REVIEW

Efficacy of 5-Grass Pollen Liquid Sublingual Allergen Immunotherapy for Seasonal Allergic Rhinoconjunctivitis: A Systematic Review and Meta-analysis

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Abstract

The efficacy and safety of allergen immunotherapy (AIT) have been demonstrated in randomized controlled trials (RCTs). However, differences in study protocols, populations, and AIT products lead to variability in outcomes. The World Allergy Organization and the European Academy of Allergy and Clinical Immunology advise assessing individual AIT products rather than assuming a universal class effect. We conducted a meta-analysis on the efficacy and safety of 5–grass pollen liquid sublingual immunotherapy (SLIT) (5-grass SLIT-liquid) in patients affected by allergic rhinoconjunctivitis (ARC) with and without asthma. We searched computerized databases (MEDLINE, ISI Web of Science, LILACS, the Cochrane Library, ClinicalTrial.gov) up to June 2023, supplemented our approach with manual literature searches, and included RCTs comparing 5-grass SLIT-liquid to placebo, irrespective of primary endpoints or treatment duration. Efficacy was assessed based on standardized mean differences (SMDs) in symptom score (SS) and medication score (MS). Subgroup analyses included age and sensitization status, while meta-regression was applied to evaluate asthma comorbidity, dose, and treatment duration. Bias and certainty of evidence were assessed using the Cochrane Risk of Bias 2 tool and the Grading of Recommendations Assessment, Development and Evaluation approach. Data from 8 RCTs for SS (621 patients) and 6 RCTs for MS (507 patients) showed a significant benefit for SLIT over placebo in SS (SMD, –0.34; 95%CI, –0.62 to –0.06; $P<.05$) and MS (SMD, –0.54; 95%CI, –0.97 to –0.10; $P<.05$). Subgroup analyses showed no differences based on age or sensitization status. Meta-regression revealed no significant impact of cumulative dose, treatment duration, or asthma on efficacy. No safety issues were observed.
This meta-analysis confirms that 5-grass SLIT-liquid offers significant clinical benefits and is safe, providing an effective option for treating the cause of ARC in patients with and without asthma.

Key words: Grasses. Meta-analysis. Pollen. Randomized controlled trials. Allergic rhinitis. Seasonal. Sublingual immunotherapy. Systematic review.

Resumen

La eficacia y seguridad de la inmunoterapia con alérgenos (AIT) han sido demostradas en ensayos clínicos aleatorizados (ECA). Sin embargo, las diferencias en los protocolos de estudio, poblaciones y productos de AIT generan variabilidad en los resultados. La Organización Mundial de Alergia (WAO) y la Academia Europea de Alergia e Inmunología Clínica (EAACI) recomiendan evaluar los productos individuales de AIT en lugar de asumir un efecto de clase universal. Realizamos un metaanálisis sobre la eficacia y seguridad de la inmunoterapia sublingual (ITSL) en solución con una mezcla de cinco pólenes de gramíneas en pacientes con rinoconjuntivitis alérgica (RCA), con o sin asma. Se realizaron búsquedas en bases de datos electrónicas (MEDLINE, ISI Web of Science, LILACS, The Cochrane Library y ClinicalTrials.gov) hasta junio de 2023, complementadas con búsquedas manuales en la literatura. Se incluyeron ECA que compararan la ITSL de cinco pólenes de gramíneas con placebo, independientemente del criterio de valoración principal o la duración del tratamiento. Para evaluar la eficacia, se emplearon diferencias de medias estandarizadas (DME) en la puntuación de síntomas (PS) y la puntuación de medicación (PM). Los análisis de subgrupos consideraron la edad y el estado de sensibilización, mientras que la metarregresión evaluó la comorbilidad asmática, la dosis y la duración del tratamiento. El riesgo de sesgo y la certeza de la evidencia se evaluaron mediante la herramienta Cochrane de riesgo de sesgo 2 y el sistema GRADE. Se analizaron datos de 8 ECA para la PS (621 pacientes) y 6 ECA la para PM (507 pacientes), evidenciando un beneficio significativo de la ITSL sobre el placebo en la PS (DME: -0,34; IC95%: -0,62 a -0,06; $p<0,05$) y en la PM (DME: -0,54; IC95%: -0,97 a -0,10; $p<0,05$). Los análisis de subgrupos no mostraron diferencias según la edad o el estado de sensibilización. La metarregresión no evidenció un impacto significativo de la dosis acumulada, la duración del tratamiento o la presencia de asma sobre la eficacia. No se observaron problemas de seguridad.

Este metaanálisis confirma que la ITSL en solución con una mezcla de cinco pólenes de gramíneas ofrece beneficios clínicos significativos y es segura, constituyendo una opción de tratamiento causal eficaz para pacientes con RCA, con o sin asma.

Palabras clave: Gramíneas. Metaanálisis. Pólenes. Ensayos clínicos aleatorizados. Rinitis alérgica. Estacional. Inmunoterapia sublingual. Revisión sistemática.

Introduction

Seasonal allergic rhinoconjunctivitis (ARC) is one of the most widespread allergic conditions in developed regions. Its symptoms wield a significant impact on daily life, disrupting sleep patterns, hindering academic and professional performance, and curtailing social engagements [1].

Allergen immunotherapy (AIT) is a well-established treatment for allergic diseases that is uniquely capable of altering disease course by directly addressing the underlying immunological mechanisms.

In clinical practice, treatment is administered mainly via the subcutaneous and sublingual routes, albeit with notable global disparities. While subcutaneous immunotherapy (SCIT) has historically been the mainstay for management of ARC, a recent trend towards sublingual immunotherapy (SLIT), notably in Europe, has been observed. In this region, SLIT is now prescribed almost as frequently as SCIT and is favored over SCIT in southern Europe, representing approximately 80% of immunotherapy treatments [1].

Most randomized controlled trials (RCTs) have demonstrated the efficacy of AIT in reducing symptoms and medication usage among allergic patients [2-6]. Meta-analyses have confirmed the overall efficacy of AIT but have also identified significant variability among individual study results [2-7]. This variability could stem from differences in the populations studied, in trial protocols and duration, and in the efficacy of specific AIT products. Such discrepancies may impact the overall conclusions drawn from meta-analyses. Consequently, the World Allergy Organization and the European Academy of Allergy and Clinical Immunology advocate for meta-analyses tailored to specific AIT products owing to their pronounced heterogeneity [8].

This article presents findings from a targeted comparison of the efficacy of SLIT with that of placebo for ARC with and without asthma. To address the heterogeneity observed in prior meta-analyses, our analysis focuses on RCTs with a singular commercially available liquid formulation designed for grass pollen allergy and containing a mixed allergen extract from 5 grass pollens.

Methods

Search Strategy and Selection Criteria

We undertook and reported this systematic review and meta-analysis in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses [9-10]; Grading of Recommendations, Assessment, Development and Evaluation (GRADE) [11-12]; and Cochrane guidelines [13].

