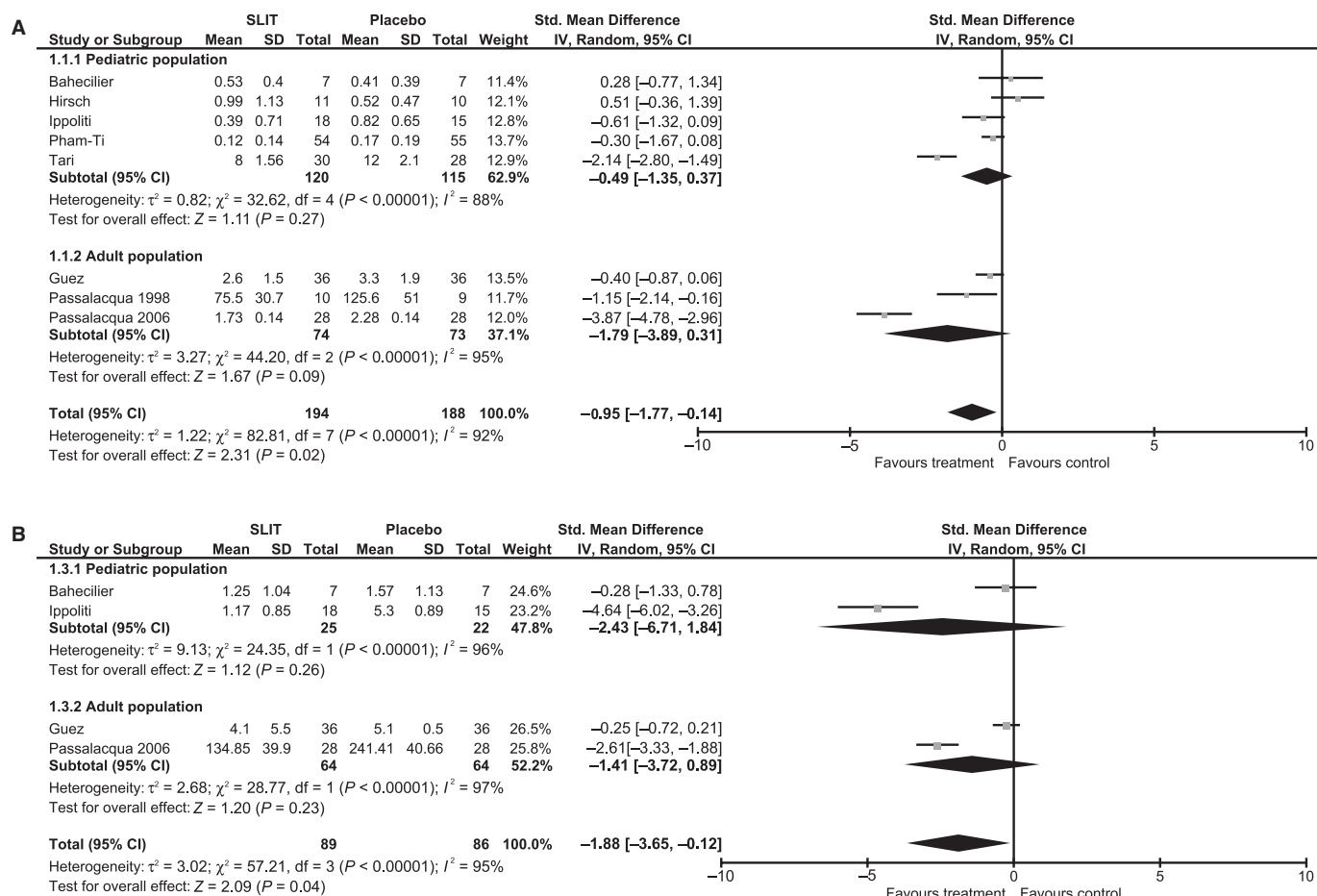


Figure 2. Efficacy of HDM SLIT in AR. Effect on total symptoms score (A) and medication requirement (B).