This study is registered at the International Prospective Register of Systematic Reviews (number CRD42023464731). No testing was performed on humans. The meta-analysis is based on published data from clinical trials with their respective ethics evaluation and approvals.

From inception to July 30, 2023, we searched PubMed/MEDLINE, the Cochrane Library, the ISI Web of Science, and the ClinicalTrial.gov databases for published and unpublished RCTs assessing the efficacy of a 5–grass pollen liquid SLIT formulation (5-grass SLIT-liquid) in patients with ARC.

A full list of the search terms is available in the protocol and the appendix (Table S1 in Supplementary Materials). Studies were included in the meta-analysis if they (*1*) included patients with ARC to grass with or without mild-to-moderate asthma, (*2*) included patients who were prescribed 5-grass SLIT-liquid (Staloral, Stallergenes Greer) for ARC, and (*3*) assessed the relevant outcome measures of the treatment effect, regardless of whether these were the primary endpoints, and with any treatment duration. Studies were excluded if they did not report the required information.

We did not apply language restrictions. We checked all reference lists and articles citing included studies and recent reviews or meta-analyses for any additional relevant studies. We also asked the study sponsor to help provide a complete list of RCTs on 5-grass SLIT-liquid for ARC in order to obtain additional data.

Data Collection

We screened titles and abstracts, reviewed full texts, extracted data, and assessed risk of bias and study quality independently in duplicate (DDB, GP) using a standardized prepiloted form (https://www.rayyan.ai). We resolved disagreements by consensus adjudication. We collected study characteristics, setting, eligibility criteria, population studied, intervention, and outcomes.

Outcomes

Consistent with the established approach for AIT, we prioritized patient-important outcomes in ARC as informative of treatment efficacy and safety [14].

We finally chose the following critical/important outcomes: symptom severity assessed as the symptom score (SS) or visual analog score (VAS); decrease in symptomatic drug use assessed as the medication score (MS) or VAS; and adverse events (AEs).

Data Analysis

We pooled summary measures using both random-effects and fixed-effect models [15]. However, we presented data

from the random-effects model, since the variations in study protocols, duration, and populations suggest that the true effect size differs across studies. We combined continuous outcomes across studies (SS, MS, VAS) using the standardized mean difference (SMD), as the outcomes were measured with different scales.

For studies evaluating the effect over several pollen seasons, we included the last year of observation under treatment in the meta-analysis. Some studies did not report standard deviations (SDs). For these, we estimated the SDs using methods based on summary statistics (minimum, maximum, lower quartile, upper quartile, median, *P* values) [16]. When the standard error (SE) was reported, the SD was obtained using the following formula: $SD=SE\sqrt{n}$. For studies not reporting means and SE, these were obtained from the graphs or provided by the study sponsor.

We assessed the risk of bias (RoB) of RCTs using version 2 of the Cochrane Risk of Bias tool for randomized trials (RoB 2) [17]. The domains included in RoB 2 cover all types of bias that are currently understood to affect the results of RCTs, as follows: (*1*) bias arising from the randomization process; (*2*) bias due to deviations from intended interventions; (*3*) bias due to missing outcome data; (*4*) bias in measurement of the outcome; and (*5*) bias in selection of the reported result.

The judgement can indicate 'Low' or 'High' risk of bias or express 'Some concerns". A study is judged to be at low risk of bias if it is at low risk of bias in all domains for this result. A study is judged to be at high risk of bias if it is at high risk of bias in at least 1 domain or has some concerns in multiple domains in a way that substantially lowers confidence in the result.

We evaluated the certainty (quality) of evidence using the GRADE approach [11]. GRADE defines evidence as follows: high certainty, when confidence that the true effect lies close to that of the effect estimate is very high; moderate certainty, when confidence in the effect estimate is moderate (ie, the true effect is likely to be close to the estimate, although there is a possibility that it is substantially different); low certainty, when the confidence in the effect estimate is limited (ie, the true effect might be substantially different from the effect estimate); and very low certainty, when confidence in the effect estimate is very low (ie, the true effect is likely to be substantially different from the effect estimate).

We tested between-study heterogeneity using the χ^2 test (threshold P=.10) and quantified it using the I^2 statistic, which describes the percentage of variability due to heterogeneity rather than sampling errors [18]. The sources of heterogeneity were explored by removing possible study outliers and conducting prespecified subgroup and sensitivity analyses. Outliers were determined using the Baujat plot, which illustrates each study's contribution to the overall Q-test statistic for heterogeneity on the horizontal axis against the study's influence on the vertical axis, defined as the standardized squared difference between the overall estimate with and without the respective study included in the model [19]. The selection of characteristics defining subgroups/explanatory variables was motivated by clinical and methodologic hypotheses. Meta-regressions were also used to explore heterogeneity and predict the size of the outcome variable according to the values of one or more continuous explanatory variables. Sensitivity analyses to test the robustness of the findings included a fixed-effect meta-analysis and subgroups defined by (*1*) estimated vs available data, (*2*) study sample size (above the median of the mean study sample size), (*3*) trial quality, and (*4*) exclusion of duplicate samples.

Then, we excluded each study in turn to ensure that no single study would be solely responsible for the significance of any result (robust analysis). We assessed publication bias using funnel plots, the Egger linear regression test [20], and fail-safe calculation, a simple procedure by which one can estimate whether publication bias (if it exists) may be safely ignored. A fail-safe number indicates the number of insignificant, unpublished (or missing) studies that would need to be added to a meta-analysis to reduce an overall statistically significant result to insignificance. If this number is large relative to the number of studies observed, one can feel confident in the summary conclusions [13].

We used GRADEpro GDT (available from gradepro.org) to create the summary of findings tables [21]. We performed all the meta-analyses and statistical analyses using R (R Foundation) with the metafor statistical package (accessed January 2024) [22] and the RevMan 5.0 [23] and ProMeta 3.0 software applications [24].

Results

Our bibliographic searches yielded 187 records. After initial screening, we reviewed 98 studies and included 9 RCTs (Figure 1). Data on the SS were available in 8 RCTs (621 patients) [25-32]. Data on the MS were available in 6 RCTs (507 patients) [26-31]. The study by Sieber et al [33], which presents safety data from the ECRIT study published by Ott et al [27], did not provide useful data for the meta-analysis and only contributed to safety.

The characteristics of the studies are summarized in the Table. All the studies were conducted in Europe. The study completion rate ranged from 70% [28] to 100% [25,26,30]. There was only 1 multicenter study [27]. The risk of bias was estimated as high in 1 RCT, moderate in 3 RCTs (some concerns), and low in the remaining 4 RCTs (Figures 2D, 4D).

The sample size of the studies included in the meta-analysis varied greatly, ranging from 30 patients [29] to 183 patients [27]. Three studies were conducted in children [28-30]. The mean (SD) age of patients from the individual studies was 24.7 (17.8) years (8.5 [0.7] years in the children's studies). Five studies included patients monosensitized to grass [28-32]. The proportion of patients with asthma was low (on average 8.5%), with a mean FEV_1 of 97.4% (5.1). Treatment duration varied from 4 months [25,32] to 36 months [27,31]. The study by Ott et al (ECRIT) [27] also evaluated SS and MS over 1 follow-up season, although the data were not included in this meta-analysis. The cumulative AIT dose ranged from 4500 IR [25] to 108 560 IR (Table) [29].