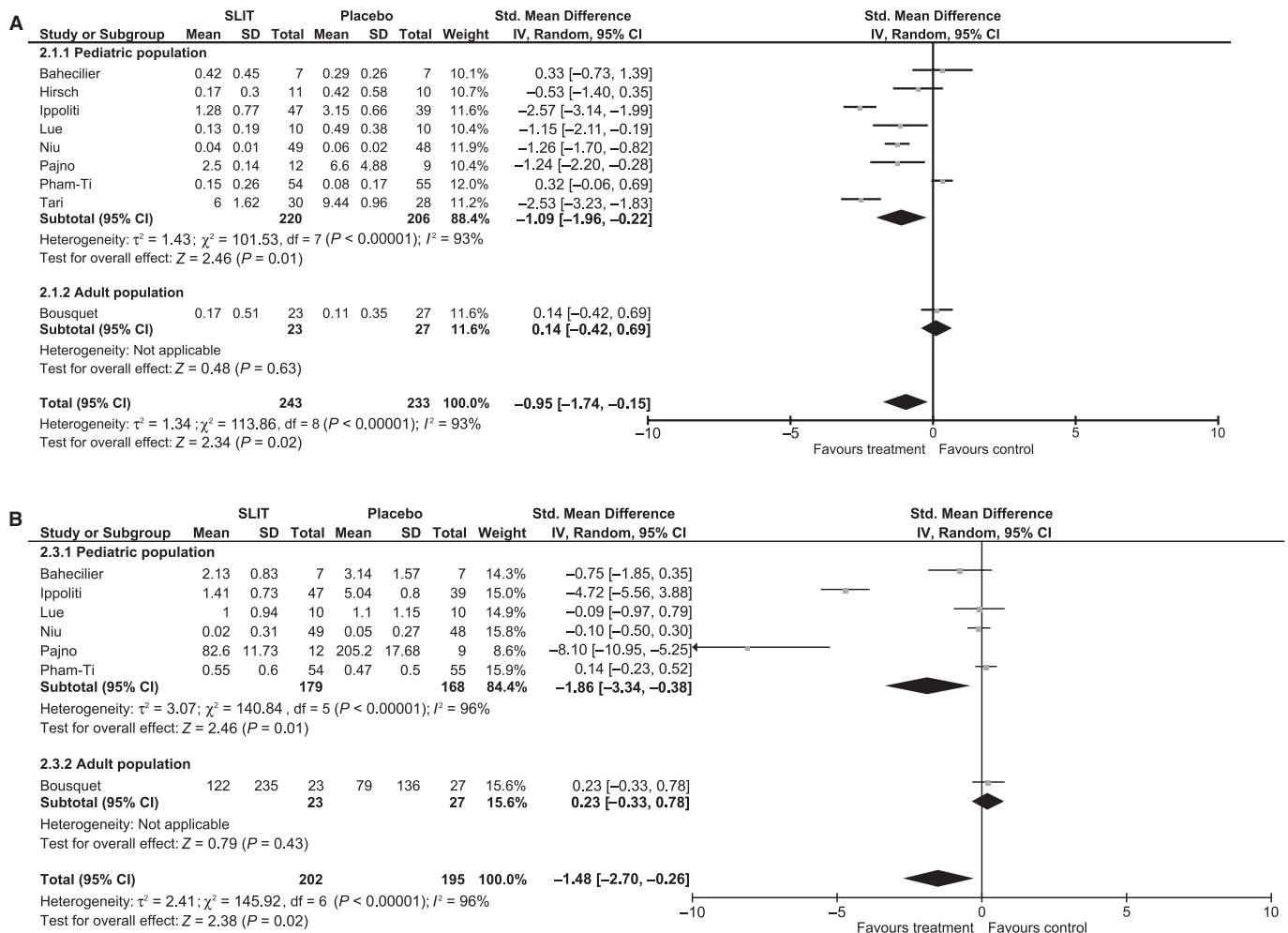


Figure 3. Efficacy of HDM SLIT in AA. Effect on total symptoms score (A) and medication requirement (B).