The effect of 5-grass SLIT-liquid on SS is shown in Figure 2A. The 3-arm study by Stelmach et al [30] in 2012 was considered as 2 different studies, since there were 2 active treatment arms (based on 2 different administration protocols), which were compared with placebo. This led to duplication of the 18 patients in the placebo arm. However, a sensitivity

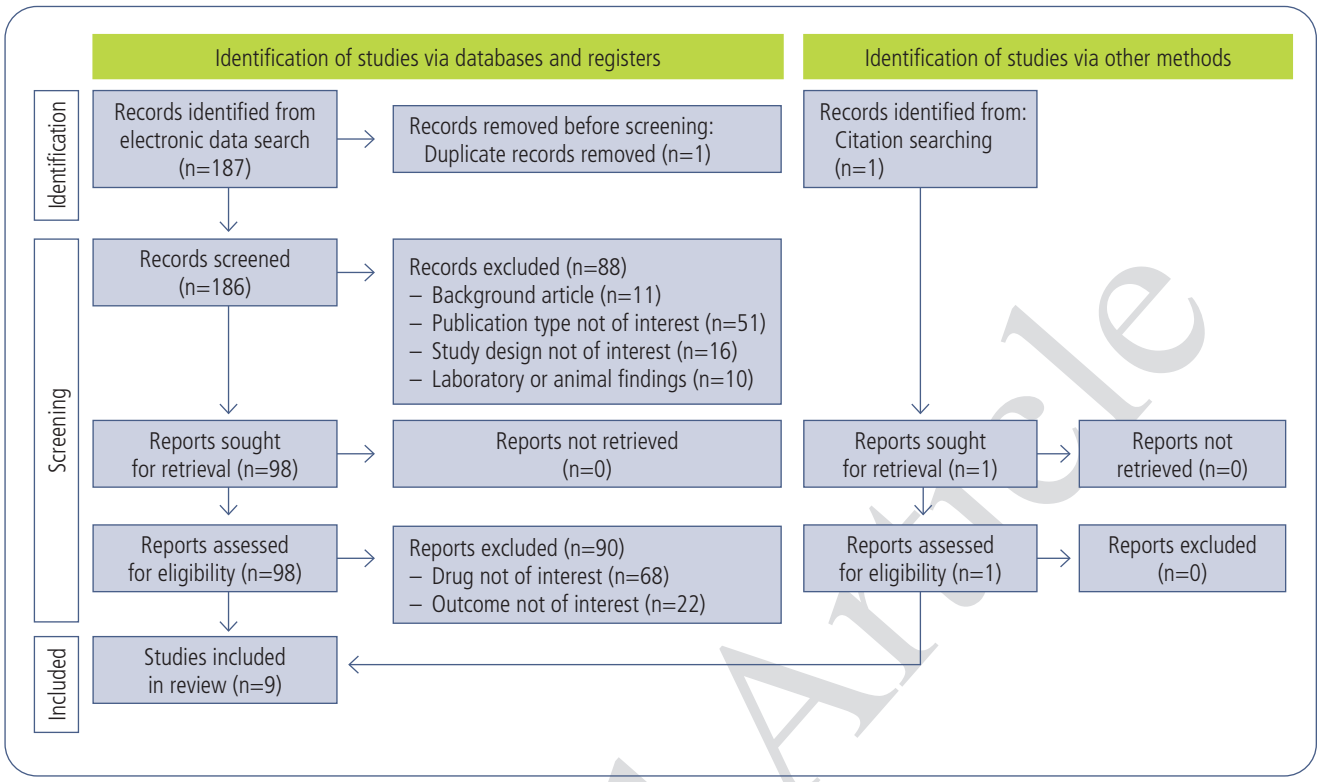

Figure 1. Flow diagram of study selection.

Table. Patient and Study Characteristics

Study, year, country	Patients	Male, %	Age, y[a]	Mono-/ Poly-sensitized	Rhinitis, No. (%)	Asthma, No. (%)	Duration, mo	Cumulative dose
Sabbah, 1994 France	AIT 29→29 C 29→29	31 (53.4)	23 (10 [13-43]) 27 (12 [13-51])	27/31	29 (100) 29 (100)	NR NR	4	4 500 IR (13 500/y)
Clavel, 1998 France	AIT 62→62 C 58→58	71 (59.2)	29 (13 [9-55]) 26 (12 [8-55])	26/94	62 (100) 58 (100)	10 (16) 16 (27.6)	6	40 700 IR (81 400/y)
Ott, 2009 Germany	AIT 123→99 C 60→46	71 (38.8)	33.2 (11.0) 33.7 (9.1)	66/69	99 (100) 46 (100)	14 (14.1) 5 (10.9)	36	81 140 IR (27 000/y)
Stelmach, 2009 Poland	AIT 25→20 C 25→15	22 (44)	9.1 (2.4 [6-17]) 8.5 (2.8)	35/0	NR NR	20 (100) 15 (100)	24	43 800 IR (21 900/y)
Kałuzińska, 2011 Poland	AIT 15→13 C 15→12	19 (63.3)	8.3 (3.3 [6-18]) 8.1 (3.3)	30/0	15 (100) 15 (100)	4 (30) 3 (25)	24	108 560 IR (54 280/y)
Sieber, 2012 Germany	AIT 142→132 C 67→63	NR	(7.9-64.7)	NR	142 (100) 67 (100)	NR NR	36	66 000 IR (22 000/y)
Stelmach, 2012 Poland	pre-co 17→17 cont. 19→19 C 18→18	36 (66.7)	8.3 (5-17) 10.1 (3-16) 8.1 (4-15)	54/0	17 (100) 19 (100) 18 (100)	6 (35) 5 (26) 5 (18)	24	43 200 IR (21 600/y) 87 600 IR (43 800/y)
Bozek, 2014 Poland	AIT 41→38 C 37→34	41 (52.6)	63.18 (3.12) 64.13 (2.92)	78/0	41 (100) 37 (100)	3 (7.32) 2 (5.4)	36	64 000 IR (21 300/y)
Kralimarkova, 2014 Bulgaria	AIT 28→21 C 28→24	33 (58.9)	30.3 (12.6) 30 (12.5)	51/0	25 (100) 26 (100)	10 (36) 10 (36)	5	45 000 IR (108 000/y)

Abbreviations AIT, allergen immunotherapy; C, controls; cont., continuous treatment; IR, index of reactivity; NR, not reported; pre-co, pre-coseasonal treatment; →, number of patients from enrolment to the observation time-point.
[a]Values shown as mean (SD [range]) or mean (SD).

analysis halving the number of patients in the placebo arm in each study did not show any significant difference (Table S2). The pooled SMD for the treatment effect was –0.34 (95%CI, –0.62 to –0.06; *P*<.05), indicating a statistically significant benefit of SLIT over placebo. The analysis using the fixed-effect model yielded comparable results. A substantial degree of heterogeneity between the results of individual studies was reported (Q=108.1; df=8; *P*<.00001; I^2=61%) [11], although this decreased to I^2=0% after the exclusion of 2 outlying studies (Figure 2C and Table S2) [29,32]. Notably, 1 of these 2 studies was judged as being of medium quality (Figure 2D) [32].