Secondary outcomes. The sub-analysis for efficacy in pediatric population, through the selection of included trials, failed to identify a significant difference from placebo in symptom scores of AR (SMD -0.52 ; IC 95% -1.77 to 0.72 $P = 0.41$) in 66 patients (Fig. 2A) and in reduction of medication use (SMD -2.43 ; IC 95% -6.71 to 1.84 $P = 0.26$) in 25 patients (Fig. 2B). A significant reduction was found for symptom scores of AA (SMD -1.09 ; IC 95% -1.96 to -0.22 $P = 0.01$) in 220 children (Fig. 3A) and medication use (SMD -1.86 ; IC 95% -3.34 to -0.38 $P = 0.01$) in 179 children (Fig. 3B).

No significant difference was found in the sub-analysis for adult patients when the symptoms of AR and medication use were considered (SMD -1.79 ; IC 95% -3.89 to 0.31 $P = 0.09$; SMD -1.41 ; IC 95% -3.72 to 0.89 $P = 0.23$) (Fig. 2A,B). Only one study (26) dealt with AA symptoms and medications in adults, with no significant results (Fig. 3A,B).

Sensitivity analysis. By using Funnel Plots we detected an apparent asymmetry for the considered outcomes, suggesting a reasonable source of bias or systematic

heterogeneity between smaller and larger studies. Neither these plots nor statistical tests could be performed because unreliable with less than 10 studies (31).

Post hoc sensitivity analyses using the fixed-effects model not substantially changed the overall significance for AR symptoms (SMD -0.74 ; IC 95% -0.96 to -0.52 $P < 0.00001$) and medication scores (SMD -1.11 ; IC 95% -1.46 to -0.75 $P < 0.00001$), for AA symptoms (SMD -0.79 ; IC 95% -0.99 to -0.59 $P < 0.00001$) and medication scores (SMD -0.64 ; IC 95% -0.91 to -0.36 $P < 0.00001$). When a sensitivity analysis in which the smallest studies were excluded, we did not find significant changes for all the primary outcomes of AR (SMD -0.77 ; IC 95% -1.00 to -0.54 $P = 0.03$ and SMD -2.43 ; IC 95% -4.73 to -0.12 $P = 0.04$ respectively) and of AA (SMD -1.09 ; IC 95% -1.94 to -0.25 $P = 0.01$ and SMD -1.62 ; IC 95% -2.99 to -0.20 $P = 0.02$, respectively). Analogous results when excluding outlying trials for AR symptoms (SMD -0.73 ; IC 95% -1.35 to -0.12 $P = 0.02$) and medication scores (SMD -1.88 ; IC 95% -3.65 to -0.12 $P = 0.04$), and for AA symptoms (SMD -0.87 ; -1.66 to -0.08 $P = 0.03$) and medication scores (SMD -1.90 ;

Table 2. Characteristics of study treatments

Source	Allergen	Vehicle	Cumulative dose	Duration	Manufacturer
Tari (18)	Mites	AE and phenol	365 STU	18 months	ALK
Hirsch (19)	Mites	Glycerol	570 mcg Der p1	1 year	AJG
Passalacqua (20)	Monoid mites	Tablets	10.000 AU	2 years	LOF
Guez (21)	Mites	Glycerol	90 000 IR, 2.2 mg Der p 1	2 years	STA
Bahcecilier (22)	Mites	Glycerol	7.000 IR, 0.56 mg Der p1	6 months	STA
Ippoliti (23)	Mites	Glycerol	57 mcg Der p1	6 months	ALK
Passalacqua (25)	Mites	Tablets	Allergoid	2 years	LOF
Bousquet (26)	Mites	Glycerol	104 000 IR, 4.2 mg Der p 1	2 years	STA
Pajno (27)	Mites	Glycerol	360 mcg Der p1	2 years	ALK
Niu (28)	Mites	Glycerol	Cumulat 1.7 mg Der p and 3 mg Der f	6 months	STA
Pham-Thi (30)	Mites	Tablets	155 000 IR, 6.9 mg Der p 1 and 14.7 mg Der f 1.Cumulat. 1.7 mg Der p	18 months	STA
Lue (29)	Mites	Glycerol		6 months	STA

STU, Specific treatment units; IR, Index of reactivity; AU, Allergic units; BU, Biologic units; ALK, Alk-Abellö; AJG, Allergopharma J. Ganzer; LOF, Lofarma; STA Stallergenes.

IC95% -3.53 to 0.28 $P = 0.02$). With the exclusion of Pham-Thi's study (30), no significant changes in effect sizes occurred (SMD -1.06 ; IC95% -2.06 to -0.05 $P = 0.04$ for AR symptoms; SMD -1.13 ; IC95% -1.89 to -0.36 $P = 0.004$ for AA symptoms; SMD -1.90 ; IC95% -3.53 to -0.28 $P = 0.02$ for asthma medications use).

Excluding those studies with drop out rate superior to 20% (21, 26), the reduction was statistically significant for AR symptoms (SMD -1.04 ; IC95% -2.04 to -0.04 ; $P = 0.04$), for rhinitis medication (SMD -2.47 ; IC95% -4.40 to -0.34 ; $P = 0.02$), for asthma symptoms (SMD -1.33 ; IC95% -2.05 to -0.62 ; $P = 0.0002$) and for asthma medication (SMD -2.47 ; IC95% -4.63 to -0.31 ; $P = 0.03$).

Discussion

The results of meta-analyses on SLIT are overall in agreement and suggest that this treatment is effective in relieving symptoms and in reducing the need for medications. Nevertheless, all the existing reviews included studies with different allergen extracts, by assuming that, in the case of immunotherapy, the mechanisms are the same, independent of the allergen. This assumption raised several criticisms and encouraged us to perform the first attempt to make an allergen-by-allergen analysis. In order to reduce the risk of bias, we decided to investigate SLIT with HDM extracts with the intention of ensuring homogeneity among studies, although differences in dosage remain. The available meta-analyses on SLIT showed inconclusive results when sub-analyses were conducted by selecting HDM trials. This is most likely due to the few studies included, since the small number of patients in some studies increases the probability of underestimating the treatment efficacy (type II error). Thus we performed an updated analysis with the recently published studies until March 31, 2008.

By including a larger sample size, a statistically significant evidence of efficacy was achieved for the

explored population, at least for symptoms and medication use. These two outcomes, usually planned as primary endpoints, should anyway be considered together with objective outcomes, mainly for AA, and with the immunological changes that SIT is expected to produce. In this regard, an increase from baseline in HDM-specific IgE levels was observed in active groups in five studies (19, 21, 26, 29, 30), while specific IgG-4 changed in four (19, 26, 29, 30); none of these changes was found in four trials (18, 22, 27, 28). SIT is also expected to involve the inflammatory response: some studies found a decrease in inflammatory cells, eosinophils, adhesion molecules, ECP, CD 40⁺ B-cells, IL 13 and prolactine (20, 23, 29). Changes in skin reactivity were observed only in three studies (22, 29, 30).

For AA, two trials demonstrated improvement to bronchial challenge (18, 26) and two did not (19, 22). Lung function tests were investigated only in five trials: PEF improvement in active group was found by Bousquet and Bahcecilier, associated to a reduction in asthma exacerbation rate; FVC, FEV 1 and PEF improved also in Niu's study; improvement for both active and placebo was detected by Pham-Thi and Lue.

Only one study (18) investigated the effect of SLIT on nasal challenge and inspiratory peak nasal flow, demonstrating less nasal obstruction and reactivity. These results are summarized in Table 3.

In most of the trials, SLIT was associated to different approaches of environmental measures, aimed to keep comparable allergen loads at the same level of baseline for the whole study duration. This aspect should be considered when observing no difference between active and placebo groups exposed to a low allergen load, responsible for low scores at baseline.