The visual inspection of the funnel plots and the Egger test did not show evidence of publication bias (Figure 2B), and the fail-safe number was sufficiently high (n=31) to confirm the robustness of these results against publication bias.

Subgroup analysis by age and sensitization status did not reveal significant differences between the subgroups (Figure 3A). Meta-regressions showed no significant difference according to cumulative yearly AIT dose received, study duration, or asthma status (Figure 3B, C, D).

Figure 4A shows data on MS. The pooled SMD was –0.54 (95%CI, –0.97 to –0.10; *P*<.05), with considerable between-study heterogeneity (I^2=79%) [11]. The exclusion of 2 influential studies (Figure 4C) led to a significant reduction in heterogeneity (I^2=45%) without affecting the final result (Table S2). There was no evidence of publication bias (Figure 4B).

Like the SS, subgroup analysis for the MS did not show significant differences based on age or sensitization status (Figure 5A), and meta-regressions did not show significant differences in the outcome according to cumulative yearly AIT dose received, duration of treatment, or asthma status (Figure 5B, C, D).

The robustness of the findings was confirmed using sensitivity analyses conducted by (*1*) removing influential studies, (*2*) removing studies with duplicated controls, (*3*) comparing studies with smaller and larger sample sizes (above/below the median value of 57), (*4*) evaluating study quality, and (*5*) comparing studies with available data to those

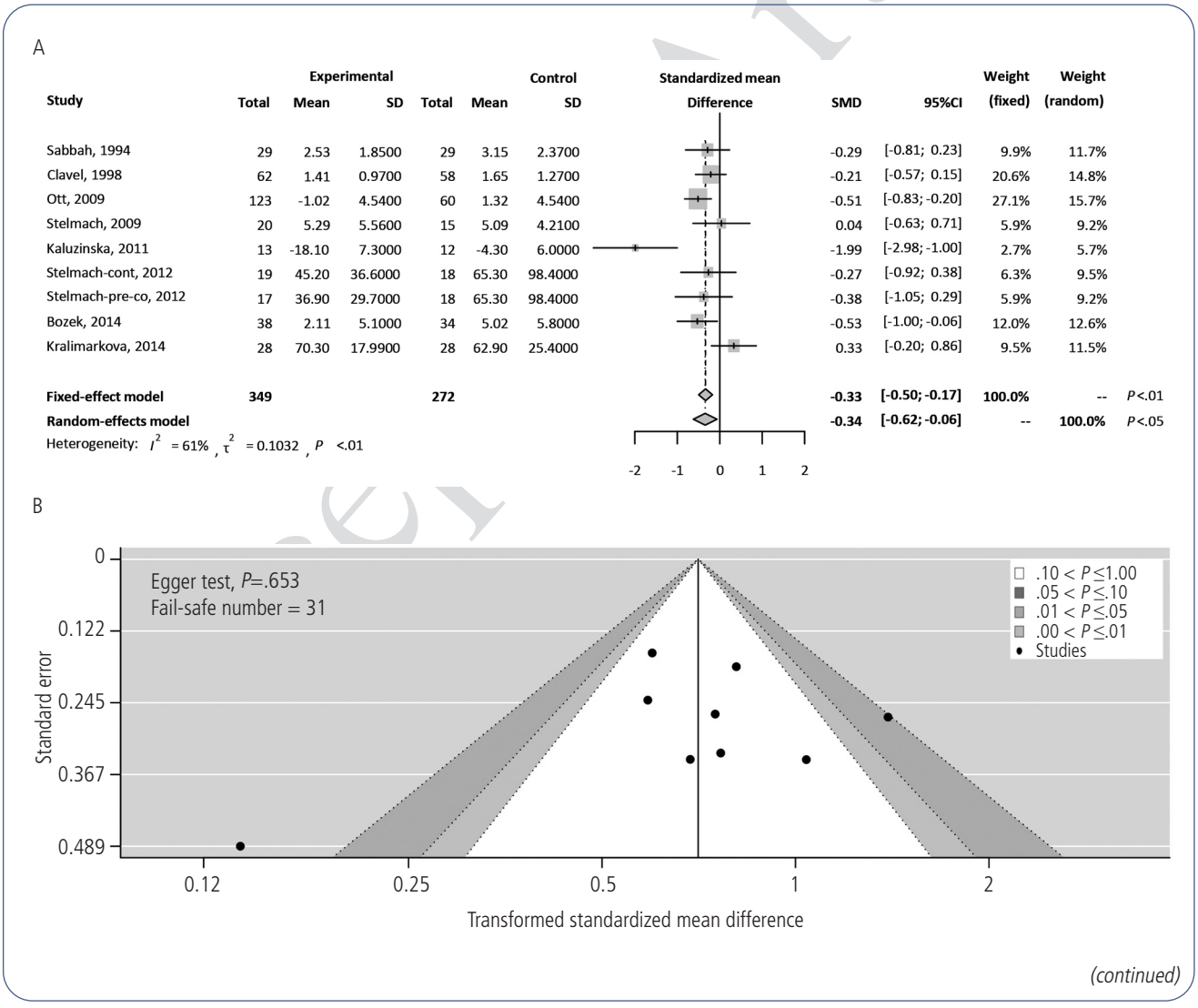

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Figure 2.