SLIT was associated with the administration of rescue medications as well (antihistamines, cromones, nasal steroids for AR; inhaled ICS, beta-2-short acting for AA, with recourse to oral steroids in case of severe symptoms); they were registered in diary cards for calculating their as-needed administration. Pham-Thi's

Table 3. Further outcomes measured in individual trials

Study	Outcomes explored	Findings
Tari (18)	Nasal peak inspiratory flow (PNIF), nasal test-rhinomanometry, bronchial specific and aspecific challenge, serum HDM-IgG and IgE	In active group: increased morning PNIF; improvement in nasal test; increased threshold for aspecific and specific bronchial challenge; not changed HDM-IgE; increase in HDM-IgG after 12–18 months
Hirsch (19)	Nasal allergen challenge-rhinomanometry, bronchial aspecific challenge, serum total and specific IgE and IgG, skin sensitivity	Improvement in nasal reactivity in PL group; no difference in bronchial challenge; increase in total IgE in both active and PL; increase in specific HDM-IgE in active and drop in specific HDM IgG4 in PL group; no difference in skin sensitivity
Passalacqua (20)	Inflammatory cells and ICAM1 expression after ocular challenge, serum ECP	Decrease in ocular inflammatory cells and ICAM 1, decrease in serum ECP
Guez (21)	Serum HDM IgE and IgG4	Increase in HDM specific IgE in active, no changes in IgG4 for active and PL
Bahcecilier (22)	Skin reactivity, serum total IgE, lung function test, bronchial aspecific challenge	In active group reduced skin reactivity end point, PEF improvement and reduced asthma exacerbation, no changes in bronchial reactivity, no changes in IgE level
Ippoliti (23)	Serum CD 40 + B-cells, ECP, IL 13, prolactine, ACTH	Decrease in IL13, ECP, PRL in active, no changes in ACTH and CD 40
Passalacqua (25)	QoL, personal satisfaction	No changes in SF-36 questionnaire (normal level at baseline)
Bousquet (26)	PEF, bronchial challenge, QoL assessment, specific IgE and IgG4	Improvement in respiratory function in active; improvement in bronchial reactivity in active group; increase in specific IgE and IgG4 in active; QoL more improved in active
Pajno (27)	specific IgE, IgG4	No changes in immunological parameters for active and PL
Niu (29)	PEF, lung function tests, skin test, serum total and specific IgE	Improved FVC, FEV 1 and PEF in active group. No changes in skin test and IgE levels
Pham-Thi (30)	Number of asthma free days, lung function, QoL, skin test, specific IgE and IgG4	Improved lung function in both groups; improved skin reactivity and increased specific IgE and IgG4 in active group, improved QoL in active
Lue (29)	PEF, skin test, lung function, total serum IgE, ECP, eosinophil count, specific IgE and IgG4	Skin reactivity reduced in both groups, FEV 1 improved in both groups. Increased total IgE and specific IgG4 in active group. Reduced eosinophil-count in active only

study planned to associate SLIT with a step-down-drug approach (based on the asthma symptom severity and lung function) to achieve a minimum dose of inhaled corticosteroid; establishing whether immunotherapy might be a useful add-on treatment was the target. So in this circumstance only patients receiving treatment with an inhaled corticosteroid (>200 and <1000 $\mu\text{g/day}$ /equivalent budesonide) daily and continuously for at least 6 months during the previous 12 months were admitted. Despite this conduction, we decided to include this trial in the pooled analysis for two reasons. We considered more relevant its contribution to increase the explored popu-

lation in respect to the difference between the reduction of a mandatory inhaled-steroids-treatment and the reduction of a on-demand inhaled-steroids-treatment (whose cumulative dose is not known as well, because in other trials patients' selection was not based on asthma severity). Therefore, we preferred a more conservative approach by including Pham-Thi's negative findings on AA, although this trial resulted underpowered and impaired by low baseline scores for symptoms and medications.

The results of Tonnel's study showed efficacy of active treatment vs placebo on rhinitis score and skin reactivity,

but we did not include them because reported in median and quartiles, so weak and not suitable for pooled analysis.

Results remained inconclusive when a sub-analysis was conducted on the selected population of children and adults; a significant reduction was found only for symptoms of pediatric AA. This apparent paradox seems to be related to the small sample sizes. The effect size of a meta-analysis represents a conservative measure, weighed on individual study features, that does not reach statistical significance until a sufficient sample size and grade of confidence are reached. Thus, for those sub-analyses, the previous parameters were probably not sufficiently robust but when considering together the adult and pediatric population we could have more reasonable reliability. Despite it was positive, the effect size of drug intake for AR should be considered with caution as based on a very small population.

As meta-analyses allow to assign an high grade of evidence to a certain phenomenon, these findings highlight the importance of investigating a large population. This aspect is desirable in order to focus the evidences on selected subgroups of patients, mainly for children, that, in the prospective of modifying the natural history of allergic disease, should represent the best target population for immunotherapy.

On the other hand, although one of the potential sources of bias has been reduced by making uniform the kind of allergen extract, the inter-study heterogeneity remains high in all the performed analysis. This impairment represents a weak point together with the small number of included studies; in these conditions the risk for the small studies with extreme outcome to influence the overall results is very high. This is the reason why we attempted a sensitivity analysis with the exclusion of the smallest and outlying studies but confirming the consistency of the results, even when a fixed effect model was adopted. In order to control inter-study heterogeneity, we adopted the SMD, used in meta-analysis for combining in a uniform scale the continuous data, and REM, a conservative approach that elaborates data considering a large grade of variability.

The possible sources of bias of the present evaluation should potentially be due to selection biases (delayed publication bias, location biases, selective outcome reporting), poor methodological quality leading to spuriously inflated effects in smaller studies, poor methodological design, inadequate analysis, true heterogeneity, statistical art-factual (31).

The studies we compared showed an high Jadad score and responded to the Consort recommendation for methodological quality and analysis, with a medium risk of bias in the overall assessment, with no relevant differences in the intensity of interventions or differences in underlying risk between studies of different sizes. Nevertheless owing to the fact that most studies were performed many years ago, unclear results particularly for blinding and allocation procedures could be detected.

Only two studies had a percentage of patients lost at follow-up barely superior to 20%; the same percentage referred to the global population was 12.43% for AR and 11.01% for AA.

Thus we can assume that the modest level of methodological quality, together with the publication bias, the high inter-study heterogeneity, the different doses administered (sometimes not reaching the recommended amount) and the small sample size, represent the main influencing factors in this evaluation.

In this regard, publication bias is an important drawback of systematic reviews, difficult to avoid. Although, we searched for articles in the most important electronic databases available, it is possible that not all the studies have been included. Therefore, some of the observed variability in treatment effects may be explained by variable responses to treatment, according to the type of extract used, the age of studied subjects or the dose and duration of treatment.

The only satisfactory way to address reporting bias and the inadequate quality of individual trials is through prospective registration of trials (32) and improvements in the quality of conducting, analyzing and reporting of the studies (33).

This aspect should be taken into account when resuming the take-home messages in order to avoid an overestimate of intervention effect. For all these reasons, the results of this analysis should be interpreted with great caution. These considerations should prompt to conduct further clinical trials administering HDM SLIT in different selected subgroups of population, following specific recommendations addressed to uniform outcomes, endpoints, time-points, scales of measurement and dosages but also to optimize strategies in patients' selection, treatment allocation and power calculation. A first step in this perspective has been made by World Allergy Organization (WAO), promoter of the first recommendation in this matter, but additional guidelines to perform clinical trials are needed (34). Anyway, one of the roles of the EBM is to explore the existing evidences and identify the 'grey areas' where the research should further expand.

Although the mentioned limitations and the contrasting results, on immunological and functional evaluations, represent an important issue, we here provide encouraging results in the research of the clinical evidence for efficacy of SLIT in HDM allergy. The final conclusion will come from more homogeneous and well designed large clinical trials, exploration of influence statistics, and investigation of objective and consistent outcomes.