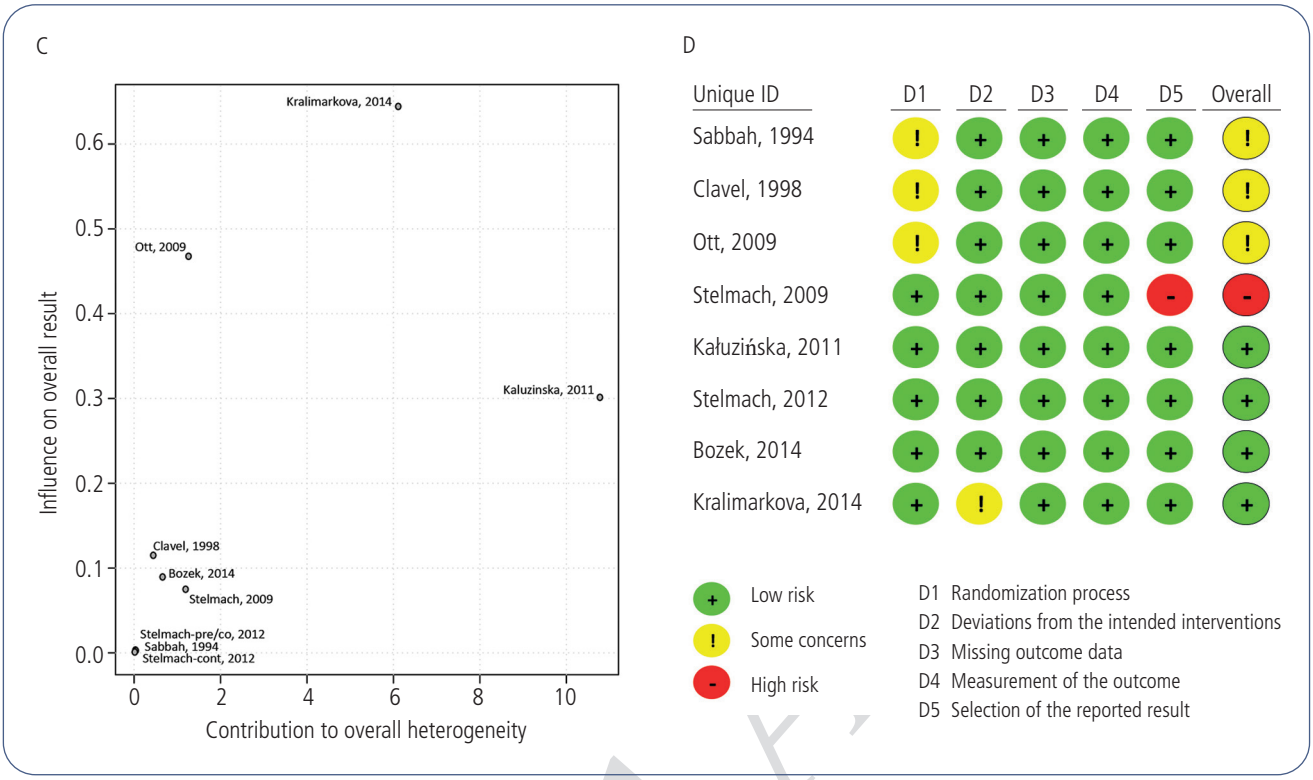


Figure 2. Meta-analysis of 8 randomized controlled trials of sublingual immunotherapy versus placebo for allergic rhinoconjunctivitis using the random-effects model and the fixed-effect model. The SMD and 95% CI for the effect of SLIT on symptom score are plotted on the graph. Studies of each group are arranged by publication year. The Stelmach et al. 2012 study was considered as 2 different studies as there were two active treatment arms (cont [continuous treatment], and pre-co [pre-coseasonal treatment]) vs. placebo. For studies assessing SS across different pollen seasons, results of the last treatment year were reported: Sabbah 1994, Clavel 1998, and Kralimarkova 2014: first treatment year; Stelmach 2009, Kałuzińska 2011, Stelmach 2012: second treatment year; Ott 2009 and Bozek 2014: third treatment year. Data from the Sabbah et al. and Kałuzińska et al. studies were estimated from the graphs. Data for the Clavel et al. study were kindly provided by the study sponsor (A). Contour-enhanced funnel plot, which displays areas of statistical significance in the funnel plot. Asymmetry particularly in the area of non-significance adds further credence that it can be caused by publication bias (B). Baujat plot, showing the relationship between each study's contribution to heterogeneity (x-axis) and its impact on the pooled estimate (y-axis) (C). Study risk of bias assessed by the RoB2 tool (D). SMD indicates standardized mean difference *(continuation)*

in which the data for the analysis were estimated (see legend of Figures 2 and 4) (Table S2).

The overall certainty of evidence for the main outcomes was rated as moderate for SS after excluding the influential study by Kaluzinska et al [29] and low for MS after excluding the influential study by Stelmach et al (2012) (Table S3) [30].

Data on AEs were available for 335 patients in the SLIT group and 217 in the placebo group, as not all studies reported all the relevant data. A total of 69 patients under SLIT (20.6%) and 38 under placebo (17.5%) reported AEs (P=.46) (Table S4). Most of the treatment interruptions were for reasons other than AEs and were significantly more frequent in the placebo group than in the SLIT group (P=.04). Treatment was discontinued because of AEs in 3% of the patients in the SLIT group and in 1.8% of those in the placebo group (P=.41).

Discussion

This meta-analysis of data from 8 RCTs including more than 600 patients with ARC to grass pollen receiving a single specific AIT product, 5-grass SLIT-liquid, provides evidence of the efficacy of the treatment in reducing symptoms and the need for symptomatic medication, with no safety issues. The data agree with those of other reports showing that SLIT is effective and safe in patients with ARC with or without mild-to-moderate asthma. The overall effect size seems slightly greater than that of other liquid SLIT products and comparable to SLIT tablets [2,3].

Subgroup analyses revealed no discernible age effect on the efficacy of 5-grass SLIT-liquid. The results remained consistent across adult and child subgroups (Figures 3A, 5A). Notably, the children's studies, all of which were long-term (spanning 24 months) [28-30], exhibited a low dropout rate, ranging from 0% to 30%. Furthermore, the adult studies, with 2 spanning 36 months [27,31], had a dropout rate ranging from 0% to 21% (Table), suggesting strong adherence to the treatment regimen. Despite the controlled environment of RCTs, which inherently foster better adherence owing to rigorous monitoring, the enduring nature of these studies underscores the product's favorable safety and tolerability profile. This assertion is reinforced by the comparable withdrawal rates between active and placebo groups, with discontinuations not related to adverse

events being more prevalent in the placebo arm (Table S4). This difference may stem from the perceived lack of efficacy in the placebo group, which prompted cessation of treatment.

Ensuring adherence and maintaining treatment persistence are pivotal considerations in AIT. Premature discontinuation of treatment, occurring before the recommended minimum 3-year duration, is linked with enduring loss of efficacy in symptom management and the prevention of new asthma cases and allergic sensitizations [34,35]. The dropout/withdrawal rate observed in studies involving adults mirrored that of studies involving children, affirming consistent findings regarding tolerability and adherence across both age groups. These results were reflected similarly in both 3-year studies [27,31].

Subgroup analysis based on sensitization status found no distinction between monosensitized and polysensitized patients, despite certain studies demonstrating the highest efficacy among monosensitized patients, in terms of both SS and MS (Figures 3A, 5A). However, it is important to note that polysensitized patients included in RCTs are likely those primarily allergic to grass pollen or whose symptoms were predominantly driven by grass pollen. This could explain the absence of differences in the overall effect based on patients' sensitization status.