Acknowledgments

This study has been partially supported by GA²LEN (Global Allergy and Asthma European Network) and ARMIA (Associazione Ricerca Malattie Immunologiche e Allergiche).

References

1. Shekelle PG, Woolf SH, Eccles M, Grimshaw J. Clinical guidelines: developing guidelines. *BMJ* 1999; **318**:593–596.
2. Jørgensen AW, Hilden J, Gotzsche PC. Cochrane reviews compared with industry supported meta-analyses and other meta-analyses of the same drugs: systematic review. *BMJ* 2006; **333**:782. Epub 2006 Oct 6. Review.
3. Bousquet J, Khaltaev N, Cruz AA, Denburg J, Fokkens WJ, Togias A 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**:8–160 (Review).
4. Penagos M, Compalati E, Tarantini F, Baena-Cagnani R, Huerta J, Passalacqua G et al. Efficacy of sublingual immunotherapy in the treatment of allergic rhinitis in pediatric patients 3 to 18 years of age: a meta-analysis of randomized, placebo-controlled, double-blind trials. *Ann Allergy Asthma Immunol* 2006; **97**:141–148.
5. Penagos M, Passalacqua G, Compalati E, Baena-Cagnani CE, Orozco S, Pedroza A et al. Meta-analysis of the efficacy of sublingual immunotherapy in allergic asthma in pediatric patients, 3 to 18 years of age. *Chest* 2008; **133**:599–609.
6. Wilson DR, Lima MT, Durham SR. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. *Allergy* 2005; **60**:4–12.
7. Calamita Z, Saconato H, Pela AB, Atallah AN. Efficacy of sublingual immunotherapy in asthma: systematic review of randomized-clinical trials using the Cochrane collaboration method. *Allergy* 2006; **61**:1162–1172.
8. Olaguibel JM, Alvarez Puebla MJ. Efficacy of sublingual allergen vaccination for respiratory allergy in children. Conclusions from one meta-analysis. *J Investig Allergol Clin Immunol* 2005; **15**:9–16.
9. Dawson B. Research questions about one group. In: Dawson B, editors. *Basic & clinical biostatistics*, 4th edn. New York, NY: McGraw-Hill Medical, 2004:118.
10. Alderson P, Green S Cochrane Collaboration open learning material for reviewers. Version 1.1, 2002. Available at: <http://www.cochrane.org>. Accessed September 10, 2005.
11. Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials* 1996; **17**:1–12.
12. Moher D, Cook DJ, Jadad AR, Tugwell P, Moher M, Jones A et al. Assessing the quality of randomized controlled trials: implications for the conduct of meta-analyses. *Health Technol Assess* 1999; **3**:12.
13. Moher D, Jadad A, Nichol G, Penman M, Tugwell T, Walsh S. Assessing the quality of randomized controlled trials: an annotated bibliography of scales and checklists. *Control Clin Trials* 1995; **16**:62–73.
14. Alderson P, Green S Cochrane Collaboration open learning material for reviewers. Version 1.1, 2002. Available at: <http://www.cochrane.org>. Accessed September 10, 2005.
15. Deeks JJ, Altman D, Bradburn MJ. Statistical methods for examining heterogeneity and combining results from several studies in meta-analysis. In: Egger M, Davey Smith G, Altman DG, editors. *Systematic reviews in health care*. London, UK: BMJ Publishing, 2001:285–312.
16. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327**:557–560.
17. RevMan 4.2.7 program [computer program]. Oxford, England: Cochrane Collaboration, 2004.
18. Tari MG, Mancino M, Monti G. Efficacy of sublingual immunotherapy in patients with rhinitis and asthma due to house dust mite. A double blind study. *Allergol Immunopathol* 1990; **18**:277–284.
19. Hirsch T, Sahn M, Leupold W. Double blind placebo controlled study of sublingual immunotherapy with house dust mite extracts in children. *Pediatr Allergy Immunol* 1997; **8**:21–27.