Meta-regression analysis based on study duration revealed no significant difference in the effect size according to treatment duration, affirming the efficacy of SLIT from the outset of the pollen season and its sustained efficacy over time. Similarly, meta-regression analysis based on cumulative yearly dose demonstrated no variation in efficacy between studies applying different dosages (Figures 3B, 5B). This suggests that

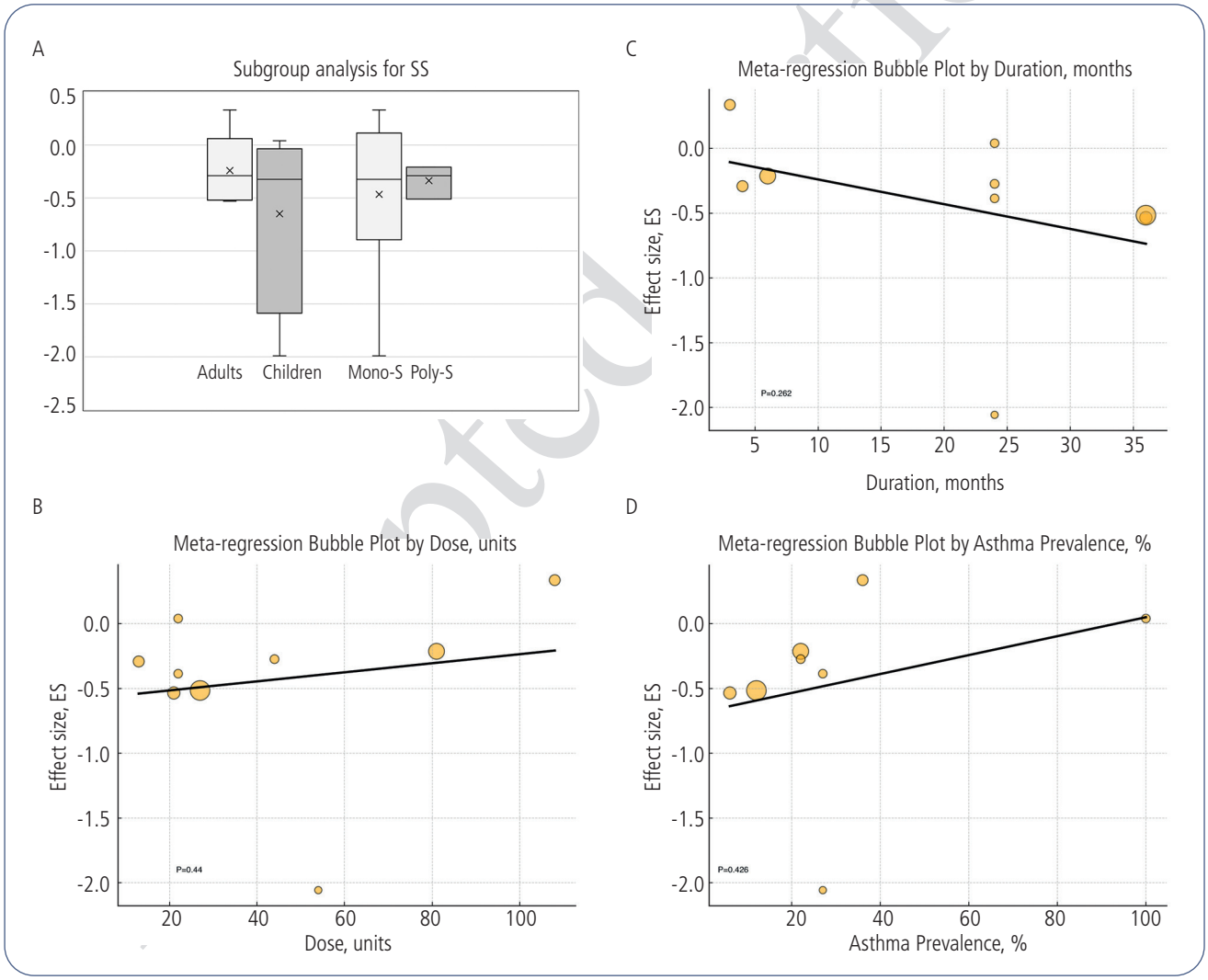


Figure 3. Subgroup analyses of symptom score by age and sensitization status of sublingual immunotherapy vs placebo. Box plots include the middle 50% of the data. The horizontal bars inside the boxes represent the median standardized mean differences. The solid lines to the whiskers extend to the most extreme data points, which are no more than 1.5 times the interquartile range from the box (A). Meta-regression analyses of symptom score for the efficacy of sublingual immunotherapy depending on the cumulative yearly dose administered (B), treatment duration (C), and asthma prevalence (D). The plots show the relationship between study characteristics (covariates: dose administered, treatment duration, and asthma prevalence) and effect estimates across studies. Each circle represents an individual study, with the x-axis displaying the covariate of interest and the y-axis the effect size. The size of the circles reflects the study sample size. The regression line indicates the relationship between the covariate and the effect size. ES indicates estimate.

the dose of 5-grass SLIT-liquid can be safely adjusted for better adverse event management without compromising treatment outcomes. This flexibility makes it possible to tailor treatment according to the patient's condition while addressing their needs and expectations. Subsequently, it facilitates achieving the recommended minimum 3 years of treatment, thereby enhancing efficacy.

Finally, no differences in the efficacy of 5-grass SLIT-liquid were observed based on asthma prevalence (Figures 3D, 5D), indicating that asthma does not appear to influence patients' perception of ARC symptoms.

There was marked variation in reporting AEs, and most studies did not report them. Few patients withdrew from treatment, even in long-term studies, and there were no significant differences in withdrawal for AE between SLIT and placebo, suggesting that the treatment is well tolerated. The treatment was also safe, as no cases of anaphylaxis were reported. It should also be noted that while the effect of 5-grass SLIT-liquid after treatment cessation was not the focus of this meta-analysis, one of the qualifying studies, the ECRIT study by Ott et al [27] showed a significant reduction in SS during the follow-up season, indicating a carry-over effect of this treatment following 3 consecutive seasons of therapy.

Strengths and Limitations

Firstly, focusing on a particular product led to a noteworthy decrease in heterogeneity, particularly following the removal of influential studies. This led to consistent estimates between random-effects and fixed-effect models, thus enhancing the reliability of the finding that the product is efficacious. Of note, the clinical benefits of 5-grass SLIT-liquid in the short term and long term and after treatment have been further confirmed in real-world studies in more than 1800 patients [36-45]. Moreover, studies showed a significant improvement in patients' quality of life after 3 years of 5-grass SLIT-liquid, indicating a meaningful effect of this treatment beyond the statistically significant results of RCTs [43-45].

A

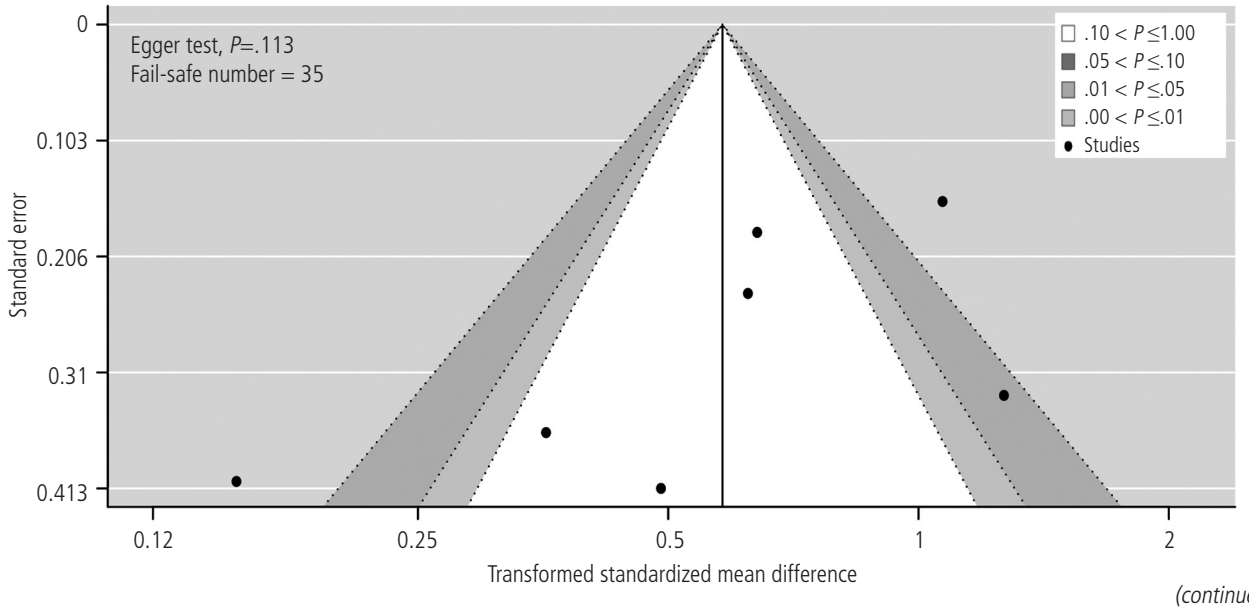
Study	Experimental Total	Mean	SD	Control Total	Mean	SD	SMD	95%CI	Weight (fixed)	Weight (random)	
Clavel, 1998	62	0.81	0.9400	58	1.33	1.3500	-0.45	[-0.81; -0.08]	25.4%	17.1%	
Ott, 2009	123	-0.28	11.5500	60	-0.92	2.4700	0.07	[-0.24; 0.38]	35.0%	17.7%	
Stelmach, 2009	20	5.10	1.7700	15	7.48	2.7800	-1.03	[-1.75; -0.31]	6.5%	12.7%	
Kaluzinska, 2011	13	-4.60	1.6100	12	0.00	8.8600	-0.71	[-1.53; 0.10]	5.0%	11.6%	
Stelmach-cont, 2012	19	11.90	4.3000	18	10.80	4.8000	0.24	[-0.41; 0.88]	8.0%	13.6%	
Stelmach-pre-co, 2012	17	3.80	1.6000	18	10.80	4.8000	-1.89	[-2.70; -1.08]	5.1%	11.6%	
Bozek, 2014	38	0.43	0.7100	34	0.88	1.1700	-0.47	[-0.94; 0.00]	15.1%	15.8%	
Fixed-effect model	**292**			**215**			**-0.34**	**[-0.52; -0.16]**	**100.0%**	--	*P*<.01
Random-effects model							**-0.54**	**[-0.97; -0.10]**	--	**100.0%**	*P*<.05

Heterogeneity: I^2 = 79%, τ^2 = 0.2542, *p* <.01

Standardized mean difference

-2 -1 0 1 2

B



(continued)

Figure 4.

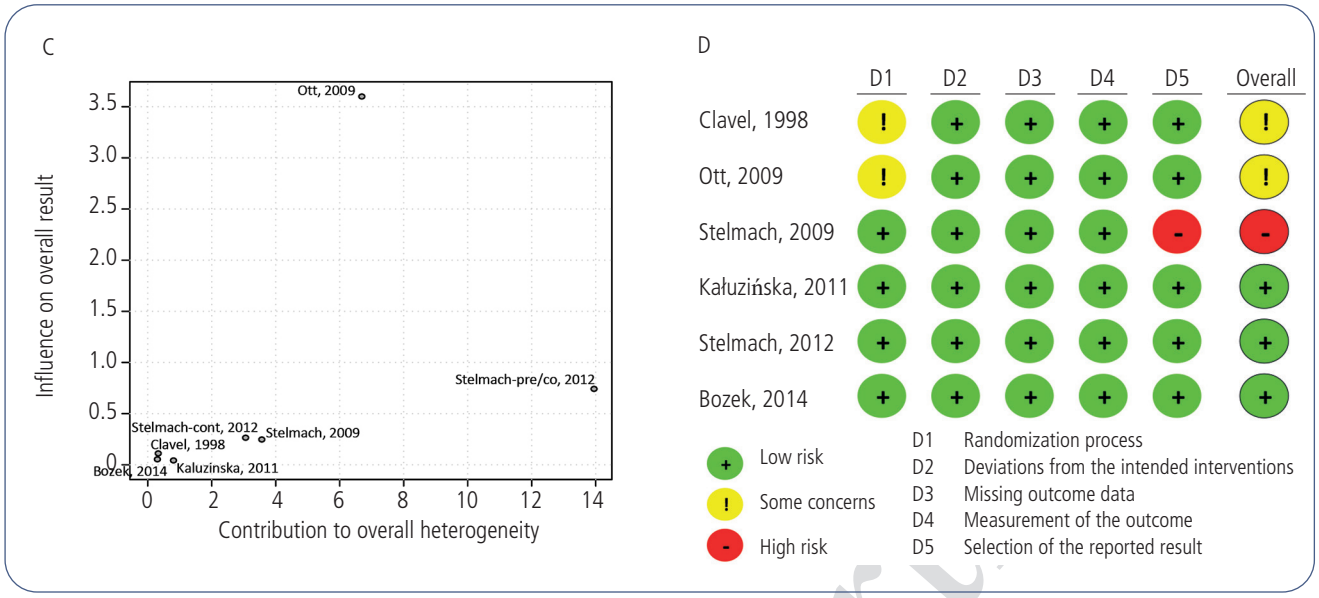


Figure 4. Meta-analysis of 6 randomized controlled trials of sublingual immunotherapy versus placebo for allergic rhinoconjunctivitis using the random-effects model and the fixed-effect model. The SMD and 95% CI for the effect of SLIT on medication score are plotted on the graph. Studies of each group are arranged by publication year. The Stelmach et al. 2012 study was considered as 2 different studies as there were two active treatment arms (cont [continuous treatment], and pre-co [pre-coseasonal treatment]) vs. placebo. For studies assessing medication scores across different pollen seasons, results of the last treatment year were reported: Clavel 1998: first treatment year; Stelmach 2009, Kałuzińska 2011, Stelmach 2012: second treatment year; Ott 2009 and Bozek 2014: third treatment year. Data from the study by Kałuzińska et al were estimated from the graphs. Data for the Clavel et al. study were kindly provided by the study sponsor (A). Contour-enhanced funnel plot, which displays areas of statistical significance in the funnel plot. Asymmetry particularly in the area of non-significance adds further credence that it can be attributed to publication bias (B). Baujat plot, showing the relationship between each study's contribution to heterogeneity (x-axis) and its impact on the pooled estimate (y-axis) (C). Study risk of bias assessed by the RoB2 tool (D). SMD indicates standardized mean difference *(continuation)*.