20. Passalacqua G, Albano M, Fregonese L, Riccio A, Pronzato C, Mela GS et al. Randomised controlled trial of local allergoid immunotherapy on allergic inflammation in mite induced rhinoconjunctivitis. *Lancet* 1998; **351**:629–632.
21. Guez S, Vatrinet C, Fadel R, Andre' C. House dust mite sublingual swallow immunotherapy in perennial rhinitis: a double blind placebo controlled study. *Allergy* 2000; **55**:369–375.
22. Bahcecilier NN, Isik U, Barlan IB, Basaran N. Efficacy of sublingual immunotherapy in children with asthma and rhinitis: a double-blind, placebo-controlled study. *Pediatr Pulmonol* 2001; **32**:49–55.
23. Ippoliti F, De Sanctis W, Volterrani A, Lenti L, Canitano N, Lucarelli S et al. Immunomodulation during sublingual therapy in allergic children. *Pediatr Allergy Immunol* 2003; **14**:216–221.
24. Tonnel AB, Scherpereel A, Douay B, Mellin B, Leprince D, Goldstein N et al. Allergic rhinitis due to house dust mites: evaluation of the efficacy of specific sublingual immunotherapy. *Allergy* 2004; **59**:491–497.
25. Passalacqua G, Pasquali M, Ariano R, Lombardi C, Baiardini I, Majani G et al. Randomized double-blind controlled study with sublingual carbamylated allergoid immunotherapy in mild rhinitis due to mites. *Allergy* 2006; **61**:849–854.
26. Bousquet J, Scheinmann P, Guinnee MT, Perrin-Fayolle M, Sauvaget J, Tonnel AB et al. Sublingual swallow immunotherapy (SLIT) in patients with asthma due to house dust mites: a double blind placebo controlled study. *Allergy* 1999; **54**:249–260.
27. Pajno GB, Morabito L, Barberio G, Parmiani S. Clinical and immunological effects of longterm sublingual immunotherapy in asthmatic children sensitized to mite: a double blind study. *Allergy* 2000; **55**:842–849.
28. Niu CK, Chen WY, Huang JL, Lue KH, Wang JY. Efficacy of sublingual immunotherapy with high-dose mite extracts in asthma: a multi-center, double-blind, randomized, and placebo-controlled study in Taiwan. *Respir Med* 2006; **100**:1374–1383.
29. Lue KH, Lin YH, Sun HL, Lu KH, Hsieh JC, Chou MC. Clinical and immunologic effects of sublingual immunotherapy in asthmatic children sensitized to mites: a double-blind, randomized, placebo-controlled study. *Pediatr Allergy Immunol* 2006; **17**:408–415.
30. Pham-Thi N, Scheinmann P, Fadel R, Combebias A, Andre C. Assessment of sublingual immunotherapy efficacy in children with house dust mite-induced allergic asthma optimally controlled by pharmacologic treatment and mite-avoidance measures. *Pediatr Allergy Immunol* 2007; **18**:47–57.

31. Alderson P, Green S, Higgins JPT. Cochrane Reviewers' Handbook 4.2.2 [updated December 2003]. In: Alderson P, Green S, Higgins JPT, editors The Cochrane Library, Issue 1, 2004. Chichester, UK: John Wiley & Sons, Ltd.
32. Dickersin K, Scherer R, Lefebvre C. Systematic Reviews: Identifying relevant studies for systematic reviews. *BMJ* 1994;**309**:1286–1291.
33. Moher D, Horsley T, Morgan K, Tricco A, Tsertsvadze A. Blinded versus unblinded assessments of risk-of-bias in studies included in a systematic review (Protocol). *Cochrane Database Syst Rev* 2007, Issue 4, Art. No.: MR000025. DOI: 10.1002/14651858.MR000025.
34. Canonica GW, Baena-Cagnani CE, Bousquet J, Bousquet PJ, Lockey RF, Malling HJ et al. Recommendations for standardization of clinical trials with Allergen Specific Immunotherapy for respiratory allergy. A statement of a World Allergy Organization (WAO) taskforce. *Allergy* 2007;**62**: 317–324.