Secondly, the minimal risk of publication bias and the results of the sensitivity analyses confirmed the robustness of the findings. However, we acknowledge that it is challenging to rule out publication bias entirely in a meta-analysis.

The main limitation of this analysis is the small sample size of most of the studies included (median sample size, 57 patients), which is likely one of the main reasons for the inconsistency and heterogeneity recorded among the individual studies, resulting in imprecision of the pooled effect. These limitations contributed to reducing the certainty of evidence to moderate for the SS and low for the MS (Table S3). Additionally, the small sample size in most of the studies included diminished the statistical power of the meta-analysis.

Nonetheless, it is noteworthy that the sensitivity analysis comparing studies with sample sizes above and below the median revealed similar outcomes and that heterogeneity was eliminated within the subgroup of larger studies evaluated for the SS.

Variations in dosages and treatment durations across studies could represent a potential limitation. While we attempted to account for these factors using meta-regression analyses, they remain a source of heterogeneity that could affect the consistency of the results.

Finally, the notable variation in reporting of adverse events across studies, with some trials lacking sufficient data, hindered a comprehensive evaluation of the safety profile of 5-grass SLIT-liquid.

Conclusions

This meta-analysis confirms that 5-grass SLIT-liquid is effective in improving the symptoms of ARC and reducing the need for symptomatic medication compared with placebo. Efficacy is not affected by major comorbidity (eg, bronchial asthma), age, treatment duration, or cumulative dose administered. The effect size is comparable to that of other immunotherapy products, with low rates of AEs and withdrawals due to AEs or for reasons other than AEs, suggesting good tolerability and adherence.

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Conflicts of Interest

DDB declares having received fees from Stallergenes Greer in relation to this work. GP declares having received payment or honoraria for lectures, presentations, speaker's bureaus, manuscript writing, or educational events from AstraZeneca, GlaxoSmithKline, and LoFarma. JCS and SS are employees of Stallergenes Greer. GWC declares having received consulting fees, and/or payment or honoraria for lectures, presentations, speaker's bureaus, manuscript writing, or educational events,

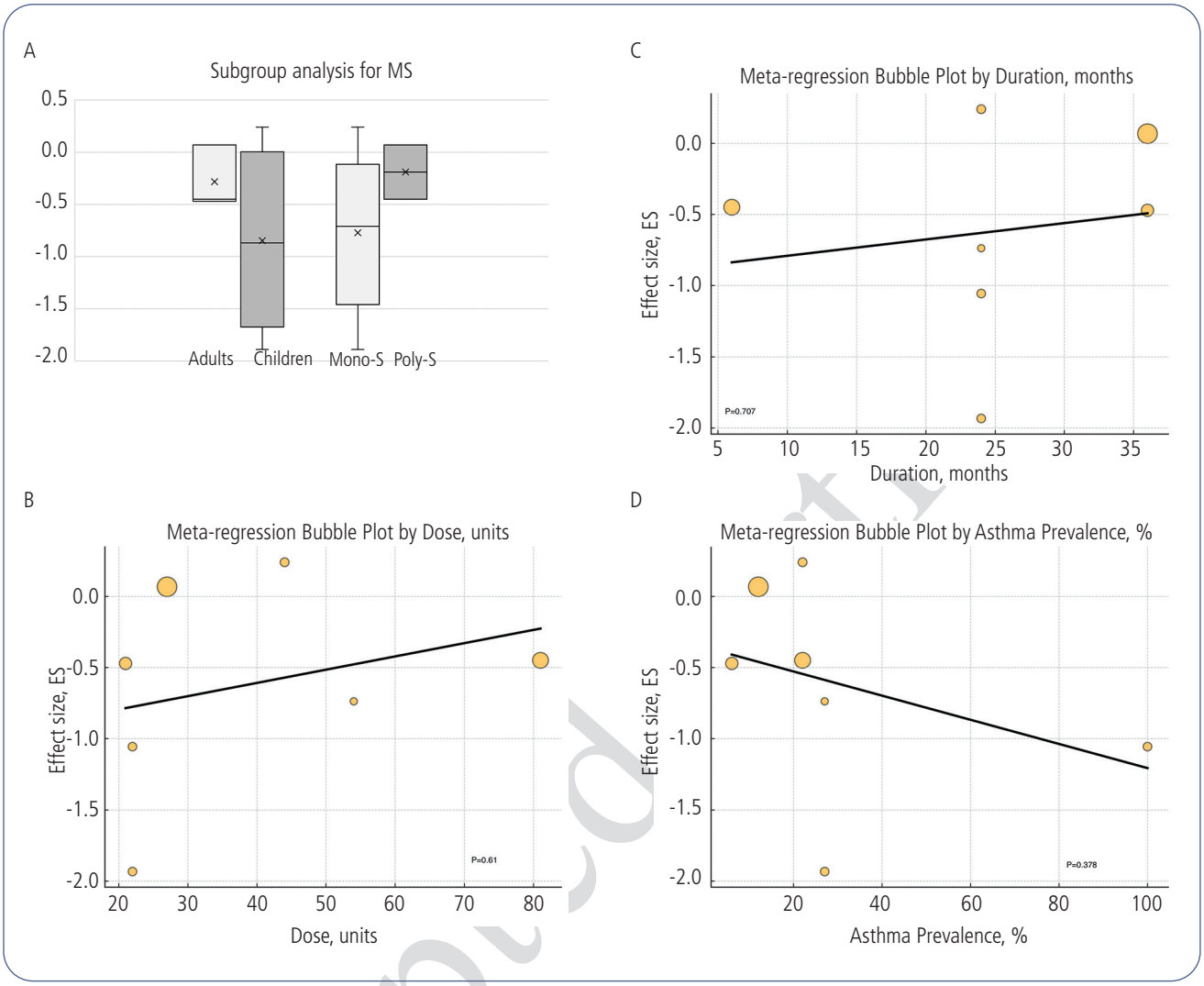

Figure 5. Subgroup analyses of medication score by age and sensitization status of sublingual immunotherapy vs placebo. Box plots include the middle 50% of the data. The horizontal bars inside the boxes represent the median standardized mean differences. The solid lines to the whiskers extend to the most extreme data points, which are no more than 1.5 times the IQR from the box. A, Meta-regression analyses of medication score for the efficacy of sublingual immunotherapy depending on the cumulative yearly dose administered (B), treatment duration (C), and asthma prevalence (D). The plots show the relationship between study characteristics (covariates: dose administered, treatment duration, and asthma prevalence) and effect estimates across studies. Each circle represents an individual study, with the x-axis displaying the covariate of interest and the y-axis showing the effect size. The size of the circles reflects the study sample size. The regression line indicates the relationship between the covariate and the effect size. ES indicates estimate.

and/or support for attending meetings and/or travel, and/or participation on data safety monitoring boards or advisory boards from Allergy Therapeutics, Anallergo, Hal Allergy, and Stallergenes Greer. GS declares that he has no conflicts of interest.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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